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INFLUENCE OF PEPTIDES ON REDUCED RESPONSE OF RATS TO ELECTRIC FOOTSHOCK AFTER ACUTE ADMINISTRATION OF MORPHINE

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Acute treatment of rats with morphine (10 mg/kg) resulted in a marked reduction of motor response to inescapable electric footshock (EFS). Nalorphine (2 mg/kg) antagonized this action of morphine. Pretreatment with synthetic ACTH 1-24 (10 IU) 60 min prior to testing also inhibited this morphine-induced reduction, whereas other ACTH-like peptides, lacking corticotrophic activity, were ineffective. ACTH 1-24 had no effect on the response of adrenalectomized rats to EFS after morphine. In intact rats dexamethasone pretreatment 4 hr prior to testing also antagonized the action of morphine on EFS. Taken together these findings suggest that ACTH 1-24 interferes with the antinociceptive action of morphine and that the integrity of the adrenal is essential for demonstration of this antagonism.

Response to EFS ACTH Dexamethasone Morphine Adrenalectomy

1. Introduction

Morphine (M) has profound effects on pituitary-adrenal activity (Selye, 1936; Briggs and Munson, 1955; Munson, 1973; Zimmermann and Critchlow, 1973; De Wied et al., 1974). Considering the importance of pituitary peptides to brain function (De Wied, 1974), one might anticipate that adrenocorticotrophic hormone (ACTH) would modify the analgesic actions of M on the nervous system as demonstrated by Winter and Flataker (1951) and by Paroli (1967). The present studies were undertaken to determine if ACTH 1-24, a synthetic polypeptide which has neurotropic and corticotrophic actions in common with ACTH (De Wied, 1969), also antagonizes the influence of M on the response of rats to unescapable electric footshock (EFS). When it was found to do so, the research was extended and attempts were made to elucidate the mechanism.

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2. Materials and methods

2.1. Animals and surgery

Female, albino rats of an inbred Wistar strain (TNO, Zeist, The Netherlands), weighing 140—160 g were used. In experiments involving

adrenalectomized rats, bilateral adrenalectomy or sham operation was performed under ether anesthesia. Adrenalectomized rats were given a solution of 0.9% NaCl instead of water to drink and all animals were studied on the third day after surgery.

2.2. Injected materials

Morphine · HCl, nalorphine · HCl and dexamethasone phosphate were dissolved in saline. Morphine was injected i.p. either 10 mg/kg or 5.6 mg/kg in a concentration of 1 mg/ml. Nalorphine (2 mg/kg in 0.3 ml) and dexamethasone (50 μg/0.3 ml/rat) were injected s.c. Synthetic ACTH 1-24 (10 IU/100 μg/rat), synthetic ACTH 1-10 (100 μg/rat) and porcine β-MSH (100 μg/rat) were dissolved in a drop of 0.01 M HCl and further diluted with saline for s.c. injection (0.3 ml).

2.3. Test procedure

Response to EFS was measured in a test session of approximately 8 min using the method described by Evans (1961) as modified by Gispen et al. (1970, 1973). Each EFS was presented for 1 sec and the interval between EFSs was 20 sec. The EFSs were varied in intensity from 33 to 300 μA and 22 EFSs were presented in two sequential series of 11 different intensities, programmed in a fixed random order. The following responses were distinguished and counted: 'no response', 'flinch' and 'jerk-run-jump'. For each EFS presentation only one type of response was recorded. Evidence validating the grading system has been presented (Gispen et al., 1973). The responses obtained for each rat were expressed as the percent of the total number of responses obtained during a testing session and these percents were used for statistical analysis using Mann Whitney U-test. A difference was considered statistically significant when $p < 0.05$ (two tailed).

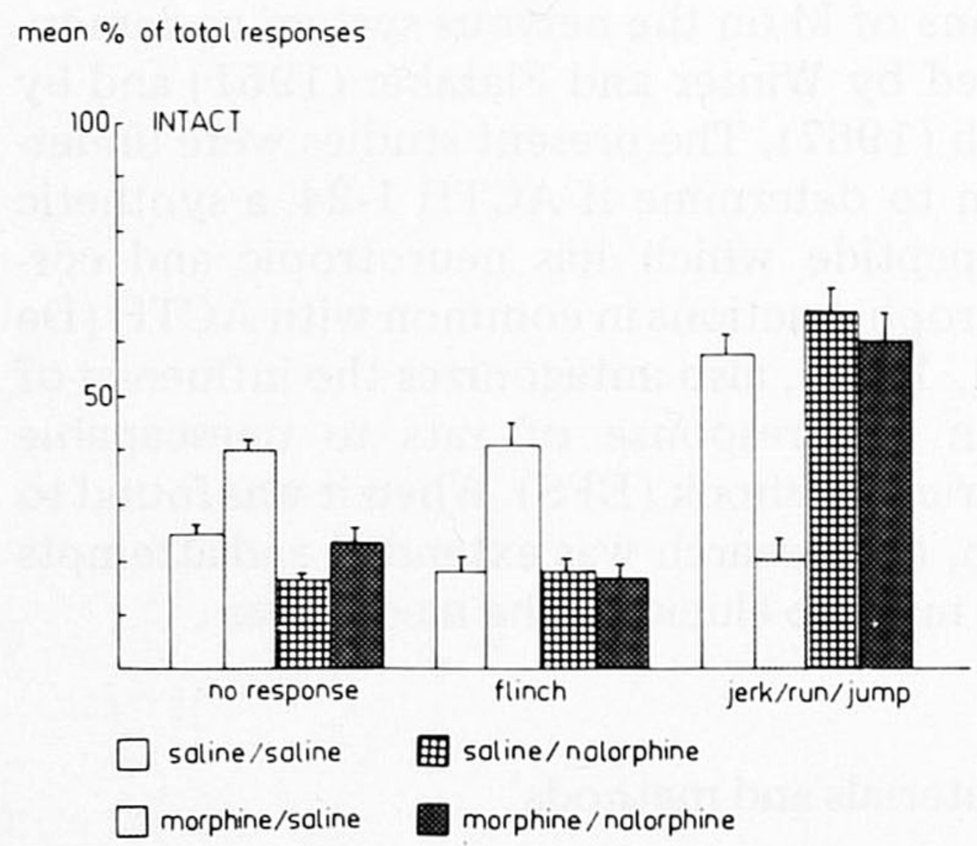

Fig. 1. Morphine-induced reduction of response to EFS; antagonism with nalorphine. Saline/saline vs. morphine/saline: no response $p < 0.02$; flinch $p < 0.02$; jerk, run, jump $p < 0.02$. Saline/nalorphine vs. saline/saline: no significant differences. n in all groups = 6. For further information see text.

3. Results

3.1. Morphine reduction of response to EFS; antagonism with nalorphine

A group of 24 rats was divided into 4 subgroups of 6 rats each. All rats received 2 injections, the first one 15 min and the second one 5 min prior to EFS. The first injection was i.p. and the second was s.c. The first subgroup received saline followed by saline, the second subgroup received morphine (10 mg/kg) followed by saline, the third subgroup received saline followed by nalorphine (2 mg/kg) and the fourth subgroup morphine (10 mg/kg) followed by nalorphine (2 mg/kg).

The influence of the various treatments on response to EFS is presented in fig. 1. Morphine/saline treatment resulted in a reduction of the jerk, run, jump response to approximately one-third of that observed in saline/saline-treated rats (20 vs. 56%). Concomittant with this, a marked increase was observed in 'flinch' and in 'no response'. Comparing the 2 groups treated with nalorphine, it is evident that pretreatment with morphine does not affect the response to EFS in the presence of this antago-

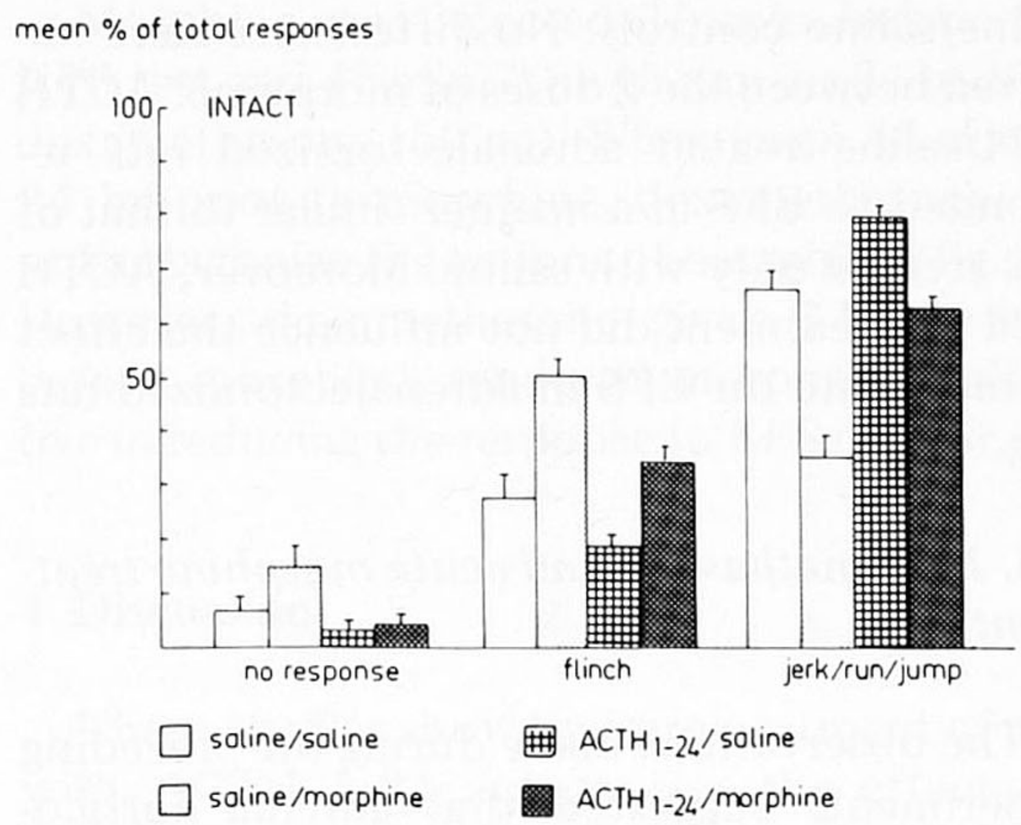

Fig. 2. Effect of ACTH 1-24 on morphine-induced reduction of response to EFS. Saline/morphine vs. ACTH 1-24/morphine: no response $p < 0.05$; flinch $p < 0.05$; jerk, run, jump $p < 0.02$. n in all groups = 6. For further information see text.

nist. Nalorphine per se did not influence the response.

3.2. *Effect of ACTH 1-24 on morphine-induced reduction of response to EFS*

This experiment was designed to determine if ACTH 1-24 influences the morphine-induced reduction of response to EFS. Therefore, ACTH 1-24 (10 IU/rat) was injected s.c. 60 min prior to testing, and morphine (10 mg/kg) or its vehicle was injected i.p. 15 min prior to testing. 4 groups of 6 rats each were used. The first group received saline for both injections; the second group received saline and then morphine; the third group received ACTH 1-24 followed by saline; the fourth group received ACTH 1-24 followed by morphine.

Fig. 2 illustrates the marked inhibitory influence of morphine on the 'jerk, run, jump' component of the response to EFS compared to the saline/saline control group. However, when rats were pretreated with ACTH 1-24, morphine had no observable influence on the response to EFS. ACTH 1-24 injection per se resulted in a small but statistically insignificant increase in the percent of 'jerk, run, jump' responses.

3.3. *Effect of ACTH 1-10 and of β-MSH on morphine-induced reduction of response to EFS*

The previous experiment showed that ACTH 1-24 is able to antagonize the morphine-induced reduction of response to EFS. In order to differentiate between a direct peptide effect and an indirect effect through the adrenal cortex, the previous experiment was repeated using 2 peptides related to ACTH, namely ACTH 1-10 and β-MSH. These 2 peptides share, with ACTH 1-24, the sequence ACTH 4-10. However, the lack of the 11-24 sequence (ACTH 1-10) or the substitution of other sequences at the N- and C-terminal sides of ACTH 4-10 (β-MSH) results in a loss of corticotrophic activity such that in the doses used, no appreciable stimulation of the adrenal cortex is observed (De Wied, 1969; Gispen and Schotman, in preparation). Using the experimental design described for the preceding experiments, pretreatment with ACTH 1-10 (100 μg/rat) or β-MSH (100 μg/rat) appeared to be ineffective in counteracting morphine-induced reduction of response to EFS (fig. 3). Moreover, neither peptide given alone affected the observed response to EFS.

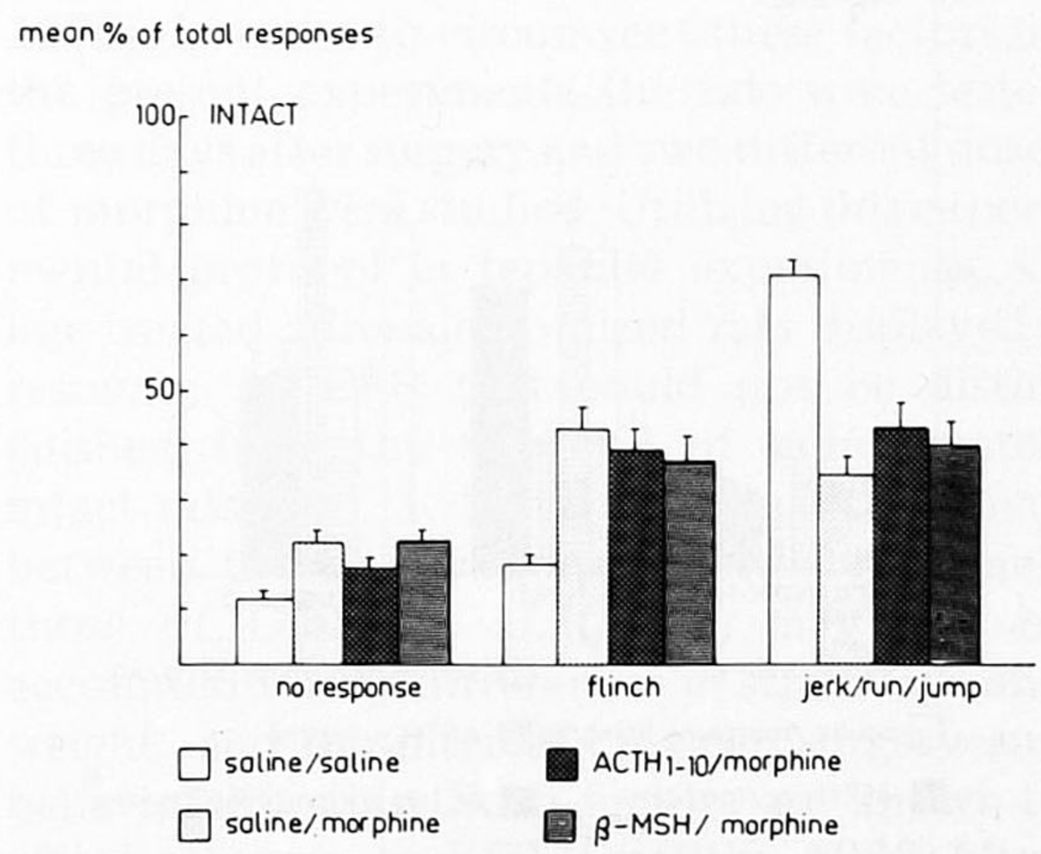

Fig. 3. Effect of ACTH 1-10 and β-MSH on morphine-induced reduction of response to EFS. No significant differences between saline/morphine, ACTH 1-10/morphine and β-MSH/morphine. n in all groups = 6. For further information see text.

3.4. Effect of ACTH 1-24 and morphine on response to EFS in adrenalectomized rats

Adrenalectomized rats were used 3 days after surgery, at a time when they would be expected to have low endogenous circulating ACTH levels (Dallman et al., 1972). The experimental protocol was the same as that described in the second experiment, i.e. 4 groups of 6 animals, with each group subjected to one of the following treatments: saline/saline; ACTH 1-24 (100 μg)/saline; saline/morphine (10 mg/kg); ACTH 1-24 (100 μg)/morphine (10 mg/kg). However, since Gebhardt and Mitchell (1972) found that 0.56 × the dose used for intact rats was needed to produce equi-analgesic effects in adrenalectomized rats, 2 additional groups were included, namely saline/morphine (5.6 mg/kg) and ACTH 1-24 (100 μg)/morphine (5.6 mg/kg). Both 5.6 and 10 mg/kg of morphine produced marked inhibition of the response to EFS, as evidenced by the decrease in scores for 'jump, run, jerk', concomittant with a rise in scores for 'flinch' and 'no response' (fig. 4) compared to the saline/saline controls. No differences were observed between the 2 doses of morphine. ACTH 1-24/saline-treated adrenalectomized rats responded to EFS in a manner similar to that of rats treated only with saline. Moreover, ACTH 1-24 pretreatment did not influence the effect of morphine on EFS in adrenalectomized rats (fig. 4).

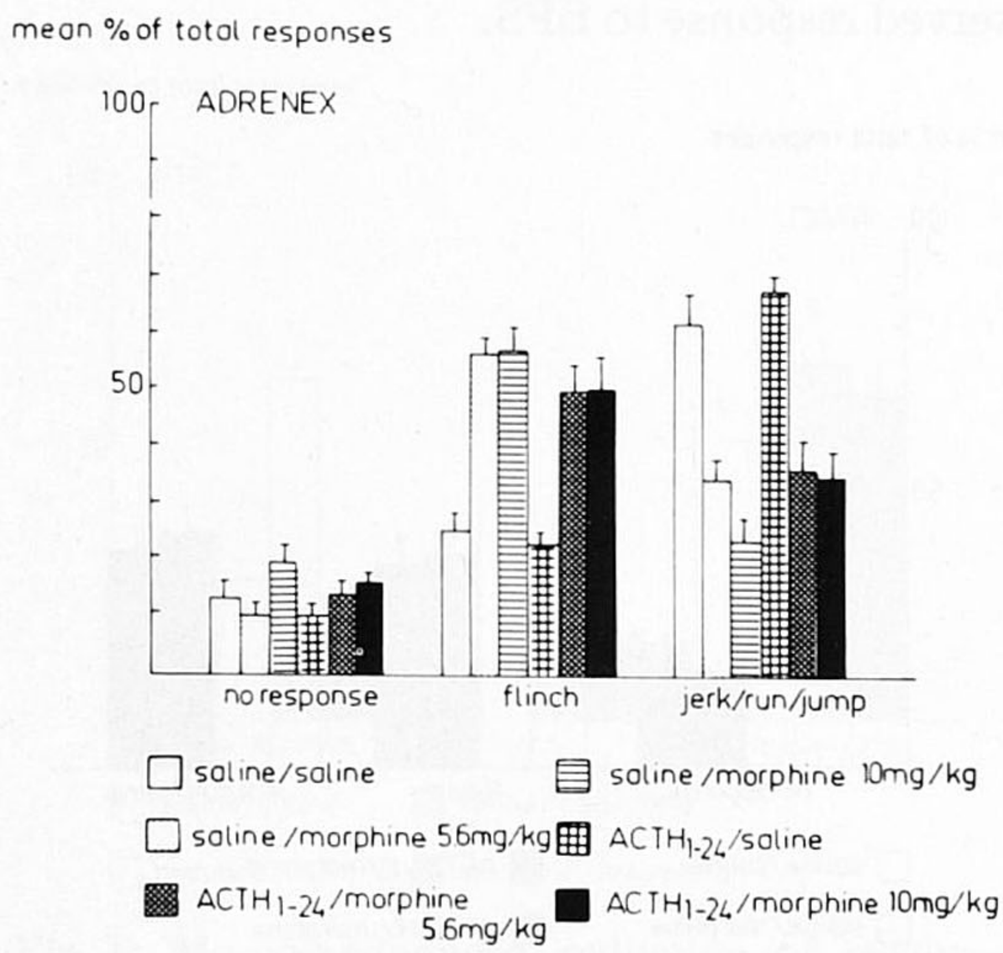

Fig. 4. Effect of ACTH 1-24 and morphine on response to EFS in adrenalectomized rats. Saline/morphine groups vs. ACTH 1-24/morphine groups: no response: n.s.; flinch n.s.; jerk, run, jump n.s. For further information see text.

3.5. Dexamethasone and acute morphine treatment

The observations made during the preceding experiments suggested that adrenal corticosteroids may be involved in the capacity of ACTH 1-24 to antagonize the effect of morphine on the response to EFS. Therefore, in this experiment a potent synthetic glucocorticosteroid, dexamethasone, was administered so as to study the effect of a glucocorticosteroid on the morphine-induced reduction of response to EFS.

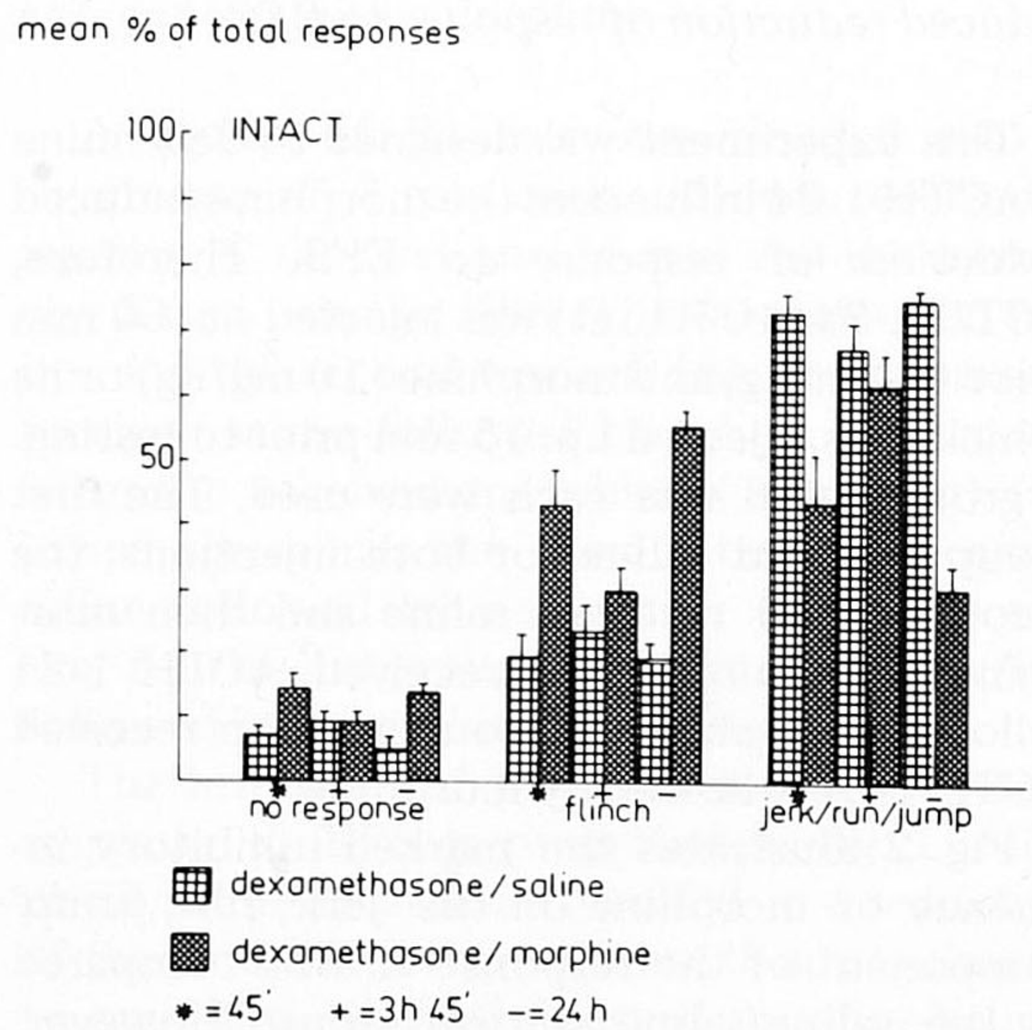

Fig. 5. Effect of dexamethasone on morphine-induced reduction of response to EFS. 45′: dex./sal. vs. dex./morphine: no response $p < 0.05$; flinch $p < 0.05$; jerk, run, jump $p < 0.05$. 3 hr 45′: no significant differences; 24 hr: no response $p < 0.05$; flinch $p < 0.02$; jerk, run, jump $p < 0.01$. For further information see text.

Morphine was injected 15 min before the EFS test and 45 min, 3 hr 45 min, or 24 hr after dexamethasone (50 μg). When given 45 min or 24 hr prior to morphine, dexamethasone did not antagonize the actions of morphine (fig. 5). However, dexamethasone given 3 hr 45 min before morphine, rendered morphine ineffective in reducing the response to EFS (fig. 5).

4. Discussion

These studies show that pretreatment of rats with ACTH 1-24 antagonizes the effects of morphine on EFS (fig. 2). These observations are consistent with those of Winter and Flataker (1951) and of Paroli (1967), who found that ACTH antagonizes the analgesic action of morphine. ACTH 1-24 per se slightly increased the 'jerk, run, jump' response of the animals. However, this small direct effect of ACTH 1-24 cannot explain the marked effect of ACTH 1-24 in antagonizing morphine (fig. 2).

The present experiments indicate that ACTH can antagonize an analgesic effect of morphine in the presence of adrenal gland since adrenalectomy prevents the antagonism. These observations are in agreement with those of Paroli (1967).

The observation that β-MSH did not antagonize the influence of morphine on EFS was unexpected since, ACTH 1-24 and β-MSH have similarities in structure, each is able to modify acquisition of conditioned avoidance responses (De Wied, 1969) and each is able to antagonize the actions of morphine on the segmental reflex of the cat (Krivoy et al., 1974). Since their major difference is that β-MSH lacks the corticotrophic activity of ACTH 1-24 we tentatively interpret the failure of β-MSH to modify the influence of morphine on EFS as further evidence that the capacity of ACTH 1-24 to antagonize this action of morphine depends upon the integrity of the adrenal cortex. The fact that β-MSH does not antagonize the action of morphine on EFS, but does antagonize the action of morphine on the segmental reflex of the cat (Krivoy et al., 1974) underscores the fact that one must exercise caution when comparing data from two different test systems. Similarly, caution must be urged in comparing the fact that ACTH 1-24 antagonizes the action of morphine on completely isolated frog spinal cord (Zimmermann and Krivoy, 1973, 1974), but does not antagonize the actions of morphine on EFS of adrenalectomized rats.

Primary evidence for the role corticosteroids play in antagonizing morphine-induced reduction of response to EFS was obtained by using adrenalectomized rats. However, in using adrenalectomized rats one must recognize several factors: (i) adrenalectomized rats are more susceptible to the analgesic effect of morphine treatment than intact rats (Gebhart and Mitchell, 1972; Wei, 1973). It has been observed that an equi-analgesic dose of morphine in adrenalectomized rats is 0.56× that used for intact rats (Gebhart and Mitchell, 1972). (ii) A long interval between adrenalectomy and testing results in a considerably increased level of endogenous ACTH, which may counteract the acute effects of morphine. (iii) Although under certain experimental conditions adrenalectomy does not alter the response to EFS (Gispen et al., 1973), under other conditions adrenalectomy is followed by an elevation of the threshold levels for motor responses (Gibbs et al., 1973). In order to circumvent these factors, in the present experiments the rats were tested three days after surgery and two different doses of morphine were studied. Utilizing this experimental protocol in separate experiments, saline-treated adrenalectomized rats displayed a response to EFS that could not be distinguished from the response of saline-treated intact rats (figs. 1, 2 and 4). The discrepancy between the observations reported here, and those of Gibbs et al. (1973) may well be accounted for by differences in strain, age and weight, and the interval between surgery and behavioral testing. Such factors are known to affect response to EFS (Griffiths, 1962; Misanin and Campbell, 1969; Paré, 1969).

Both doses of morphine, i.e., 5.6 and 10 mg/kg, reduced the response of adrenalectomized rats to EFS to the same degree (fig. 4).

We cannot explain this observation except for the likelihood that the doses of morphine used are close to the top of the dose—response curve. If this is the case, it may also explain the failure of β-MSH to have produced a demonstrable antagonism in intact rats, and of ACTH 1-24 to have produced one in adrenalectomized rats.

Although treatment of intact rats with dexamethasone resulted in a transient inhibition of morphine-induced reduction of response to EFS, the fact that the effective interval for dexamethasone did not correspond to that for ACTH 1-24 warrants caution in interpretation. Thus, the observation that both dexamethasone and ACTH 1-24 antagonize morphine could be taken as evidence that ACTH 1-24 antagonizes morphine by releasing corticosteroids. On the other hand, whereas the temporal dissimilarity in this action of ACTH 1-24 and dexamethasone might be due to a different time course of the effect of subcutaneously administered dexamethasone compared to that of an endogenous corticosteroid whose release is induced by ACTH 1-24, the temporal discrepancy may also be related to possible differences in the mechanism of action of ACTH 1-24 and of dexamethasone. It is of interest that the effective interval between administration of dexamethasone and its influence on the morphine-induced changes in responses to EFS corresponds to the interval necessary for maximal uptake of radioactive dexamethasone in the brain (De Kloet et al., 1974), to that of feedback inhibition of ACTH-release (Smelik, 1969) and to that of extinction of active avoidance behavior (Van Wimersma Greidanus, 1970). It is of further interest that the effect of ACTH analogues on avoidance conditioning occur 1 hr after injection (De Wied, 1971; Van Wimersma Greidanus et al., 1974).

The results of the study with dexamethasone treatment do not support the finding of Brown and Garrett (1972), who used a tail-flick method to determine analgesia in rats and found that dexamethasone acts synergistically with morphine. Our observation that dexamethasone can antagonize certain actions of morphine is consistent with the observations of Zimmermann and Critchlow (1973) and of Zimmermann et al. (1974a; 1974b).

Finally, one must question the significance of an altered response to EFS. The present study corroborates that of Evans (1961) by illustrating the potential utility of EFS in screening for analgesic drugs and their antagonists. The ability of morphine to modify response to EFS is likely due to an action on the central nervous system (Herz, 1972; Teschemacher et al., 1972). However, it is unlikely that EFS exclusively measures the animals' response to pain (Gispen et al., 1973). Other factors, such as motivation, locomotor activity and integrity of reflex pathways also influence the response to EFS.

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