

## Further Evidence for the Participation of an $\alpha_2$ -Adrenoceptor Mediated Mechanism in the Production of Forced Swimming-Stress Induced Analgesia in Mice

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Subanalgesic dose, 0.01 to 0.25 mg/kg, of clonidine (CLO), an  $\alpha_2$ -adrenoceptor agonist, potentiated forced swimming (SW) stress induced analgesia (SIA) and suppressed psychological (PSY)-SIA in a dose dependent manner but did not affect foot-shock (FS)-SIA. Daily exposure to each stress rapidly developed tolerance, and the development was suppressed by daily concomitant subanalgesic dose of CLO in SW-SIA but not in FS- and PSY-SIA. Meanwhile, SW-stress, applied after injection of CLO, 1 mg/kg, potentiated the analgesic effect of CLO and suppressed the development of tolerance to the effect. On the other hand, FS- and PSY-stress did not affect CLO analgesia and failed to block the tolerance development. These results provide further evidence that  $\alpha_2$ -adrenergic mechanism is involved in the production of SW-SIA.

**Keywords** — stress-induced analgesia (SIA); clonidine; tolerance;  $\alpha_2$ -adrenoceptor

### Introduction

In a series of studies on stress-induced analgesia (SIA), that is, foot-shock (FS)-, psychological (PSY)-, and forced swimming (SW)-SIA, we found that the underlying mechanism for their production is common in part, but distinct from each other. For instance, the induction of the SIAs mentioned above are completely blocked by pretreatment of the animals with reserpine.<sup>1)</sup> On the other hand, SW-SIA is insensitive to naloxone but FS- and PSY-SIA are antagonized by naloxone,<sup>2,3)</sup> and diazepam specifically suppresses PSY-SIA but not FS- and SW-SIA.<sup>4)</sup>

The fact that SW-SIA as well as the analgesic effect of clonidine (CLO), an  $\alpha_2$ -adrenoceptor agonist, is completely blocked by pretreatment with yohimbine, an  $\alpha_2$ -adrenoceptor antagonist,<sup>5)</sup> suggests the participation of a common mechanism between SW-SIA and CLO analgesia for their production. Furthermore, the distinctiveness of SW-SIA from FS- and PSY-SIA was shown by the fact that the development of analgesic tolerance to morphine was completely suppressed by concomitant exposure to FS- and PSY-stress but not to SW-stress.<sup>6)</sup> The present study was carried out to characterize more precisely the involvement of  $\alpha_2$ -mediated mechanism in the production of SW-SIA, in comparison with FS- and PSY-SIA, and to

demonstrate a unique blocking effect of SW-SIA on the development of analgesic tolerance to CLO.

### Materials and Methods

**Animals** — Male ddY strain mice (Otsubo Exp. Animals, Nagasaki) weighing 18—20 g, were housed as a group of 20 animals. They were kept in a room maintained at an ambient temperature of  $22 \pm 1$  °C and were given normal laboratory diet and tap water *ad libitum*. After their body weight reached 23 to 28 g, they were used for experiments.

**Drug** — Clonidine-HCl (Boehringer) was dissolved in saline to contain the dose in a volume of 0.1 ml/10 g of body weight, and was administered i.p.

**Methods of Stress Exposure** — SW-stress: Mice were forced to swim in a water bath at  $20 \pm 1$  °C. FS-stress: Animals were exposed to an inescapable and unsignaled FS (2 mA, 0.2 Hz, 1 s duration) through the floor grid. PSY-stress: The communication box which consists of 9 compartments was used. Namely, animals were placed individually into the compartments and an electric shock was delivered through the floor. Animals placed in the compartments in which the floor is covered with a plastic plate were prevented from receiving the shock, but they were ex-

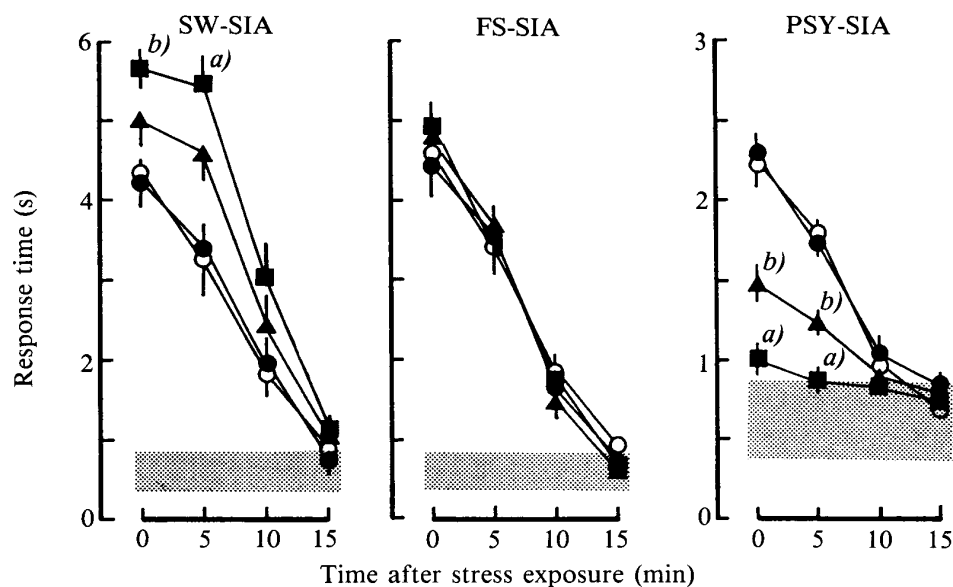


Fig. 1. Effect of CLO on SW-, FS- and PSY-SIA

Mice were exposed to forced swimming (SW)-, foot shock (FS)- or psychological (PSY)-stress for 5, 30 and 5 min, respectively. The analgesic effect was measured every 5 min from immediately after the termination of the stress exposure. Clonidine (CLO), 0.01 (●), 0.1 (▲), 0.25 (■) mg/kg, was administered i.p. 30 min before each stress. The control group (○) was given saline instead of CLO. Each point is the mean ± S.E. of the data obtained from 10–12 mice. Dotted area indicates response time before exposure to stress a)  $p < 0.01$ , b)  $p < 0.05$ , compared with the saline pretreated group.

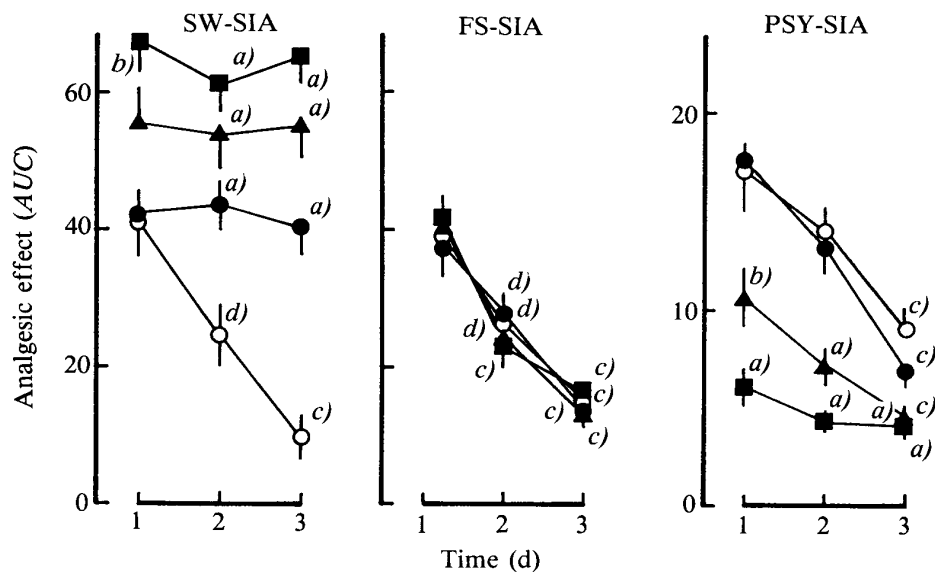


Fig. 2. Effect of CLO on the Development of Tolerance to SIA

CLO, 0.01 (●), 0.1 (▲), 0.2 (■) mg/kg, was administered i.p. 30 min before the daily stress exposure. Control group (○) was given saline instead of CLO. Each point is the mean ± S.E. of the data obtained from 10–12 mice, a)  $p < 0.01$ , b)  $p < 0.05$ , compared with the saline pretreated group. c)  $p < 0.01$ , d)  $p < 0.05$ , compared with the value on 1st day. For other details, refer to the legend of Fig. 1.

posed to PSY-stress by watching and hearing the struggle, jumping and vocalization of the shocked animals. Details of the exposure to each stress have been described elsewhere.<sup>1-3)</sup>

**Assessment of Analgesic Effect** — The analgesic effect was measured at the interval of 5 min immediately after termination of the stress exposure, and every 15 min after injection of CLO by the modified Haffner's method,<sup>7)</sup> tail pinch test (TP), with a cutoff time of 6 s.

**Evaluation of Tolerance** — Analgesia induced by each stress and CLO, 1 mg/kg, was measured daily for 3 and 5 d, respectively. The analgesic effect was expressed as area under the curve (AUC) by plotting the increase in response time (s) 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 Analysis** — Results were expressed as mean  $\pm$  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 differ-

ence was considered to be significant at  $p < 0.05$ .

## Results

### Effect of CLO on SW-, FS- and PSY-SIA

Animals were pretreated with a subanalgesic dose, 0.01 to 0.25 mg/kg, of CLO 30 min before exposure to each stress. CLO potentiated SW-SIA and suppressed PSY-SIA in a dose dependent manner but did not affect FS-SIA at the dose employed (Fig. 1).

### Effect of CLO on the Development of Tolerance to Each SIA

CLO, 0.01 to 0.25 mg/kg, completely suppressed the development of tolerance to SW-SIA, but did not influence the development of tolerance to FS- and PSY-SIA (Fig. 2).

### Effect of Concomitant Stress Exposure on CLO Analgesia and Development of Tolerance to the Effect

The analgesic effect of CLO, 1.0 mg/kg, was potentiated by concomitant exposure to SW-stress, whereas FS- and PSY-stress did not significantly modify the CLO analgesia. Daily injection of CLO developed tolerance to its

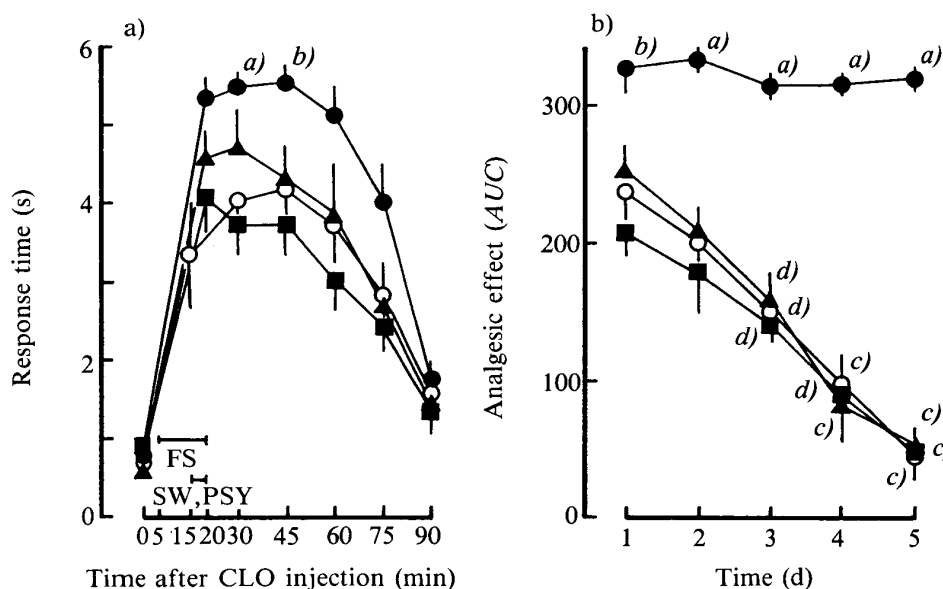


Fig. 3. Effect of Stress Exposure on CLO Analgesia and the Development of Tolerance to CLO

a) After injection of CLO, 1 mg/kg, animals were exposed to SW-, FS- or PSY-stress at the time expressed by horizontal bars.

b) Combined treatment with CLO plus stress was repeated daily for 5 d. CLO alone (○), CLO in combination with SW (●), FS (▲) or PSY (■). Each point indicates the mean  $\pm$  S.E. of the data obtained from 12–14 animals. a)  $p < 0.01$ , b)  $p < 0.05$ , compared with the group treated with CLO alone. c)  $p < 0.01$ , d)  $p < 0.05$ , compared with the value on 1st day. For other details, refer to text.

analgesic effect and the effect was lost by 5 d repetitions. Daily combined exposure to SW-stress with CLO completely suppressed the development of analgesic tolerance to CLO during 5 daily repetitions. However, concomitant exposure to FS- and PSY-stress did not affect the development of tolerance to CLO (Fig. 3).

### Discussion

In our preceding paper,<sup>5)</sup> we have shown the difference of the underlying mechanism for the production of SW-SIA and FS- and PSY-SIA, and suggested the involvement of a common adrenergic  $\alpha_2$ -mediated mechanism in the production of SW-SIA and CLO analgesia. The difference of SW-SIA from FS- and PSY-SIA in the participation of an  $\alpha_2$ -mediated mechanism was apparent. As shown in the present study, the pretreatment of the mice with a subanalgesic dose of CLO only potentiated SW-SIA but not the other SIAs. These did not affect FS-SIA and rather suppressed PSY-SIA.

The characteristic of SW-SIA was also evident in the effect of CLO on the development of tolerance to each SIA. As reported previously,<sup>3,5)</sup> daily exposure to FS-, SW- or PSY-stress rapidly developed tolerance to the effect, and almost no analgesia was induced on the 3rd day. The development of tolerance to SW-SIA was completely blocked by the simultaneous treatment with a subanalgesic dose of CLO. However, the same dose of CLO did not influence the development of tolerance to FS- and PSY-SIAs although the analgesia induced by PSY-stress was dose-dependently suppressed by CLO. The suppressive effect of CLO on PSY-SIA may be due to the anti-anxiety effect of CLO as reported by Rudolf *et al.*<sup>8)</sup> and Rudolf.<sup>9)</sup>

In the experiment on cross tolerance between SW-SIA and CLO analgesia, we found that CLO analgesia was enhanced in SW-SIA tolerant animals.<sup>5)</sup> The blockade by CLO in the development of tolerance to SW-SIA, obtained in the present study, however, is not due to the potentiating effect of CLO on SW-SIA since in the animals treated with the same dose of CLO for 3 d the intensity of SW-SIA was not altered from that appearing in normal animals.

On the other hand, SW-stress, applied after injection of CLO, potentiated CLO analgesia, and daily repetitions of the same treatment suppressed the development of analgesic tolerance to CLO. Similar treatment with FS- and PSY-stress affect neither CLO analgesia nor the development of tolerance to the effect. We have reported that FS-<sup>2)</sup> and PSY-SIA<sup>3)</sup> are antagonized by naloxone, an opioid antagonist, but SW-SIA<sup>5)</sup> is insensitive to naloxone. This suggests the involvement of an opioid mediated mechanism in the production of FS- and PSY-SIA but not in SW-SIA. Furthermore, both the FS- and PSY-SIA but not SW-SIA completely blocked the development of analgesic tolerance to morphine.<sup>6)</sup> Likewise, the development of tolerance to CLO analgesia, which is insensitive to naloxone and mediated through an  $\alpha_2$ -adrenoceptor, was blocked by SW-SIA. Thus, we demonstrated here additional evidence that SW-SIA and CLO analgesia share a common  $\alpha_2$ -mediated mechanism for their production as we have suggested in our previous report.<sup>5)</sup>

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