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PARTICIPATION OF AN α_2-MEDIATED MECHANISM IN THE PRODUCTION OF FORCED SWIMMING-STRESS INDUCED ANALGESIA IN MICE

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In mice, both swimming-stress induced analgesia (SW-SIA) and clonidine (CLO) analgesia were dose dependently antagonized by yohimbine, an α_2-adrenoceptor antagonist, but not by naloxone, an opioid μ-antagonist. SW-SIA was potentiated by subanalgesic dose of CLO, and CLO analgesia was enhanced by SW-SIA. Animals tolerant to CLO analgesia were tolerant to SW-SIA, in contrast, CLO analgesia was potentiated in SW-SIA tolerant mice. Thus, SW-SIA and CLO analgesia partially share a common α_2-adrenergic-dependent mechanism, for their production.

KEYWORDS — forced swimming-stress induced analgesia; SW-SIA; clonidine; cross-tolerance; α_2-adrenoceptor

INTRODUCTION

We have previously shown that stressful stimuli, such as electric foot-shock (FS)[1], psychological (PSY)[2] and forced swimming (SW)[1] stress, induce analgesic effects and such stress induced analgesia (SIA) is completely suppressed by pretreatment of mice with reserpine, a catecholamine depletor.[3] These facts suggest that catecholaminergic function plays an important role in the production of the SIAs. Both FS- and PSY-SIA are antagonized by naloxone (NX), an opioid antagonist, indicating the involvement of opioid mechanisms in the production of these SIAs,[1,2] however, SW-SIA is insensitive to naloxone suggesting that its induction is not mediated through an opioid-dependent mechanism.

On the other hand, clonidine (CLO), an α_2-adrenoceptor agonist, produces a potent analgesia which is not antagonized by naloxone in rodents.[3,4] In the present investigation, participation of an α_2-adrenoceptor mediated mechanism in the production of SW-SIA was investigated by comparing the effect with that

of CLO analgesia.

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 were given a normal laboratory diet and tap water *ad libitum*. After their body weights reached 23 to 28 g, they were employed for experiments.

Drugs - Clonidine-HCl (Boehringer), yohimbine-HCl (YOH, Nacalai Tesque) and naloxone-HCl (Sigma) were dissolved in sufficient saline to contain the dose in a volume of 0.1 ml/10 g of body weight, to be given i.p.

Assessment of Analgesic Effect - Analgesia was measured by a modified Haffner's method, with a cut-off time of 6 sec (5). Measurement was made every 5 min immediately after termination of the stress (forced swimming in water at 20° C for 5 min) or every 15 min after CLO injection.

Evaluation of Tolerance and Cross Tolerance - Analgesia induced by SW-stress or CLO (1 mg/kg) was measured daily for 3 days. A significant decrease of analgesia, compared with that of the 1st day, indicated the development of tolerance. In animals rendered tolerant by 3 daily treatments with CLO or SW-stress, the analgesic effect of SW-SIA in CLO tolerant mice, and that of CLO in SW-SIA tolerant animals were estimated on the 4th day to assess the development of cross tolerance. In these experiments, the analgesic effect was expressed as the area under the curve (AUC) obtained by plotting the increase in response time (s) on the ordinate and the time interval (min) on abscissa.

Statistical Analysis - Results were expressed as means ± 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 to be significant at $P < 0.05$.

RESULTS

1. Effect of NX and YOH on SW-SIA and CLO Analgesia

SW-stress produced short lasting analgesia, and its intensity was dependent on the duration of the stress (Fig. 1a). The SW-SIA, induced by 5 min exposure, was resistant to 1 mg/kg of NX, given 5 min before stress exposure, but was dose dependently antagonized by YOH (Fig. 1b,c).

CLO produced dose dependent analgesia which was maximal 30 to 45 min after injection and persisted for about 90 min (Fig. 2a). Even a large dose of NX, 2 mg/kg, did not alter the analgesic effect of 1 mg/kg of CLO (Fig. 2b). On the other hand, pretreatment with YOH, 5 min before CLO injection, inhibited CLO analgesia dose dependently (Fig. 2c).

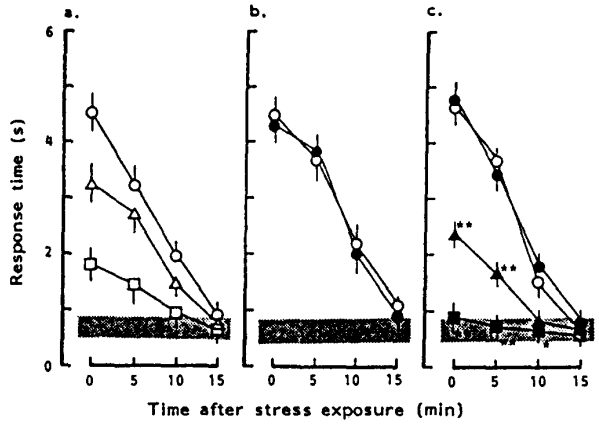

Fig. 1. Effect of Naloxone and Yohimbine on SW-SIA
a. Mice were exposed to SW-stress for 1 (□), 3 (△) and 5 (○) min. b. NX, (●) 1 mg/kg, was given 10 min before the exposure to SW-Stress. c. Mice were pretreated with YOH, 2 (●), 5 (▲), 10 (■) mg/kg, 30 min before exposure to SW-stress. [▒]: Response time of control animals. * P < 0.05, ** P < 0.01 vs SW-SIA.

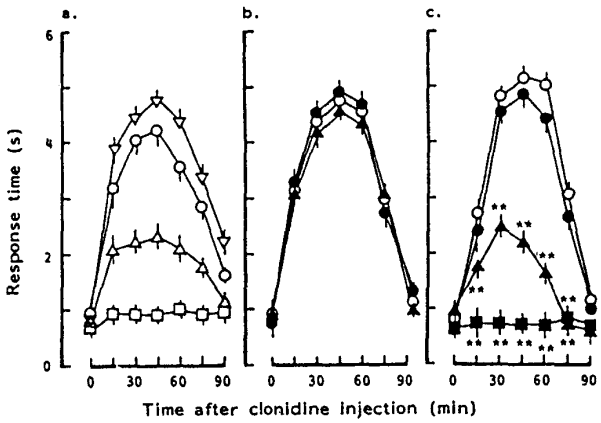

Fig. 2. Effect of Naloxone and Yohimbine on Clonidine Analgesia
a. CLO, 0.25 (□), 0.5 (△), 1 (○), 2 (▽) mg/kg. b. NX 1 (●), 2 (▲) mg/kg was given 10 min before injection of 1 mg/kg of CLO. c. YOH 2 (●), 5 (▲), 10 (■) mg/kg was given 30 min before the injection of 1 mg/kg of CLO.
** P < 0.01 vs CLO, 1 mg/kg (○) group.

2. Interaction between SW-SIA and CLO Analgesia

Pretreatment with subanalgesic doses of CLO, 0.1-0.25 mg/kg, dose-dependently potentiated SW-SIA (Fig. 3a). On the other hand, a 5-min exposure to SW-stress, applied 15 after injection of CLO, potentiated CLO analgesia (Fig. 3b).

3. Cross tolerance between SW-SIA and CLO Analgesia

Mice given daily CLO injections and daily SW-stress rapidly developed tolerance to the analgesic effect (Fig. 4a). In animals tolerant to CLO, the intensity of SW-SIA was reduced significantly. However, in SW-SIA tolerant animals, the analgesic effect of CLO was significantly potentiated (Fig. 4b).

DISCUSSION

The similarity between SW-SIA and CLO analgesia, i.e., that both are not

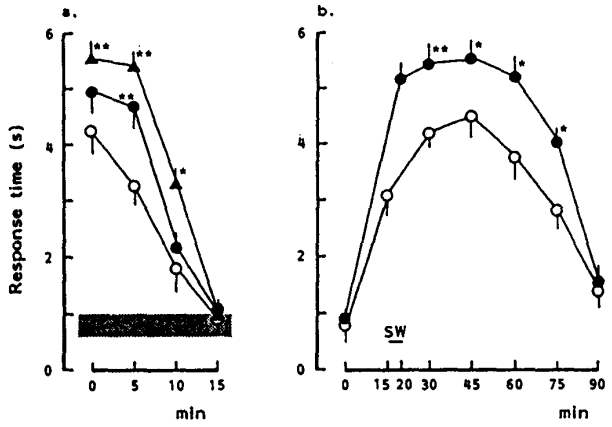

Fig. 3. Interaction between SW-SIA and Clonidine Analgesia
a. SW-SIA (○). CLO, 0.1 (●), 0.25 (▲) mg/kg was given 30 min before exposure to SW-stress. b. CLO 1 mg/kg (○), CLO plus SW-SIA (●). SW-stress was applied at the time indicated as SW after injection of 1 mg/kg of CLO. * P < 0.05, ** P < 0.01 vs SW-SIA and CLO analgesia.

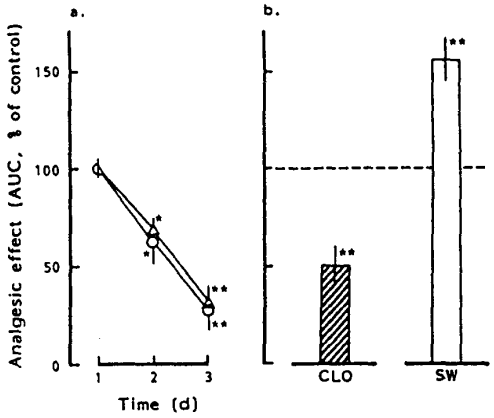

Fig. 4. Development of Tolerance to SW-SIA and Clonidine Analgesia and Cross Tolerance between Them
a. Development of tolerance to SW-SIA (○) and CLO analgesia (△). * P < 0.05, ** P < 0.01 vs AUC on the 1st day.
b. SW-SIA (▨) and CLO analgesia (□) were measured respectively in CLO (CLO) and SW-SIA (SW) tolerant animals on the 4th day. ** P < 0.01 vs Control (dotted line).

antagonized by NX but are completely abolished by reserpine,[3] stimulated our interest, and a comparative study was made between SW-SIA and CLO analgesia and tolerance to their analgesic effects.

In the present study, we reconfirmed our previous data that both SW-SIA and CLO analgesia were insensitive to NX pretreatment, and found that they were antagonized by YOH, an α_2-adrenergic antagonist, in a dose dependent manner. We also demonstrated that CLO potentiated SW-SIA, and vice versa. These results are consistent with the report of Bodnar et al. that cold water swim induced analgesia is potentiated by systemic administration of CLO,[6] indicating that a catecholaminergic mechanism, especially an α_2-adrenoceptor mediated mechanism, is involved in the production of SW-SIA. Synergism between SW-SIA and CLO analgesia may also suggest that both share a common mechanism for their production.

Tolerance to daily SW-SIA and repetitive CLO analgesia developed rapidly. Interestingly, CLO tolerant animals were also tolerant to SW-SIA, however, analgesic effects of CLO were significantly potentiated in SW-SIA tolerant animals, namely, tolerance between SW-SIA and CLO analgesia was one-way. This fact may suggest that the mechanism for production of SW-SIA and CLO analgesia is common in part but also underlied by different one.

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