Role of Adrenal Glucocorticoids in the Blockade of the Development of Analgesic Tolerance to Morphine by Footshock Stress Exposure in Mice

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Abstract—To elucidate the mechanism for the suppression by concurrent footshock (FS) exposure of the development of morphine tolerance, the effect of adrenalectomy and a possible participation of glucocorticoids in the mechanism were examined. The analgesic effect of morphine was potentiated in adrenalectomized (ADX) mice, and further enhancement of the effect was shown by the simultaneous exposure to FS (2 mA, 0.2 Hz, 1 sec duration for 15 min) stress, while no such effects were observed in sham-operated (Sham) animals. Daily morphine treatment developed tolerance in Sham and ADX mice. The combined treatment with FS stress suppressed the development of morphine tolerance in Sham mice, whereas such suppression was abolished by adrenalectomy. The suppression of tolerance development was restored in ADX mice by supplement of prednisolone. In contrast to FS stress which produces analgesia through an opioid receptor, forced swimming stress which exerts analgesia through a non-opioid mechanism did not affect the development of morphine tolerance in both Sham and ADX mice. Thus, an opioid mediated stress, FS, could prevent the development of morphine tolerance, and adrenal glucocorticoids play an essential role in the mechanism.

In our previous experiments which were undertaken to examine the effect of the concurrent exposure to various stressful stimuli on the development of morphine tolerance in mice, we found that footshock (FS) stress, which produces analgesia through an opioid receptor, only blocked the development of morphine tolerance (1). Meanwhile, there are several reports which suggest that the analgesic actions induced by stress exposure have relevance to the endocrine mechanisms: namely, stimulation of the pituitary-adrenal axis (e.g., hypophysectomized rat attenuated analgesia following FS, cold water swimming and immobilization) (2); footshock stress-induced analgesia was enhanced by adrenalectomy in mice (3); and the analgesic effect induced by an opioid mediated stress such as FS-stress under certain conditions was suppressed by the treatment with dexamethasone or by adrenalectomy (4). These stress induced analgesia are probably produced by activating the intrinsic pain-inhibitory system as an adaptive response to stress (5). If this is the case, the blockade by stress of the development of morphine tolerance may be a result of the emergency responses to such stress, through hormonal systems, since there is a general acceptance that adaptive responses to stress are closely related to the pituitary-adrenal system (6).

In this context, we examined a possible role of the adrenal system in the blockade of the development of analgesic tolerance to morphine by the concurrent exposure to FS-stress, and we also examined the role of the system in the effect of forced swimming (SW) stress which is a non-opioid mediated stress and has no effect on the development of morphine tolerance.

Materials and Methods

Animals: Male mice of the ddY strain weighing 18–20 g (Otsubo Exp. Animals,
Nagasaki) were housed as a group of 20 animals in plastic cages with free access to food and water. They were kept in a temperature controlled room at 22±1°C, and they were used for the experiments after reaching 22 to 24 g. Adrenalectomy or sham operation was performed by bilateral dorsal incisions under ether anesthesia. Both adrenalectomized (ADX) and sham operated (Sham) mice were given 0.9% NaCl as drinking water, with normal laboratory diet, and allowed 14 days to recover from the surgical trauma.

Exposure to FS-stress and SW-stress: ADX or Sham animals were exposed to an inescapable and unsignaled FS (2 mA, 0.2 Hz, 1 sec duration) through the floor grid for 15 min (FS-stress) or they were stressed by being placed in a water bath (40Lx35Wx20H) with 15-cm deep water at 20°C for 5 min (SW-stress). FS- and SW-stresses were applied from 5 min and 15 min, respectively, after morphine injection.

Assessment of antinociception: The measurement of the antinociceptive effect was started from 30 min after morphine injection and then done at intervals of 15 min for the following 60 min, by the modified Haffner’s method (7), using a 6 sec cut-off time to avoid tissue damage due to longer application.

Evaluation of the development of tolerance: Injection of morphine, 10 mg/kg, and concurrent exposure to the stress was repeated for 5 days, and the analgesic effect was measured daily. The effect was expressed as the area under the curve (AUC) by plotting the increases in response time (sec) on the ordinate and the time intervals (min) on the abscissa, and compared with the effect of the 1st treatment.

Drugs and administration schedule: The following compounds were used: Prednisolone sodium succinate (Predonin, Shionogi) and morphine-HCl (Takeda). These drugs were diluted to appropriate concentrations or dissolved in saline and administered in a volume of 0.1 ml/10 g of body weight. Prednisolone, 2.5 mg/kg and 5 mg/kg for supplemental doses, was administered s.c. twice a day (9:00 and 17:00) from 24 hr after the adrenalectomy or sham operation for 2 weeks and during the test days (chronic treatment) or during only the test period (acute treatment). Morphine was administered 1 hr after the prednisolone injection on the test days.

Statistical analysis: 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.

Results

The analgesic effect of morphine was potentiated in ADX mice, and it was further enhanced by concurrent exposure to FS-stress. In Sham mice, no substantial changes in the analgesic effect of either morphine alone or morphine with concurrent FS-stimuli were observed (Fig. 1).

In Sham mice, daily injection of morphine gradually developed tolerance; however, the concurrent exposure to FS-stress with morphine suppressed the development of morphine tolerance without affecting the analgesic effect on the 1st day. In ADX mice, daily morphine developed tolerance as well as in the Sham group. In contrast with the Sham group, the concurrent FS-stress exposure did not suppress the development of morphine tolerance in ADX animals (Fig. 2).

The suppression by FS-stress of tolerance development was restored in ADX mice by the supplemental treatment with 2.5 and 5 mg/kg prednisolone persistently during post-operation and the test days (chronic), although the analgesic effect of morphine with FS-stress was dose-dependently reduced. When the application of prednisolone was withdrawn from the 6th day, the analgesic effect was maintained reasonably on the 6th day; however, the further treatment with morphine and FS developed tolerance as rapidly as that in ADX mice treated without prednisolone (Fig. 3).

Meanwhile, either acute or chronic treatment with prednisolone did not cause any appreciable changes in morphine analgesia and its tolerance development in Sham mice. Chronic prednisolone treatment did not affect the development of morphine tolerance in ADX mice, although the compound reduced morphine analgesia. Acute treatment with
Fig. 1. Effect of concurrent footshock stress exposure on morphine analgesia in sham-operated and adrenalectomized mice. Animals were exposed to footshock (FS) stress 5 min after morphine injection (10 mg/kg, i.p.) for 15 min. Left: morphine alone (○) and morphine+FS-stress (□) in sham-operated (Sham) mice; morphine alone (●) and morphine+FS-stress (■) in adrenalectomized (ADX) mice. Right: analgesic effect in the left panel transformed into the area under the curve (AUC) by plotting the increase in response time (sec) on the ordinate and the time intervals (min) on the abscissa. Each point indicates the mean±S.E. of 8–16 animals. *P<0.05, **P<0.01, compared with the Sham group treated with morphine alone. *P<0.05, compared with the ADX group treated with morphine alone.

Fig. 2. Effect of daily footshock stress on the development of tolerance to morphine analgesia in sham-operated and adrenalectomized mice. Daily changes of analgesic effect was expressed as the area under the curve (AUC). Morphine alone (○, ●), morphine+FS-stress (□, ■) for 5 days, in Sham mice (left panel) and in ADX mice (right panel). Each point indicates the mean±S.E. of 8–16 animals. **P<0.01, compared with the corresponding value on the 1st day. *P<0.05, **P<0.01, compared with the control group treated with daily morphine. For abbreviations, see the legend for Fig. 1.
prednisolone likewise did not affect morphine analgesia and its tolerance development in ADX mice (Fig. 4).

On the other hand, an acute treatment with prednisolone in ADX mice also resulted in the suppression by FS-stress of the development
of morphine tolerance, although the suppressive effect was induced on the 4th and 5th day after the initial treatment (Fig. 5). Although the analgesic effect of morphine was attenuated by concurrent exposure to SW-stress on the 1st day in both Sham and ADX mice, the development of morphine tolerance was not influenced by the stress (Fig. 6).

Fig. 5. Effect of acute prednisolone on analgesia induced by morphine and footshock stress and the development of tolerance to the effect in adrenalectomized mice. Saline (■) or prednisolone (5 mg/kg, △) was i.p. given 1 hr before the combination of morphine and FS-stress for 5 successive test days to ADX mice. The data are shown in the same way as in Fig. 2. *P<0.05, **P<0.01, compared with the corresponding value on the 1st day. ##P<0.01, compared with the control group treated with daily morphine. For abbreviations, see the legend for Fig. 1.

Fig. 6. Effect of daily forced swimming stress on morphine analgesia and the development of tolerance to the effect in sham-operated and adrenalectomized mice. Animals were exposed to forced swimming (SW) stress 15 min after morphine injection (10 mg/kg, i.p.) for 5 min. Morphine alone (○, ●), morphine+SW-stress (△, ▲) for 5 days, in Sham mice (left panel) and in ADX mice (right panel). The data are shown in the same way as in Fig. 2. *P<0.05, **P<0.01, compared with the corresponding value on the 1st day. #P<0.05, compared with the control group treated with daily morphine. For abbreviations, see the legend for Fig. 1.
Discussion

The antinociceptive effect of morphine, 10 mg/kg, given intraperitoneally to mice was enhanced by adrenalectomy. This result is consistent with earlier reports, in which increased affinity of opioid receptors (3), a decrease in morphine metabolism (8) or both of them (9) were suggested in the underlying mechanisms. We observed here that the enhanced analgesic effect of morphine in ADX mice was further potentiated by concurrent FS-stress. This mechanism was not clarified; however, as suggested above, the potentiation may be attributable to the increased affinity of opioid receptors by adrenalectomy.

The concurrent exposure to FS-stress with morphine blocked the development of morphine tolerance in Sham mice. Although several possibilities may exist for the suppressive mechanism(s) by the combined treatment, our interest has focused on adrenal glucocorticoids which are related to stress responses.

It is well-known that the exposure to stress causes various humoral and endocrine changes in animals, especially in the pituitary-adrenal system. This may increase the probability that the adrenal system may play a critical role in the blockade of the development of morphine tolerance by concurrent FS-stress. In this experiment, we observed the abolishment of the suppressive effect of FS-stress on the development of morphine tolerance in ADX animals, indicating the important role of the pituitary-adrenal system in this phenomenon.

Adrenalectomy decreases the plasma corticosterone level (10). Corticosterone has been revealed to play a critical role in the production of an opioid mediated stress-induced analgesia (SIA), which was reduced by adrenalectomy and was reinstated by the supplemental administration of corticosterone (4). If this is the case, there is a possibility that the suppression by the opioid-mediated stress of the development of morphine tolerance, and the reoccurrence of the tolerance development in ADX animals, are closely related to the levels of corticosterone. Actually, Dunn (11) reported FS-stress increased glucocorticoids concentrations, but in ADX mice, FS-stress had no influence on the concentrations, suggesting the importance of glucocorticoids in response to stress. In this report, daily administration of prednisolone to the ADX mice suppressed the tolerance development, apparently like in Sham mice, indicating that prednisolone produced the supplemental effect on the corticosterone deficiency in ADX mice, and the steroid may play an essential role in the underlying mechanism.

This effect may not result from the direct action of the glucocorticoids, since glucocorticoids when administered acutely or chronically were without effect on the development of morphine tolerance in Sham mice. Moreover, the attenuation of pain resulted from anti-inflammatory effects (12) or modulation of SIA by glucocorticoids (3, 4, 13, 14) released following FS-stress and may be unrelated to the suppression by FS-stress of the development of morphine tolerance. Indeed, FS-SIA per se did not affect morphine analgesia on the 1st day, and low current FS (0.5 mA) which did not produce any appreciable analgesic effect suppressed the morphine tolerance as well (1).

We previously reported that in contrast to FS-stress, concurrent exposure to SW-stress which had non-opioid actions (15) did not suppress the development of morphine tolerance (1). These results imply that the opioid system may be involved in the blockade of the development of morphine tolerance. Since it is reported that there exist enkephalin-like peptides in the adrenal gland (16), the blockade by FS-stress of tolerance development and consequent abolishment of the blockade after the adrenalectomy may be attributable to these endogenous peptides. Concurrent SW-stress, therefore, did not affect the development of tolerance in both Sham and ADX mice. Furthermore, we confirmed from these results that glucocorticoids work as a critical factor in the suppression of morphine tolerance by stress which produces opioid receptor mediated actions. A recent report by Hendrie (17) that pretreatment with ACTH prevented the development of tolerance to morphine may support the important role of glucocorticoids in the underlying mechanism.
The activated release and turnover in the catecholaminergic system in the brain following stress exposure (18, 19) may be attributable to the suppression by the stress of the tolerance development. This may be supported by our earlier findings that α- and β-adrenergic blockers, similar to FS-stress exposure, suppressed the development of morphine tolerance (20). However, Dunn reported (11) that the FS-stress induced increase of catecholamine turnover was not affected by adrenalectomy, suggesting no appreciable participation of catecholamine metabolism in the underlying mechanism. In any case, the involvement of the catecholaminergic mechanism in the underlying process may not be excluded.

In conclusion, the concurrent exposure to FS-stress with morphine blocked the development of morphine tolerance in Sham mice; however, the treatment was not effective in the ADX mice. Meanwhile, prednisolone clearly produced the supplemental effect on the deficiency in ADX mice. Thus, our findings may indicate the important role of the pituitary-adrenal system, especially the action of glucocorticoids, in the suppression by FS-stress of the development of morphine tolerance.

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