Involvement of Serotonergic Receptor Subtypes in the Production of Antinociception by Psychological Stress in Mice

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ABSTRACT—Besides the important role of emotional factors in the production of psychological-stress-induced analgesia (PSY-SIA), recent attention to the participation of serotonergic (5-HTergic) neurons in the fear- and anxiety-evoking mechanism led us to examine the effects of 5-HTergic ligands on PSY-SIA. Pretreatment of mice with 2.0 to 10 mg/kg of methysergide, a 5-HT receptor antagonist, or 1.0 to 10 mg/kg of buspirone, a 5-HTIA receptor partial agonist, dose-dependently suppressed the production of PSY-SIA. Ritanserin, a 5-HT receptor antagonist, 1.0 to 5.0 mg/kg, or Y-25,130, a 5-HT receptor antagonist, 0.03 and 0.1 mg/kg, also inhibited PSY-SIA dose-dependently, while (±)pindolol, a 5-HTIA/IB receptor antagonist, was ineffective at doses up to 3.0 mg/kg. Furthermore, the suppressive effect of PSY-stress on the development of antinociceptive tolerance to morphine was also antagonized by methysergide, buspirone, ritanserin and Y-25,130, but not by (±)pindolol. These results suggest that 5-HT receptor (5-HTIA, 5-HTIB and 5-HTIB but not 5-HTIB)-mediated mechanisms play an important role in the production of PSY-SIA.

Keywords: Communication box, Stress-induced analgesia (SIA), Serotonin (5-HT), Buspirone, Morphine tolerance

Benzodiazepines (BZPs) are widely used as anxiolytic drugs, and we have previously demonstrated that diazepam (DZP), an anxiolytic agent acting on BZP receptors, inhibits the psychological-stress-induced analgesia (PSY-SIA) dose-dependently (1, 2) and β-carboline-3-carboxylic acid ethyl ester, an inverse agonist on BZP receptors, potentiates the PSY-SIA (2). Likewise, we have found that concurrent exposure to PSY stress blocks the development of antinociceptive tolerance to morphine, and the effect of PSY-stress is reversed by DZP, indicating the involvement of emotional factors such as anxiety, fear and unpleasantness, which are mediated through BZP receptors in the production of PSY-SIA (2).

It is well known that administration of BZPs is often accompanied by undesirable side effects such as drowsiness, ataxia and sedation. Because of the discovery of the anti-anxiety action of buspirone, a partial agonist of serotonin (5-HTIB receptors, that is devoid of side effects of BZPs (3, 4), the importance of 5-HTergic mechanisms for the control of anxiety is now being realized. In addition, BZPs are known to reduce 5-HT turnover and decrease the activity of the central 5-HT neurons (5), suggesting the interaction between the BZP and the 5-HT system.

The present study was carried out to clarify the involvement of the 5-HT receptor-mediated mechanisms in the production of PSY-SIA, using typical compounds selective for the 5-HT receptor subtypes.

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. They were maintained in an ambient temperature (22±1°C) and relative humidity (55±5%) controlled room with free access to laboratory diet (MF, Oriental Yeast, Tokyo) and tap water. After reaching 23 to 28 g, they were used for the experiments.

Drugs and administration schedules
Morphine-hydrochloride (Takeda Pharm. Co., Osaka) and methysergide maleate, a 5-HT receptor antagonist (U.S.P.C., Inc., Rockville, MD, USA) were commercially obtained. The following drugs were generously donated: buspirone-hydrochloride, a 5-HTIB receptor partial agonist (Bristol-Myers Co., Evansville, IN, USA); (±)pin-
dolol, a 5-HT1A/1B receptor antagonist (Sandoz, Berne, Switzerland); ritanserin tartrate, a 5-HT2 receptor antagonist (Janssen-Kyowa Pharm. Co., Tokyo) and Y-25,130 ((±)N-(1-azabicyclo[2,2,2]oct-3-yl)-6-chloro-4-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-8-carboxamide hydrochloride), a 5-HT3 receptor antagonist (Yoshitomi Pharm. Co., Fukuoka).

Methysergide was dissolved in 25% polyethylene glycol, and ritanserin was ultrasonically dispersed in 10% dimethyl sulfoxide. Other drugs were dissolved in saline. They were administered i.p. in a volume of 0.1 ml/10 g of body weight, and the dose was expressed in terms of the respective salt.

Morphine and other drugs were injected 15 min and 30 min, respectively, before exposure to PSY-stress.

**Exposure to PSY-stress**

Using the communication box with a slight modification of the method of Ogawa and Kuwahara (6), animals were exposed to PSY-stress by watching and hearing the struggle, jumping and vocalization of the footshocked animals for 5 min. The protocol and techniques for exposure to PSY-stress were described in our previous report (7).

**Assessment of antinociceptive effect**

The antinociceptive effect was measured by the modified Haffner's method (8), which is a tail pinch test (TP), with a cutoff time of 6 sec to avoid any damage to the tail, every 5 min from immediately after the termination of the stress exposure for 15 min or every 15 min after the administration of morphine for a period of 90 min. In the case of concurrent exposure to PSY-stress with morphine, the 1st measurement was performed at 20 min after the morphine injection.

**Evaluation of morphine tolerance**

Injection of morphine at the dose of 10 mg/kg, i.p. was repeated daily for 5 days. The effect was expressed as the area under the curve (AUC) obtained by plotting the increase in response time (sec) on the ordinate and the time intervals (min) on the abscissa. A significant decrease of AUC, compared with that of the 1st day, indicated the development of tolerance.

**Statistical analyses**

The results were expressed as the mean ± S.E. Following 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. A difference was considered significant at P < 0.05.

![Fig. 1. Effect of methysergide and buspirone on PSY-SIA.](image-url)

The figure shows the effect of methysergide and buspirone on PSY-SIA. Mice were exposed to psychological (PSY)-stress for 5 min. The antinociceptive effect was measured by the tail pinch (TP) method, every 5 min from immediately after the termination (0 min) of the PSY-stress exposure. Methysergide and buspirone were administered i.p. 30 min before PSY-stress. Each group consisted of 12–14 animals, and each point indicates the mean ± S.E. (vertical bar). The dotted area indicates the response time before exposure to stress. The control group (C) was treated with vehicle instead of these drugs at the corresponding time. (a) methysergide, 2.0 (A), 5.0 (■) or 10 (●) mg/kg; (b) buspirone, 1.0 (▲), 3.0 (■) or 10 (●) mg/kg. **P<0.01, *P<0.05, compared with the control group.
RESULTS

Effect of methysergide, buspirone, (±)pindolol, ritanserin and Y-25,130 on PSY-SIA

Exposure to PSY-stress produced short-lasting antinociception. Pre-treatment of mice with methysergide (2.0, 5.0 and 10 mg/kg) or buspirone (1.0, 3.0 and 10 mg/kg) dose-dependently suppressed PSY-SIA (Fig. 1). Ritanterin at the doses of 1.0 to 5.0 mg/kg or Y-25,130 at the doses of 0.03 and 0.1 mg/kg inhibited PSY-SIA dose-dependently, while (±)pindolol was ineffective at doses up to 3.0 mg/kg (Fig. 2). Throughout the experiments, these drugs at the doses employed here did not affect the response time before the exposure to PSY-stress (data not shown).

Effect of methysergide, buspirone, (±)pindolol, ritanterin and Y-25,130 on the blockade by PSY-stress of the development of morphine tolerance

Daily injection of morphine at 10 mg/kg developed tolerance to its antinociceptive effect, and the effect was remarkably reduced by 5 repetitions. Concurrent exposure to PSY-stress completely suppressed the development of antinociceptive tolerance to morphine, and the suppressive effect was antagonized by methysergide at 2.0 mg/kg; buspirone at 1.0 or 3.0 mg/kg (Fig. 3); ritanterin at 1.0 and 2.0 mg/kg; and Y-25,130 at 0.03 and 0.1 mg/kg. However, (±)pindolol at doses up to 3.0 mg/kg did not reverse the suppressive effect of PSY-stress (Fig. 4). Such a pretreatment with these drugs had no effect on the morphine antinociception and its tolerance development (data not shown).

DISCUSSION

In a series of studies, we found that in the production of PSY-SIA, various receptor-mediated systems such as opioid (7, 9), catecholamine (10), BZP (1, 2) and GABA (11) are involved. Based on these findings, in this study, the participation of 5-HTergic mechanisms was examined using drugs selective for the 5-HT receptor subtypes.

It is reported that buspirone, a 5-HT1A receptor partial agonist, produces an anxiolytic effect in several anxiety model animals (12–14). Complete inhibition of PSY-SIA by buspirone at doses of 3.0 and 10 mg/kg indicates the participation of 5-HT1A receptors in the production of PSY-SIA. Buspirone, however, possesses appreciable affinity for dopamine D2-receptors as well as 5-HT1A receptors, and consequently, the involvement of dopamine D2 receptors in the production of PSY-SIA could not be neglected (15, 16).

It is well known that (±)pindolol has an antagonistic activity on 5-HT1A and 5-HT1B receptors in addition to β-adrenoceptors (17, 18). In this experiment, PSY-SIA was not affected by (±)pindolol. Failure of (±)pindolol to suppress PSY-SIA suggest that 5-HT1B receptors have little importance in the mediation of PSY-SIA in contrast to 5-HT1A receptors and, possibly, that (±)pindolol and...
Fig. 3. Blockade of the development of antinociceptive tolerance to morphine by PSY-stress and reversal of the effect by methysergide and buspirone. Fifteen minutes after the administration of morphine (10 mg/kg, i.p.) to mice, they were exposed to PSY-stress for 5 min. The antinociceptive effect was expressed as the area under the curve (AUC) obtained by plotting the increase in response time (sec) on the ordinate and the time intervals (min) on the abscissa. Methysergide and buspirone were administered i.p. 15 min before morphine injection. (a) PSY without drug (△), PSY with methysergide, 2.0 mg/kg (▲); (b) PSY without drug (△), PSY with buspirone, 1.0 (▲), 3.0 (■) mg/kg. **P < 0.01, *P < 0.05, compared with the control group treated daily with morphine (○); #P < 0.01, #P < 0.05, compared with the corresponding value on the 1st day. For other details, refer to the legend of Fig. 1.

Fig. 4. Blockade of the development of antinociceptive tolerance to morphine by PSY-stress and reversal of the effect by (±)pindolol, ritanserin and Y-25,130. (a) PSY without drug (△), PSY with (±)pindolol, 1.0 (▲), 3.0 (■) mg/kg; (b) PSY without drug (△), PSY with ritanserin, 1.0 (▲), 2.0 (■) mg/kg; (c) PSY without drug (△), PSY with Y-25,130, 0.03 (▲), 0.1 (■) mg/kg. **P < 0.01, *P < 0.05, compared with the control group treated daily with morphine (○); #P < 0.01, #P < 0.05, compared with the corresponding value on the 1st day. For other details, refer to the legends of Figs. 1 and 3.
buprioner act on $5\text{-HT}_{1A}$ receptors in a different manner.

Ritanserin has been characterized as a potent and long-acting central $5\text{-HT}_2$ receptor antagonist (19). As regards to its anti-anxiety effect, however, no distinct conclusion has been reached (20–23). In the present study, ritanserin showed an apparent suppressive action on PSY-SIA. Therefore, it is suggested that $5\text{-HT}_2$ receptors participate in the production of PSY-SIA in addition to $5\text{-HT}_{1A}$ receptors. Although Rodgers et al. (23) and Barber et al. (24) have reported that 5.0 mg/kg of ritanserin induces antinociception, we found that administration of 5.0 mg/kg of ritanserin, i.p. did not induce the antinociceptive effect in the TP method (data not shown). The discrepancy between the two reports depends on the differences of the animal and test method employed.

Recently, Rodgers et al. (25) have demonstrated that GR 38032F (ondansetron), a $5\text{-HT}_3$ receptor antagonist, has an anti-anxiety effect and $5\text{-HT}_2$ receptors play an important role in the generation of anxiety. Similarly, Y-25,130, a $5\text{-HT}_3$ receptor antagonist (26), blocked PSY-SIA. Thus, our results support the possibility of the involvement of $5\text{-HT}_2$ receptors in the production of PSY-SIA.

Vonvoigtlander et al. (27) and Ho and Takemori (28) have shown that $5\text{-HT}_3$ergic mechanisms participate in the production of antinociception by U-50,488H, a $\kappa$-opioid receptor agonist. Our previous reports (7, 9) revealed that PSY-SIA involved the mediation by $\kappa$-opioid receptor mechanisms, and in this experiment, we showed that $5\text{-HT}_3$ergic-mediated mechanisms were also involved in PSY-SIA. Taken together, it is suggested that both $5\text{-HT}_3$ergic mechanisms and $\kappa$-opioid receptor-mediated mechanisms are closely related to each other for the production of PSY-SIA.

On the other hand, we have shown that concurrent exposure to PSY-stress blocked the development of antinociceptive tolerance to morphine, and the suppressive effect was antagonized by DZP (2). Expectantly, the suppressive effect of PSY-stress on the development of morphine tolerance was reversed by methysergide, buspirone, ritanserin and Y-25,130 but not by ($\pm$)pindolol. Further suggesting the relationship between $5\text{-HT}_2$ receptors and PSY-SIA, Yamamoto et al. (29) have reported that the development of tolerance to morphine is blocked by the concomitant treatment with U-50,488H. Thus, not only $5\text{-HT}_3$ergic but also $\kappa$-opioid receptor-mediated mechanisms may underlie the stress effect on the tolerance development.

Recently, $5\text{-HT}$ receptors have been classified into four subtypes, $5\text{-HT}_{1A}$, $5\text{-HT}_{1B}$, $5\text{-HT}_2$, and $5\text{-HT}_3$ receptors. Furthermore, the $5\text{-HT}_3$ receptors can be divided into four distinct subtypes, $5\text{-HT}_{1A}$, $5\text{-HT}_{1B}$, $5\text{-HT}_{1C}$ and $5\text{-HT}_{1D}$ receptors (30–33). The results obtained here suggest that mechanisms mediated by $5\text{-HT}_{1A}$, $5\text{-HT}_3$ and $5\text{-HT}_2$ receptors, but not $5\text{-HT}_{1B}$ receptors, play an important role in the production of PSY-SIA.

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