The Role of the Catecholaminergic Mechanism in Foot Shock (FS) Stress- and Immobilized-Water Immersion (IW) Stress-Induced Analgesia in Mice

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Abstract—Involvement of the catecholaminergic mechanism in foot shock (FS)- and immobilized-water immersion (IW)-stress-induced analgesia (SIA) and in the development of tolerance to the effect were investigated in mice. With daily treatment with clonidine or daily exposure to stresses, tolerance developed rapidly to the analgesic effect. Clonidine-induced analgesia, which could not be antagonized by naloxone, was potentiated in the animals rendered tolerant to FS-stress, and it was attenuated in the animals tolerant to IW-SIA. On the other hand, animals tolerant to clonidine failed to show the attenuation of FS- and IW-SIA. The analgesic effect of clonidine and the development of tolerance to the effect were not influenced by reserpine. However, reserpine pretreatment completely suppressed the analgesic effect induced by FS- and IW-stresses on the 1st day; but with daily exposure to the stress, the analgesic effect gradually appeared and returned to the control level on the 5th day. These results indicate not only the differences between clonidine analgesia and SIAs but also those between each SIA. Thus, the central catecholaminergic mechanisms play an important role in these SIAs and also in the development of tolerance to the effect, although the degree of participation of these mechanisms seems to be somewhat different between FS- and IW-SIA, as indicated by the cross-tolerance between clonidine analgesia and each SIA.

In the underlying mechanisms for the production of stress induced analgesia (SIA), different mechanisms, opioid and non-opioid forms, are involved (1–8). Our previous report indicates that the endogenous opioid system is more closely associated with the mechanism of foot shock (FS)-SIA than with immobilized-water immersion (IW)-SIA; on the other hand, the non-opioid mechanism is more closely concerned in IW-SIA than in FS-SIA (9).

A series of stressful stimuli is accompanied by a change in the rate of norepinephrine (10–12) or dopamine (10, 13, 14) turnover in the brain, and the role of catecholamine could not be excluded in the production of SIA. Clonidine, a central \( \alpha_2 \)-agonist, is known to produce an analgesic effect in animals (15, 16), presumably through a central catecholaminergic mechanism. Reserpine, a catecholamine depletor, also modifies the analgesic effect of morphine (17, 18).

The aim of the present study was to elucidate the involvement of the catecholaminergic system in SIA.

Materials and Methods

Animals: Male mice of the dd-strain weighing 18–20 g were purchased and housed as a group of 10 animals in plastic cages at an ambient temperature of 22±1 °C. They were given food and water ad libitum. After reaching 23–26 g they were used in the
Foot shock stress: Animals were individually placed on the grid floor of the operant chamber (10 (D) × 10 (W) × 30 (H) cm) where they received inescapable and unsignaled foot shock (2 mA, 1 sec duration, 0.2 Hz) delivered by a scrambled electronic shock generator (Biomedica Co. Ltd., Osaka) for 30 min.

Immobilized-water immersion stress: Each animal was confined in a plastic box (4 × 4 × 10 cm) and immersed except for the head, in water (25°C) for 30 min.

Drugs and administration schedules: The following compounds were used: clonidine-HCl (gift from Boehringer Co., Ltd.), reserpine (Apoplon, Daiichi Pharmaceu. Co., Ltd.) and naloxone-HCl (a gift from the National Institute on Drug Abuse, U.S.A.). The drugs were dissolved in saline and administered in a volume of 0.1 ml/10 g of body weight. Clonidine was administered intraperitoneally at a daily dose of 1 mg/kg. Reserpine was injected intraperitoneally, 2.5 mg/kg on day 1 and 0.5 mg/kg on day 4, 4 hr before the daily exposure to each stress or the daily injection of drug.

Assessment of analgesic effect: The analgesic effect was assessed by the modified Haffner’s method (19) every 5 min from immediately after the termination of stress exposure or every 15 min after the injection of clonidine.

Evaluation of tolerance and cross-tolerance: The analgesic effect induced by 1 mg/kg clonidine or each stress was measured daily and expressed as the change of area under the curve (AUC) by plotting the increase in response threshold (sec) on the ordinate and the time intervals (min) on the abscissa. In the animals rendered tolerant by 3 daily treatments with clonidine or each stress, the analgesic effect induced by each stress or clonidine was estimated on the 4th day in order to assess the development of cross-tolerance between them.

Results

As shown in Fig. 1, 2 mg/kg of naloxone which completely blocked the analgesic effect of 10 mg/kg of morphine did not antagonize the analgesic effect of clonidine. Daily injection of clonidine resulted in the gradual loss of the analgesic effect, indicating the development of tolerance, and the effect was decreased to a half of the initial values on the 3rd day (Fig. 2).

FS- or IW-stress produced a short-lasting (15 min duration) analgesic effect (9), and daily exposure to these stresses developed tolerance to the effect.

In the mice tolerant to clonidine, both stresses produced an analgesic effect to the same extent as in normal animals. On the other hand, the effect of clonidine was potentiated in FS-SIA tolerant mice and was attenuated in IW-SIA tolerant mice (Fig. 2).

Reserpine had no effect on clonidine analgesia and on the development of tolerance to the drug (Figs. 3 and 4). On the other hand, both FS-SIA and IW-SIA were almost completely antagonized by reserpine pretreatment (Fig. 3). However, in contrast with control animals, with daily exposure of the reserpinized mice to the stress, the analgesic effect was gradually increased and returned to the control level by 5 days (Fig. 4).

Discussion

Our previous results (9) revealed that among various underlying mechanisms for the production of SIAs, the endogenous
Fig. 2. Development of tolerance to clonidine analgesia and SIAs and cross-tolerance between them. Left: Daily changes of analgesic effect of clonidine and SIAs. The analgesic effect is expressed as the area under the curve (AUC) that is obtained by plotting the increase in response threshold (sec) on the ordinate and the time intervals (min) on the abscissa. Clonidine, 1 mg/kg, i.p. (O—O); FS-SIA (□—□); IW-SIA (■—■). Each point is the mean±S.E. of 25 to 44 animals. *P<0.05, ***P<0.001, compared with the respective value on the 1st day. Right: Cross-tolerance between clonidine and each SIA. Animals were rendered tolerant by 3 daily treatments with clonidine or each stress. The analgesic effect induced by 1 mg/kg of clonidine or each stress was estimated on the 4th day. Clo, FS and IW: tolerant animals to clonidine, FS-SIA and IW-SIA, respectively. Clo (□), FS (□) and IW (□): analgesic effect of clonidine, FS-SIA and IW-SIA, respectively. Values are the mean±S.E. of 13 to 38 animals, and expressed as the percent of the effect of the control group. **P<0.01, compared with the control.

Fig. 3. Effect of reserpine on clonidine analgesia and both SIAs. Analgesic effect (response threshold, a cut-off time of 6 sec) was measured by the modified Haffner’s method, every 15 min after clonidine injection or every 5 min from immediately after stress exposure. Reserpine (2.5 mg/kg, ⋅—⋅) or saline (○—○) was injected i.p. 4 hr before clonidine (1 mg/kg, i.p.) or the exposure to each stress. Each point is the mean±S.E. of 10 to 21 animals. **P<0.01, ***P<0.001, compared with each saline pretreated group.
opioid system is more closely associated with FS-SIA than with IW-SIA; On the other hand, the non-opioid system which is not antagonized by naloxone is more closely concerned with IW-SIA than with FS-SIA.

As the non-opioid mechanism, a role for the central catecholaminergic mechanism has been suggested. Actually, clonidine, an α2-agonist, showed a potent naloxone-insensitive analgesia. Both FS- and IW-SIA were completely suppressed by pretreatment with reserpine, and furthermore, the analgesic effect of clonidine, which is directly mediated by adrenergic receptors, is not suppressed by reserpine.

Clonidine is known to act as an α2-agonist (20) at the supraspinal part of CNS at a low dose and not only as an α2-agonist but also as an α1-agonist at the spinal level in a high dose (20, 21). At the dose used in the present experiments, the analgesic effect of clonidine may be mediated through both mechanisms.

With daily treatment with clonidine or daily exposure to stresses, tolerance to the analgesic effect was rapidly developed. The fact that the analgesic effect of clonidine was attenuated in IW-tolerant animals suggests the existence of a common mechanism in IW-SIA and clonidine analgesia. However, animals tolerant to clonidine analgesia were not tolerant to IW-SIA. This may suggest that production of IW-SIA involves different mechanisms than those for clonidine-induced analgesia.

Cross-tolerance could not be demonstrated between clonidine and FS-SIA, indicating the discrepancy of mechanisms in both analgesia. The difference between FS- and IW-SIA is partially explained in our previous paper by the fact that the opioid mechanism was more closely associated to the production of FS-SIA than IW-SIA (9).

Differences between clonidine and SIAs were also demonstrated in the mechanisms for the development of tolerance in the reserpinized animals. Reserpine neither affects the analgesic effect nor the development of tolerance to clonidine. On the other hand, reserpine pretreatment completely suppressed the analgesic effect induced by both stresses on the 1st day, but during daily exposure to the stresses, the analgesic effect gradually appeared and returned to the control level on the 5th day. The apparent increase of the analgesic effect during repetition of stress exposure was completely different from the results obtained in control

Fig. 4. Effect of reserpine on the development of tolerance to clonidine analgesia and both SIAs. Reserpine (2.5 mg/kg on the 1st day and 0.5 mg/kg on the 4th day, •—•) or saline (○—○) was injected i.p. 4 hr before daily clonidine (1 mg/kg/day, i.p.) or daily exposure to each stress. Each point is the mean ± S.E. of 10 to 21 animals. *P<0.05, **P<0.01, ***P<0.001, compared with each saline pretreated group.
animals, and it seems that the development of tolerance was inhibited in reserpinized animals. These results also imply the importance of the catecholaminergic system in the development of tolerance to SIAs.

It is concluded from the present study that central catecholaminergic mechanisms play an important role in the analgesic effect induced by stresses and also in the development of tolerance to the effect. However, the degree of the participation of the catecholaminergic system in the underlying mechanisms of FS- and IW-SIA is somewhat different as indicated by the cross-tolerance between clonidine analgesia and each SIA.

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