Suppressive Effect of Dexamethasone on Morphine-Induced Adrenocorticoidogenesis in Rat

TSUKASA KUWAMURA, SHIGEYUKI NAGAO*, SHINSUKE NAKAURA*, KUNIO KAWASHIMA*, SATORU TANAKA*, YOSHIHITO OMORI
AND TAKESHI NAKAO

Department of Pharmacology, The Jikei University School of Medicine, Tokyo
*Department of Pharmacology, National Institute of Hygienic Sciences, Tokyo

Synopsis

The effect of morphine on adrenal-pituitary axis function was examined by determination of adrenal and serum corticosterone changes in rats pretreated with dexamethasone.

Increases in both adrenal and serum corticosterone concentrations induced by a single dose of 20 mg/Kg of morphine were almost completely inhibited by subcutaneous injection with 1 mg/rat of dexamethasone 3 hr prior to morphine administration. However, no suppressive effect of dexamethasone was observed when a dose of 30 µg/rat of synthetic ACTH was given.

In morphinized rats, adrenal and serum corticosterone concentrations were not so significantly increased as observed in a single dose of morphine administration. However, corticosterone concentrations after the morphinization were increased strikingly by synthetic ACTH or nalorphine administration immediately after the final treatment of morphine. But these increases after nalorphine administration were inhibited entirely by the pretreatment with dexamethasone.

On the bases of these findings, it was suggested that morphine does not act directly corticoidogenesis in adrenal cortex but affects through the central nervous system.

In rat, the adrenal and plasma corticosterone concentrations increased after a single dose of morphine (Nakao and Hiraga, 1968). On the contrary, apparent dysfunction of adrenal glands was demonstrated with chronic morphine treatment, and the interpretation of this event is not consistent among investigators; namely, morphine is assumed by some to exert a direct action on the adrenal (Paroli and Melchiorri, 1961) and by others to induce depletion of ACTH from the pituitary gland (Gillette, 1965).

On the other hand, suppressive effect of such steroids as cortisol1, prednisolone2 and dexamethasone3 on stress-induced increases in adrenal corticoidogenesis is well known (Kendall, 1961; Hedner and Relup, 1962; Sirett and Gibs, 1969). The site of corticoid feedback action has been assumed to be the hypothalamus and/or the pituitary gland. Egdahl (1964), Stark et al. (1968), Hedge and Smelik (1969), Bohus and Strashimirou (1970), Takebe et al. (1971) and Ondo and Kitay (1972) have suggested that the glucocorticoids' feedback site is in the intrahypothalamic area, while others (Arimura et al., 1967; Kendall and Allen, 1968; Russell et al., 1969) have reported that the corticoids inhibit ACTH secretion by acting directly the pituitary gland. Therefore, the authors tried to

1. 17α, 21-dihydroxy-4-pregnen-3, 11, 20-trione.
2. 11β,17,21-trihydroxy-pregna-1,4-diene-3,20-dione.
3. 9-fluoro-11β,17,21-trihydroxy-16α-methyl-pregna-1,4-diene-3, 20-dione.
determine the site of morphine action specially in relation to its effect on corticoidogenesis using dexamethasone treated rats.

Materials and Methods

Five male rats of the Wistar strain in each group were used those weighing about 230-250 g through the experiments. Morphine hydrochloride, nalorphine hydrochloride and synthetic adrenocorticotropic hormone (ACTH; Cortrosyn-Z, Organon) were dissolved in saline and dexamethasone phosphate was suspended in a 2.5% gum arabic solution. Rats given saline alone served as control. All injections were performed subcutaneously. In morphinization experiments, morphine was administered twice daily with the increasing doses; 20 mg/kg for the 1st and 2nd days, 40 mg/kg for the 3rd and 4th days and 80 mg/kg from the 5th to 10th days. Dexamethasone was given at a dose of 1 mg/rat 3 hr prior to each experiment according to the observation that the systemic administration of dexamethasone inhibits both stress-induced and resting plasma corticosterone levels 2 to 4 hr after the injection (Hedner and Rerup, 1962).

Animals were decapitated and blood was collected and then the adrenals were removed immediately.

Corticosterone was determined fluorometrically by the method of Guillemin et al. (1959) modified by Nakao and Hirai (1961).

Results

Effect of dexamethasone pretreatment on changes in adrenal and serum corticosterone concentration after morphine and ACTH treatment in rats

Twenty mg/Kg of morphine or 30 μg/rat of ACTH were given and animals were sacrificed after 1 hr.

As shown in Table 1, adrenal and serum corticosterone concentrations 3 hr after dexamethasone administration decreased below those of saline control levels. Similar results were also obtained in further experiments and such decreases in both corticosterone concentrations can be seen more clearly in Tables 2 and 3. But these concentrations were increased strikingly after the administration of morphine without pretreatment with dexamethasone. With dexamethasone pretreatment 3 hr prior to morphine administration, however, no increases in both corticosterone concentrations were observed and the levels were comparable with those of the control. On the contrary, there was no significant difference in adrenal and serum corticosterone concentrations between the groups pretreated and nonpretreated with dexamethasone 3 hr prior to ACTH administration.

Effect of dexamethasone pretreatment on changes in adrenal and serum corticosterone concentrations in the morphinized rats

Animals were treated with dexamethasone 3 hr prior to the final injection of morphine, and also 30 μg/rat of ACTH or 5 mg/Kg of nalorphine was administered immediately after morphine, and animals were sacrificed after 1 hr.

It was observed clearly that corticosterone concentrations in both adrenal and serum were decreased by dexamethasone pretreatment in non-morphinized control rats. These values were increased by a single administration of morphine or ACTH without dexamethasone, but after dexamethasone pretreatment these increases were not observed in rats receiving morphine, though ACTH produced an increase in these concentrations even after dexamethasone pretreatment (Tables 2 and 3).

An apparent increase was observed in both adrenal and serum corticosterone concentrations following the administration of nalorphine, but the effect was inhibited completely by pretreatment with dexamethasone 3 hr prior to nalorphine administration in nonmorphinized rats.

In morphinized rats without dexamethasone pretreatment, both adrenal and serum corticosterone concentrations were higher than those of the non-morphinized rats without dexamethasone pretreatment. Following the final injection of morphine without dexameth-
Table 1. Effect of dexamethasone pretreatment on changes in adrenal and serum corticosterone concentrations after a single dose of morphine and ACTH

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Corticosterone concentration</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>adrenal (µg/100 mg adr.)</td>
<td>serum (µg/100 ml)</td>
</tr>
<tr>
<td>Saline Control</td>
<td>1.22 ± 0.16</td>
<td>3.69 ± 0.58</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>1.17 ± 0.37</td>
<td>1.28 ± 0.07</td>
</tr>
<tr>
<td>Morphine</td>
<td>12.56 ± 1.38</td>
<td>86.37 ± 7.64</td>
</tr>
<tr>
<td>Dexamethasone + Morphine</td>
<td>1.11 ± 0.17</td>
<td>3.56 ± 0.98</td>
</tr>
<tr>
<td>ACTH</td>
<td>11.19 ± 0.63</td>
<td>71.57 ± 3.69</td>
</tr>
<tr>
<td>Dexamethasone + ACTH</td>
<td>9.79 ± 0.71</td>
<td>84.75 ± 12.16</td>
</tr>
</tbody>
</table>

One mg/rat of dexamethasone was administered subcutaneously 3 hr prior to subcutaneous injection of 20 mg/kg of morphine or 30 µg/rat of synthetic ACTH. Five animals in each group were sacrificed at 8:30 a.m., 1 hr after morphine or ACTH, and adrenal and serum corticosterone concentrations were determined fluorometrically. Figures in the table represent Mean ± S.E.

Table 2. Effect of dexamethasone pretreatment on changes in adrenal corticosterone concentrations in morphinized rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Corticosterone concentration (µg/100 mg adrenal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-morphinized without dex.</td>
</tr>
<tr>
<td>Saline Control</td>
<td>2.29 ± 0.37</td>
</tr>
<tr>
<td>Morphine</td>
<td>12.94 ± 1.03</td>
</tr>
<tr>
<td>ACTH</td>
<td>10.98 ± 0.60</td>
</tr>
<tr>
<td>Nalorphine</td>
<td>6.07 ± 1.25</td>
</tr>
</tbody>
</table>

In morphinized rats, morphine was injected subcutaneously 2 times daily in morning and evening for 10 days with graded increasing doses; 20 mg/kg on the 1st and 2nd days, 40 mg/kg on the 3rd and 4th days, and 80 mg/kg from the 5th to 10th days. One mg/rat of dexamethasone was administered subcutaneously 3 hr prior to the last injection of morphine, 30 µg/rat of synthetic ACTH or 5 mg/kg of nalorphine was administered subcutaneously and animals were sacrificed 1 hr later. Figures in the table represent Means ± S.E. For other legends refer to Table 1.

Table 3. Effect of dexamethasone pretreatment on changes in serum corticosterone concentrations in morphinized rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Corticosterone concentration (µg/100 ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-morphinized without dex.</td>
</tr>
<tr>
<td>Saline Control</td>
<td>4.07 ± 1.24</td>
</tr>
<tr>
<td>Morphine</td>
<td>76.28 ± 6.60</td>
</tr>
<tr>
<td>ACTH</td>
<td>71.68 ± 4.81</td>
</tr>
<tr>
<td>Nalorphine</td>
<td>6.78 ± 0.59</td>
</tr>
</tbody>
</table>

Figures in the table represent Mean ± S.E. For other legends refer Table 2.
amethasone pretreatment, the levels of corticosterone concentrations in both adrenal and serum were about one half and one third those of the single dose of morphine in non-morphinized rats, respectively. However, when ACTH or nalorphine was administered immediately after the final morphine treatment, corticosterone concentrations in both adrenal and serum were increased more markedly than those observed in non-morphinized rats. On the other hand, the levels of both adrenal and serum corticosterone concentrations after nalorphine in morphinized rats pretreated with dexamethasone were near to or below those of the respective control, though the levels of the adrenal or serum corticosterone concentration after ACTH administration were increased regardless of morphinization and of dexamethasone pretreatment.

**Discussion**

Dexamethasone has a powerful inhibitory effect on corticoidogenesis through a negative feedback action in ACTH release at the level of the pituitary and/or hypothalamus (Zimmermann and Critchlow, 1969). Therefore, if morphine acts directly the adrenal cortex in vivo, the stimulative effect of morphine on corticoidogenesis will not be blocked by pretreatment with dexamethasone. However, if morphine acts corticoidogenesis through the pituitary or the central nervous system as postulated by several investigators (Briggs and Munson, 1955; Eisenman *et al.*, 1958; Leeman *et al.*, 1962), one can expect that dexamethasone may block the action of morphine on adrenal function, even though the effect is not complete.

In the present work, elevation of the adrenal and serum corticosterone concentrations following a single dose of 20 mg/Kg of morphine was almost completely blocked by a subcutaneous injection of 1 mg/rat of dexamethasone 3 hr prior to morphine administration. The dose of dexamethasone applied was assumed to be effective to depress adrenal secretion as observed in decrease of serum corticosterone level, although a little change was observed in adrenal level in the present experiment. However, no suppressive effect of dexamethasone pretreatment was observed when a dose of 30 μg/rat of synthetic ACTH was given. Using hypophysectomized rats, Hirai *et al.* (1970) concluded that morphine does not act directly corticoidogenesis in vivo. Similar finding was also confirmed in hypophysectomized rats in our laboratory (unpublished data). The present results strongly support the Hirai's conclusion and it seems likely that the site of morphine action was localized at the level of the pituitary and/or in some area of the central nervous system through which morphine may exert its effect on the target organs.

In case of morphinization, adrenal and serum corticosterone concentrations did not indicate such significant elevation after final injection as observed in a single dose of morphine. However, when ACTH or nalorphine was given immediately after the final injection of morphine, drastic rises in these concentrations have been observed. These results provide further evidences that morphine may not act directly the adrenal cortex as ACTH does but may act the pituitary and/or through the central nervous system. The present finding which reveals an increase of corticosterone concentration in serum and adrenal after nalorphine in morphinized rats suggests that pituitary ACTH content is not significantly depleted by morphinization, contrary to the claim proposed by Gillette (1965). The elevation of adrenal and serum corticosterone concentrations was not blocked after ACTH injection even if the animals were pretreated with dexamethasone. Furthermore, the fact that the elevation in both adrenal and serum corticosterone concentrations after nalorphine treatment in morphinized rats was blocked by dexamethasone pretreatment will not be in agreement with the claim that the apparent adrenal hypofunction by mor-
phinization is due exclusively to an impairment of the target organ which became less responsive to proper corticotrophic stimulation (Paroli and Melchiorri, 1961). The work on the ACTH dynamics which suggests a partial block of its secretion from the pituitary gland after morphinization is in progress and is to be reported elsewhere (Kuwamura, 1973).

References
