Participation of GABAergic Systems in the Production of Antinociception by Various Stresses in Mice

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ABSTRACT—Based on the data that diazepam, a benzodiazepine (BZP) receptor agonist, antagonized psychological (PSY)-stress induced analgesia (SIA) without prominent action on footshock (FS)- and forced swimming (SW)-SIA and that BZP receptors are coupled with GABA receptors, we examined how the GABAergic system participates in the production of various SIAs. Muscimol, a GABA<sub>A</sub> receptor agonist, at doses of 0.25 to 1.0 mg/kg, affected each SIA differently, suppressed PSY-SIA at 0.25 mg/kg but tended to potentiate it at 1.0 mg/kg, potentiated SW-SIA dose-dependently and did not affect FS-SIA at the doses employed. Both bicuculline, a GABA<sub>A</sub> receptor antagonist, 0.5 to 2.0 mg/kg, and picrotoxin, a Cl<sup>-</sup> channel blocker, 0.25 to 1.0 mg/kg, dose-dependently suppressed PSY- and FS-SIA. Meanwhile, the effects of both drugs on SW-SIA were less than those on PSY- and FS-SIA, namely, bicuculline slightly inhibited it only at 2.0 mg/kg, and picrotoxin did not produce any appreciable effect even at the highest dose. Baclofen, a GABA<sub>B</sub> receptor agonist, at 5.0 and 10.0 mg/kg had no influence on each SIA. On the contrary, CGP 35348, a GABA<sub>B</sub> receptor antagonist at 20 to 100 mg/kg caused the dose-dependent blockade of FS-SIA, but affected neither PSY- nor SW-SIA. The production of PSY- and SW-SIA is attributable to the GABA<sub>A</sub> receptors/Cl<sup>-</sup> channel mediated mechanism alone, while that of FS-SIA involves both GABA<sub>A</sub> and GABA<sub>B</sub> receptor mediated systems. Thus, GABAergic systems play an important role in the production of each SIA; however, the participation of the receptor subtypes in the mechanism was different from each other.

Keywords: Stress induced analgesia (SIA), GABA, Muscimol, Bicuculline, Baclofen

It has been recognized that experimental animals exposed to stressful stimuli such as psychological (PSY) (1–3), footshock (FS) (4–7) and forced swimming (SW) (8–10) stress produce an antinociceptive effect. In a series of studies on these phenomena known as stress induced analgesia (SIA), we have found that the underlying mechanism for their production is common in part, but distinct from each other. For example, the induction of the SIAs described above are completely blocked by pretreatment of the animals with reserpine (4). On the other hand, FS- and PSY-SIA are antagonized by naloxone but SW-SIA is insensitive to naloxone (1, 7), and clonidine specifically potentiates SW-SIA and suppresses PSY-SIA but does not affect FS-SIA (9, 10).

Furthermore, we have demonstrated that diazepam (DZP), an anxiolytic agent acting on benzodiazepine (BZP) receptors, inhibits the PSY-SIA dose-dependently without remarkable effect on FS- and SW-SIA (2). We have also shown that β-carboline-3-carboxylic acid ethyl ester, an inverse agonist on BZP receptors, potentiates the PSY-SIA, and such potentiation is reversed by DZP and Ro 15-1788 (flumazenil), a BZP receptor antagonist (11).

It is well-established that GABA receptors are classified into two distinguishable subtypes, GABA<sub>A</sub> and GABA<sub>B</sub> receptors (12), and BZP receptors are coupled with GABA<sub>A</sub> receptors and Cl<sup>-</sup> channels (13, 14). The present study has been carried out to clarify the GABA receptor mediated mechanisms in the production of the antinociceptive effect induced by exposure to PSY-, FS- and SW-SIA in mice.

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 kept in a room maintained at an ambient temperature of 22 ± 1°C and given normal laboratory diet and tap water ad libitum. After their body weight reached 23 to 28 g, they were used for the experiments.

**Drugs**

Muscimol, a GABA<sub>A</sub> receptor agonist (Sigma, St. Louis, MO); bicuculline, a GABA<sub>A</sub> receptor antagonist (Sigma, St. Louis, MO); picrotoxin, a Cl<sup>-</sup> channel blocker (Nacalai Tesque, Kyoto); baclofen, a GABA<sub>B</sub> receptor agonist (Daiichi Pharm. Co., Tokyo); and CGP 35348, a GABA<sub>B</sub> receptor antagonist (3-amino-propane-diethoxymethyl-phosphinic acid, a gift from Ciba-Geigy Co., Hyogo). Bicuculline was dissolved in 0.1 N HCl and the pH was adjusted to about 4–5 with appropriate amount of NaOH solution, and other drugs were dissolved in saline. They were administered in a volume of 0.1 ml/10 g of body weight, and the dose was expressed in terms of the respective salt. All drugs were injected i.p. 30 min before exposure to each stress.

**Exposure to stresses**

**PSY-stress:** Using the communication box with a slight modification of the method of Ogawa and Kuwahara (15), animals were exposed to PSY-stress by watching and hearing the struggle, jumping and vocalization of the footshocked animals for 5 min (1, 2, 11).

**FS-stress:** Animals were exposed to an inescapable and unsignalled FS (2 mA, 0.2 Hz, 1 sec duration) through the floor grid for 30 min (1, 4).

**SW-stress:** Mice were forced to swim in a water bath at 20 ± 1°C for 5 min (1, 9, 10).

Details of the exposure to each stress have been described in the respective reports.

**Assessment of antinociceptive effect**

The antinociceptive effect was measured by the modified Haffner's method (16), which is a tail pinch test (TP), with a cutoff time of 6 sec to avoid the damage of the tail, every 5 min from immediately after termination of the stress exposure, for 15 min.

**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.

**RESULTS**

**Effect of muscimol on PSY-, FS- and SW-SIA**

As shown in Fig. 1, PSY-SIA was significantly suppressed by pretreatment with 0.25 mg/kg of muscimol, while the higher dose, 1.0 mg/kg, tended to potentiate it. Muscimol at the doses of 0.25 to 1.0 mg/kg potentiated SW-SIA dose-dependently, but had no effect on FS-SIA.

**Effect of bicuculline and picrotoxin on PSY-, FS- and SW-SIA**

Pre-treatment of mice with bicuculline at 0.5, 1.0 and 2.0 mg/kg or picrotoxin at 0.25, 0.5 and 1.0 mg/kg dose-dependently suppressed PSY- and FS-SIA. Meanwhile, bicuculline was less effective and picrotoxin was without effect on SW-SIA (Figs. 2 and 3).

**Effect of baclofen on PSY-, FS- and SW-SIA**

Baclofen, at doses up to 10 mg/kg had no influence on each SIA (Fig. 4).

**Effect of CGP 35348 on PSY-, FS- and SW-SIA**

CGP 35348, 20 to 100 mg/kg, dose-dependently blocked FS-SIA; however, PSY- or SW-SIA was not affected by CGP 35348 (Fig. 5).

**DISCUSSION**

In our previous paper, we have shown that the underlying mechanism in the production of PSY-, FS- and SW-SIA are mutually different, and suggested that BZP receptor mediated mechanisms are involved in the production of PSY-SIA (2, 11).

Since it is well-established that GABA receptors are classified into two subtypes, GABA<sub>A</sub> and GABA<sub>B</sub> receptors (12), and BZP receptors are coupled with GABA<sub>A</sub> receptors and Cl<sup>-</sup> channels (13, 14), we examined the effect of GABA-related drugs on each SIA in the present study. Muscimol, a GABA<sub>A</sub> receptor agonist, at the dose of 0.25 mg/kg slightly but significantly suppressed the PSY-SIA. Furthermore, 0.25 to 1.0 mg/kg of bicuculline, a GABA<sub>A</sub> receptor antagonist, and 0.25 to 1.0 mg/kg of picrotoxin, a Cl<sup>-</sup> channel blocker, dose-dependently antagonized the PSY-SIA. Baclofen and CGP 35348, a GABA<sub>B</sub> receptor agonist and antagonist (17), respectively, had no effect on the production of PSY-SIA. These results indicate that a GABAergic mechanism, especially a GABA<sub>A</sub> receptor mediated mechanism but not GABA<sub>B</sub> receptors, is involved in the production of PSY-SIA. Considering the coupling theory of BZP, GABA<sub>A</sub> receptors and Cl<sup>-</sup> channels, in addition to our previous findings that
Fig. 1. Effect of muscimol on PSY-, FS- and SW-SIA. Mice were exposed to psychological (PSY)-, foot shock (FS)- or forced swimming (SW)-stress for 5, 30 and 5 min, respectively. The antinociceptive effect was measured by the tail pinch (TP) method every 5 min from immediately after the termination of the stress exposure. Muscimol, 0.25 (○), 0.5 (□), 1.0 (△) mg/kg, was administered i.p. 30 min before each stress. The control group (●) was given saline instead of muscimol. Each point is the mean ± S.E. of the data obtained from 12–14 mice. The dotted area indicates the response time before exposure to stress. **P < 0.01, *P < 0.05, compared with the saline-pretreated group.

Fig. 2. Effect of bicuculline on PSY-, FS- and SW-SIA. Bicuculline, 0.5 (△), 1.0 (□), 2.0 (○) mg/kg, was administered i.p. 30 min before each stress. The control group (●) was given vehicle instead of bicuculline. **P < 0.01, *P < 0.05, compared with the vehicle-pretreated group. For other details, refer to the legend of Fig. 1.
Fig. 3. Effect of picrotoxin on PSY-, FS- and SW-SIA. Picrotoxin, 0.25 (△), 0.5 (□), 1.0 (○) mg/kg, was administered i.p. 30 min before each stress. The control group (●) was given saline instead of picrotoxin. **P < 0.01, *P < 0.05, compared with the saline-pretreated group. For other details, refer to the legend of Fig. 1.

Fig. 4. Effect of baclofen on PSY-, FS- and SW-SIA. Baclofen, 5.0 (□), 10 (○) mg/kg, was administered i.p. 30 min before each stress. The control group (●) was given saline instead of baclofen. For other details, refer to the legend of Fig. 1.

DZP blocked PSY-SIA, we expected the potentiation of PSY-SIA by bicuculline or picrotoxin. However, these blockers, unexpectedly failed to enhance PSY-SIA. Since it is reported that the anticonflict effect of DZP is not affected by bicuculline (18), the present data may deduce that the BZP and GABA system is not always cooperative for the production of PSY-SIA. In support of this possibility, Zhang et al. (19) have shown that cold-immobilized stress selectively decreases the number of GABA\textsubscript{A} receptor binding sites without
altering that of BZP receptor binding sites in spite of the functional coupling between GABA_\text{A} and BZP receptors.

Muscimol at a dose of 0.25 mg/kg suppressed the PSY-SIA, and a higher dose, 1.0 mg/kg, unexpectedly tended to potentiate the PSY-SIA. Since it has been reported that muscimol at a dose of 0.75 mg/kg has an antinociceptive effect (20) and indeed, we found that administration of 1.0 mg/kg of muscimol i.p. induced a weak antinociceptive effect in the TP method (data not shown), the potentiation of PSY-SIA by 1.0 mg/kg muscimol, therefore, may be attributable to the additive action. In addition to the suppressive effect of a small dose of muscimol, a GABA_\text{A} receptor antagonist and even a Cl\textsuperscript{-} channel blocker, bicuculline and picrotoxin, also blocked the PSY-SIA. The possible explanation on these conflict results remains to be elucidated.

FS-SIA is antagonized by naloxone, indicating the involvement of opioid mechanisms in the production of PSY-SIA (1, 7). On the other hand, it is reported that subconvulsive doses of bicuculline attenuate the morphine antinociceptive effect (21). In this experiment, FS-SIA was blocked by pretreatment with GABA antagonists such as bicuculline and picrotoxin. Accordingly, it is suggested that both opioidergic and GABAergic systems may be closely related in the production of FS-SIA. In fact, Sherman and Gebhart (22) have demonstrated that FS-stress and morphine increase central GABA levels. Furthermore, from the antagonism of CGP 35348 on PSY-SIA, we confirmed that not only a GABA_\text{A} receptor mediated mechanism but also a GABA_\text{B} receptor mediated one might be involved in the production of FS-SIA, differed from the production of PSY- and SW-SIA.

Skerritt et al. (23) have observed the parallelism between the time course of antinociception measured by the tail flick method and increase in GABA binding following acute cold-water swim (CWS) exposure, and that repeated exposure to CWS eliminates the swim-induced increase in GABA binding, accompanied by the dissipation of antinociception. Given the activation of GABA receptor mediated mechanism was responsible for the production of the antinociceptive response following CWS, a potentiation and an attenuation of SW-SIA by muscimol and bicuculline, respectively, would be expected. The results obtained here that muscimol resulted in the potentiation of SW-SIA appeared to be consistent with this assumption. Meanwhile, Bodnar and Sperber (24) have reported that CWS induced antinociception is not altered by systemic administration of muscimol. It is considered that this discrepancy between the two reports depends on the pharmacological variables such as dose of used drug, temperature as well as paradigm for stress exposure. However, differing from our expectation, the attenuation of SW-SIA by bicuculline was only obtained when the highest dose was employed. Therefore, a GABA_\text{A} receptor mediated mechanism seems to participate in the production

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**Fig. 5.** Effect of CGP 35348 on PSY-, FS- and SW-SIA. CGP 35348, 20 (△), 50 (□), 100 (○) mg/kg, was administered i.p. 30 min before each stress. The control group (●) was given saline instead of CGP 35348. **P < 0.01, *P < 0.05, compared with the saline-pretreated group. For other details, refer to the legend of Fig. 1.
of SW-SIA to some extent. The results obtained here suggest that GABAergic systems play an important role in the production of PSY-, FS- and SW-SIA; however, the participation of the receptor subtypes in the mechanism was different from each other.

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