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Adrenalectomy-Induced Potentiation of Morphine Analgesia: Reversal by Prednisolone

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MIYAMOTO, Y., M. OZAKI, S. KISHIOKA, T. YAMANISHI, Y. KITABATA, N. MORITA AND H. YAMAMOTO. *Adrenalectomy-induced potentiation of morphine analgesia: Reversal by prednisolone.* PHARMACOL BIOCHEM BEHAV **37**(4) 703–706, 1990.—Effects of adrenalectomy (ADX) on analgesic potency and morphine (MOR) content after SC administration of 3.5 or 7 mg/kg of MOR, and effects of prednisolone (PRED) on the ADX-induced effects were studied. ADX significantly potentiated MOR analgesia at both MOR doses, and PRED reversed the ADX-induced potentiation of MOR analgesia. ADX did not affect MOR content in brain and plasma after 3.5 mg/kg MOR, but significantly increased MOR content in brain and plasma after 7 mg/kg MOR, and PRED reversed the ADX-induced increase in the MOR content. Although the analgesic potency of 3.5 mg/kg MOR in ADX group was equipotent with those of 7 mg/kg MOR in sham-operated and PRED-treated ADX groups, MOR content in the former group was significantly lower than those in the latter two groups. These results suggest that ADX potentiates MOR analgesia through both mechanisms of the increased MOR content and the increased sensitivity to MOR, and that the lack of glucocorticoids participates in both of these ADX-induced effects.

Morphine analgesia Morphine content Adrenalectomy Prednisolone

THERE is considerable evidence that adrenalectomy (ADX) potentiates morphine (MOR) analgesia (16,22), which can be restored after replacement with glucocorticoids (9, 19, 20), but its mechanism remains to be elucidated. Holaday et al. (9) demonstrated that ADX causes a decrease in MOR metabolism which enhances MOR analgesia through an increased MOR concentration with no change in the sensitivity to MOR. On the other hand, Ratka et al. (19,20) reported that ADX potentiates MOR analgesia through an increased sensitivity to MOR in the central nervous system. Recently Miyamoto et al. (15,16) suggested that ADX potentiates MOR analgesia through both mechanisms of the decreased MOR metabolism and the increased sensitivity to MOR.

In the present study, effects of ADX on MOR analgesia and MOR content in brain and plasma were examined to elucidate the mechanism of the ADX-induced potentiation of MOR analgesia. In addition, the effects of replacement treatment with prednisolone (PRED), a representative glucocorticoid, on the ADX-induced effects were investigated to elucidate the mechanism of the glucocorticoid-reversal of MOR analgesia.

METHOD

Male Sprague-Dawley rats (Clea) weighing 220–260 g were used. They were housed two to a cage in an air-conditioned (23–24°C, 60% humidity), light-controlled (lights on from 08:00 to 20:00) room. Food (CA-1, Clea) and water were available ad lib. ADX and sham operation (SHAM) were performed by bilateral dorsal incisions under ether anesthesia. ADX rats were divided into two groups, one as ADX group and another as ADX-PRED group to which PRED (Predonine, Shionogi Pharm.) 10 mg/kg, SC was administered twice a day after surgery for replacement treatment. Final dose of PRED was 4–6 h before MOR administration. ADX and ADX-PRED rats were given 0.9% NaCl as drinking water after surgery.

MOR analgesia was estimated on the 7th day after surgery, according to the hindpaw pressure method (8) with an analgesymeter (Ugo Basile). Rats were gently handled for at least 5 days prior to the analgesic assay. Basal pain threshold was measured twice, at an interval of 10 min, just before MOR administration and the average was employed. Pain thresholds were measured every 10 min for 120 min after MOR administration (3.5 or 7 mg/kg, SC, morphine hydrochloride, Takeda Pharm.). When the weight loaded on the hindpaw reached 1750 g without any nociceptive response, the trial was terminated and the pain threshsold was determined to be 1750 g.

MOR content in brain and plasma was determined on the 7–9th day after surgery. At 50 min after MOR administration (3.5 or 7 mg/kg, SC), rats were sacrificed under sodium-pentobarbital anesthesia (260 mg/rat, IP, Somnopentyl, Pitman-Moore) and biological samples were obtained as follows. Blood was collected in a heparinized syringe by cardio-puncture, then centrifuged at 2000 × g, 4°C for 20 min to obtain a plasma sample. Brain was removed and dissected (7), and the medulla oblongata and pons, which includes the sensitive site to MOR analgesia (3,21), served as a brain sample. These samples were stored at −80°C until MOR determination by the HPLC-ECD method as previously de-

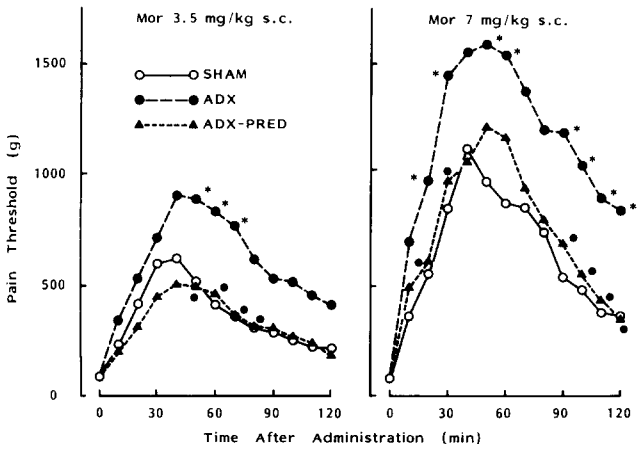

FIG. 1. Potentiation of morphine-induced increase in pain threshold by adrenalectomy, and its reversal by prednisolone. Each point represents the mean value of 7–8 rats. *Significant difference from SHAM. ●Significant difference from ADX.

scribed (16). The time of sacrifice, i.e., 50 min after MOR administration, was chosen because the content-analgesia relationship of MOR could be considered to be equilibrated, as the peak effect of MOR analgesia occurred at 30–40 min after SC administration.

Dose and content of MOR were calculated as morphine hydrochloride. Statistical analysis was performed by one-way analysis of variance followed by Newman-Keuls test, and a value of $p<0.05$ was considered significant.

RESULTS

Effects of ADX and PRED Replacement on the MOR Analgesia

The time courses of the increase in pain threshold after administration of MOR 3.5 or 7 mg/kg, SC are shown in Fig. 1. Basal pain thresholds in SHAM, ADX and ADX-PRED groups were 88±5 g (mean±S.E., n=15), 91±3 g (n=14) and 87±5 g (n=15), respectively, and there was no difference between the three groups. The increase in pain threshold produced by 3.5 mg/kg of MOR was significantly potentiated by ADX at 50–70 min after MOR administration, and that produced by 7 mg/kg of MOR was significantly or tended to be potentiated by ADX at 20–120 min after MOR administration. The ADX-induced potentiation of MOR analgesia after both doses was abolished by the replacement with PRED. Comparison of the area under the analgesic curve (AUAC) for 120 min as an index of analgesic potency revealed that ADX significantly potentiated MOR analgesia after both MOR doses, and the ADX-induced potentiation was abolished by the replacement with PRED (Fig. 2). The analgesic potency of MOR 3.5 mg/kg in ADX group was almost equipotent with that of MOR 7 mg/kg in SHAM and ADX-PRED groups (Figs. 1 and 2).

Effects of ADX and PRED Replacement on the MOR Content

Figure 3 shows MOR content in brain and plasma at 50 min after administration of MOR 3.5 or 7 mg/kg, SC. After 3.5 mg/kg of MOR, plasma MOR was not affected by ADX or PRED replacement, however, brain MOR tended to be increased by ADX (not significant), and PRED reversed the ADX-induced tendency of increase in brain MOR. In contrast, after 7 mg/kg, MOR content in both brain and plasma was significantly increased by ADX,

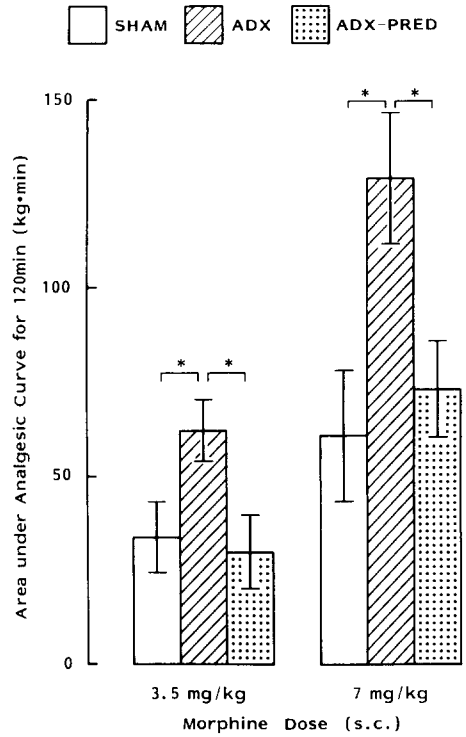

FIG. 2. Potentiation of morphine analgesia by adrenalectomy, and its reversal by prednisolone. Each value and vertical bar represent the mean and S.E.M. of 7–8 rats, respectively. These data are derived from Fig. 1. *Significant difference.

and PRED reversed the ADX-induced increase in MOR content. The brain/plasma ratios of MOR content were not affected by ADX or PRED replacement after both doses of MOR.

Comparison of MOR Content After Equi-Analgesic Doses

Figure 4 shows the comparison of the analgesic potency (AUAC) and MOR content in brain and plasma between three groups, i.e., MOR 7 mg/kg in SHAM, 3.5 mg/kg in ADX and 7 mg/kg in ADX-PRED, which showed almost equipotent analgesia (Figs. 1 and 2). MOR content in both brain and plasma of ADX group was significantly lower than those of SHAM and ADX-PRED groups.

DISCUSSION

Analgesic potency of MOR 3.5 and 7 mg/kg, SC was significantly potentiated by ADX, and PRED replacement reversed the ADX-induced potentiation of MOR analgesia (Figs. 1 and 2). Based on the equi-analgesic dose ratio, the extent of the ADX-induced potentiation was about 2-fold, being consistent with other investigators (9, 15, 22).

Holaday et al. (9) reported that ADX-induced potentiation of MOR analgesia is due to the increased MOR concentration in blood and brain. However, in their study, total radioactivity after administration of ^{3}H-MOR was measured for MOR content. In the present study, unchanged MOR was specifically determined by the HPLC-ECD method (16). ADX did not affect MOR content in brain and plasma after 3.5 mg/kg MOR and prominently increased MOR content in both brain and plasma after 7 mg/kg MOR, being consistent with our previous report (15), and PRED reversed the ADX-induced increase in MOR content (Fig. 3).

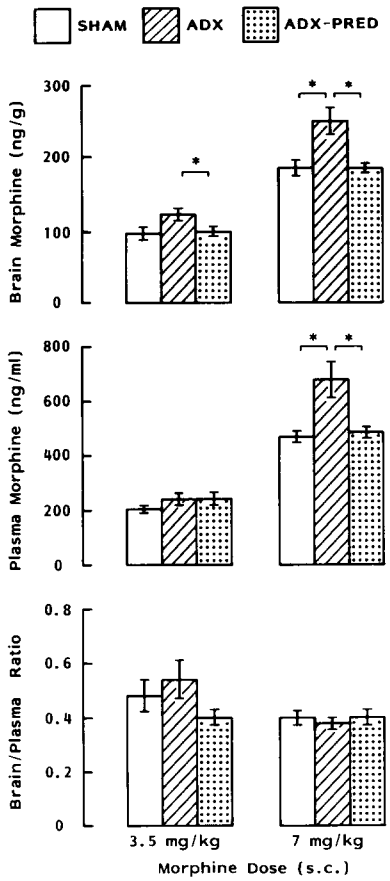

FIG. 3. Effects of adrenalectomy on the morphine content in brain and plasma, and its reversal by prednisolone. Each value and vertical bar represent the mean and S.E.M. of 8–10 rats, respectively. *Significant difference.

The activity of hepatic microsomal mixed function oxidase has been reported to be reduced by ADX (5). However, there are contradictory reports about the effect of ADX on the activity of glucuronide conjugation (2,23), which is the primary metabolic pathway of MOR (10). It is possible that ADX reduces glucuronide conjugation of MOR, because the MOR conjugation is activated by inducers of microsomal enzyme (6) and reduced by inhibitors of microsomal enzyme (12). The ADX-induced reduction of mixed function oxidase has been reported to be reversed by replacement with glucocorticoids (4). Accordingly, the present ADX-induced increase in MOR content and its reversal by PRED replacement may suggest that ADX reduces the glucuronide conjugation of MOR and this ADX-induced reduction is reversed by glucocorticoids as well as the mixed function oxidase. The ADX-induced increase in MOR content was apparent after 7 mg/kg of MOR but not after 3.5 mg/kg, being consistent with Adler et al. (2) and our previous report (15) that ADX reduces the capacity of MOR metabolism.

The comparison of MOR content after equi-analgesic doses (Fig. 4) revealed that the ADX-induced potentiation of MOR analgesia cannot be explained by the increased MOR content alone. The MOR content in brain and plasma in the ADX group (MOR 3.5 mg/kg) was significantly lower than those in the other equi-analgesic groups, i.e., SHAM and ADX-PRED groups (MOR 7 mg/kg). This result indicates that ADX increases the sensitivity to MOR, and PRED reverses this ADX-induced sensitization. Lewis et al. (11) suggested that ADX potentiates opiate analgesia by increasing the affinity of opiate receptors, and Ratka et al. (19) suggested that ADX increases the number and/or the affinity of available opioid receptor sites for MOR. Other reports (14,20) also postulated that the glucocorticoid is a critical factor in the control of an opioid-mediated pain inhibitory system in the central nervous system. However, no report could provide a direct evidence for the mechanism of the ADX-induced increase in the sensitivity to MOR, and further details of the mechanism of ADX-induced sensitization to MOR remain to be elucidated.

The brain/plasma ratios of MOR content after both MOR doses were not affected by ADX or PRED, so the possibility that ADX increases the passage of MOR through the blood-brain barrier

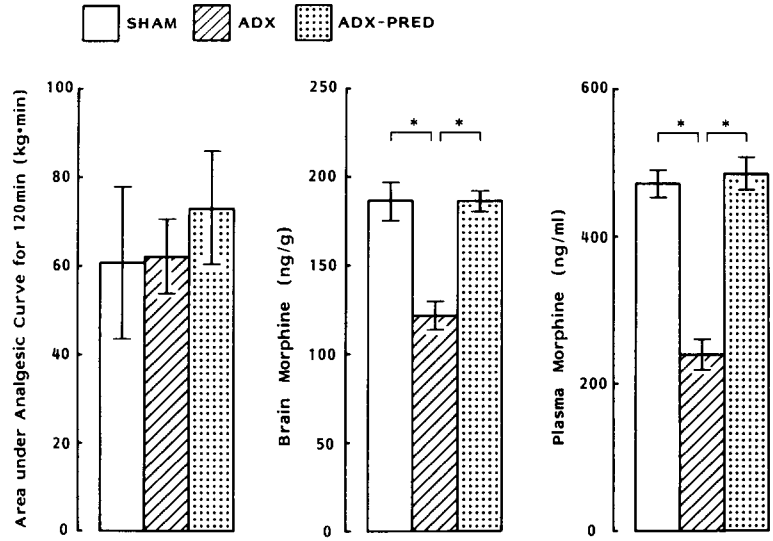

FIG. 4. Comparison of morphine content between equi-analgesic groups. SHAM, MOR 7 mg/kg in SHAM; ADX, MOR 3.5 mg/kg in ADX; ADX-PRED, MOR 7 mg/kg in ADX-PRED. These data are derived from Figs. 2 and 3. *Significant difference.

(13,15) could be excluded. Recently, it has been reported that morphine-6β-glucuronide (M6G) has a much more potent analgesic effect than MOR itself and/or enhances opioid binding to rat brain membrane (1,18). As M6G is a minor metabolite in rats (17), and it was suggested that ADX reduces the glucuronidation of MOR as described above, it seems unlikely that M6G contributes to the ADX-induced potentiation of MOR analgesia. However, in the present study, metabolites of MOR were not measured and the possibility that ADX affects the formation and/or the distribution of M6G and consequently potentiates MOR analgesia cannot be excluded.

In conclusion, ADX potentiated MOR analgesia through both mechanisms of the increased MOR content and the increased sensitivity to MOR, and replacement with PRED abolished these ADX-induced effects, suggesting that the lack of adrenal glucocorticoids participates in both of these ADX-induced effects.

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