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THE EFFECTS OF OPIOID DRUGS AND OF LITHIUM ON STEROIDOGENESIS IN RAT ADRENAL CELL SUSPENSIONS

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1 The effects of opioid drugs and of Na^+ replacement on steroidogenesis in rat adrenal cell suspensions were investigated.

2 In medium containing normal Na^+ (156 mM), opioid antagonists but not opioid agonists reduced the steroidogenic response to adrenocorticotrophic hormone$_{1-24}$ ($ACTH_{1-24}$) but not to dibutyryl adenosine 3',5' cyclic monophosphate (db cyclic AMP).

3 Replacement of 50% Na^+ in the medium by choline had no effect on steroidogenesis, but further reductions in Na^+ content reduced the steroidogenic activity of both $ACTH_{1-24}$ and db cyclic AMP.

4 In 50% Na^+ medium both opioid agonists and antagonists inhibited $ACTH_{1-24}$ induced steroidogenesis.

5 Addition of therapeutic concentrations of lithium to otherwise normal medium inhibited the steroidogenic response to $ACTH_{1-24}$ but not to db cyclic AMP.

6 The selective inhibition of $ACTH_{1-24}$-induced steroidogenesis by opioid drugs suggests some similarity between the opioid and ACTH receptors.

7 The relevance of the potent inhibitory effect of lithium to its therapeutic actions is discussed.

Introduction

The plasma corticosteroid response to ether stress is enhanced by the opioid agonist, normorphine, and abolished by the opioid antagonist, naloxone, suggesting that endogenous opioid substances may be involved in the regulation of the hypothalamus-pituitary-adrenal (HPA) system (Gibson, Ginsburg, Hall & Hart, 1977). The observations that adrenocorticotrophic hormone (ACTH) and some of its fragments can interact with opioid receptors (Terenius, 1975), and that opioid effects can be antagonized by ACTH (Gispen, Buitelaar, Weigant, Terenius & de Wied, 1976) suggested that there might be a similarity between the opioid and ACTH receptors and therefore that the adrenal cortex might be one of the sites at which opioids might act to modify the HPA system. The present study was undertaken to determine whether opioid agonists or antagonists might influence the steroidogenic response to $ACTH_{1-24}$ in dispersed cell preparations from rat adrenal glands.

Since it is known that the receptor binding of opioid agonists and antagonists is sodium-sensitive (Simon, Hillier, Groth & Edelman, 1975) experiments were also carried out to test the effect of varying the Na^+ content of the incubation medium on the adrenal cell response to $ACTH_{1-24}$ and on the ability of opioids to modify this response. These experiments led to the observation that the steroidogenic response produced by $ACTH_{1-24}$ is partially Na^+-dependent and that it is inhibited by low concentrations of lithium.

Methods

Isolated adrenal cells were prepared by a slight modification of the method of Lowry, McMartin & Peters (1973). Eight male rats (200 to 400 g; Wistar) were killed by stunning and exsanguination, the adrenal glands removed, cleared of fat and quartered. The adrenal quarters were then placed in a nylon tube containing 5 ml Hanks medium and 12.5 mg trypsin. The adrenal cells were dispersed by mechanical agitation of the tube contents with a plastic paddle attached to a Fischer-Technik MOT 8 electric motor, the tube being maintained at 37°C in a dry block. The cells were harvested by centrifugation (100 *g*; 10 min), and were resuspended in Hanks medium containing albumin (5.3 mg/ml) and lima bean trypsin inhibitor (533 μg/ml). $ACTH_{1-24}$ or dibutyryl adenosine 3',5' cyclic monophosphate (db cyclic AMP) were added in a range of concentrations to duplicate 0.6 ml aliquots of cell suspension, and after incubation

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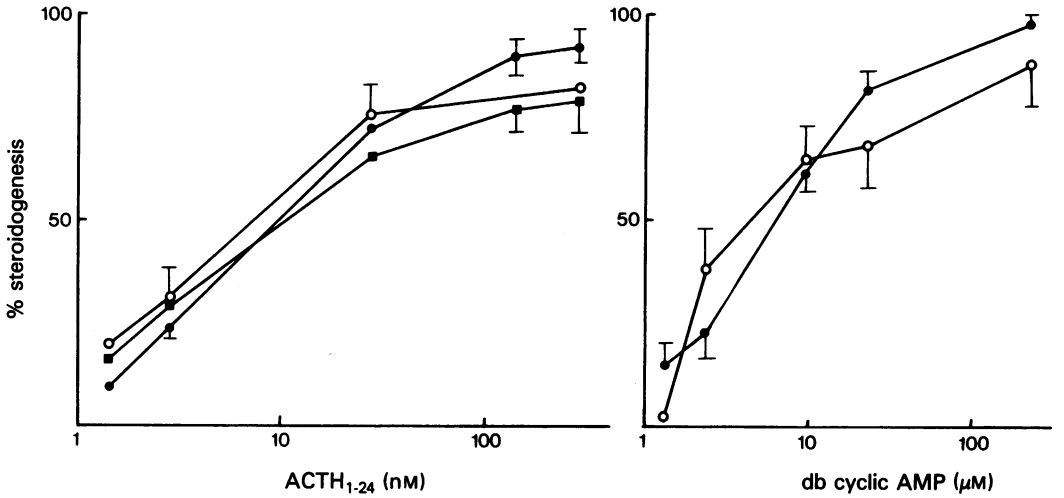

Figure 1 Dose-response curves of the steroidogenic response of rat adrenal cell suspensions to $ACTH_{1-24}$ or db cyclic AMP, acting alone (●) or in the presence of 100 μg/ml etorphine (○) or 100 μg/ml met-enkephalin (■). Each point is the mean of at least 6 observations; vertical lines show s.e. mean.

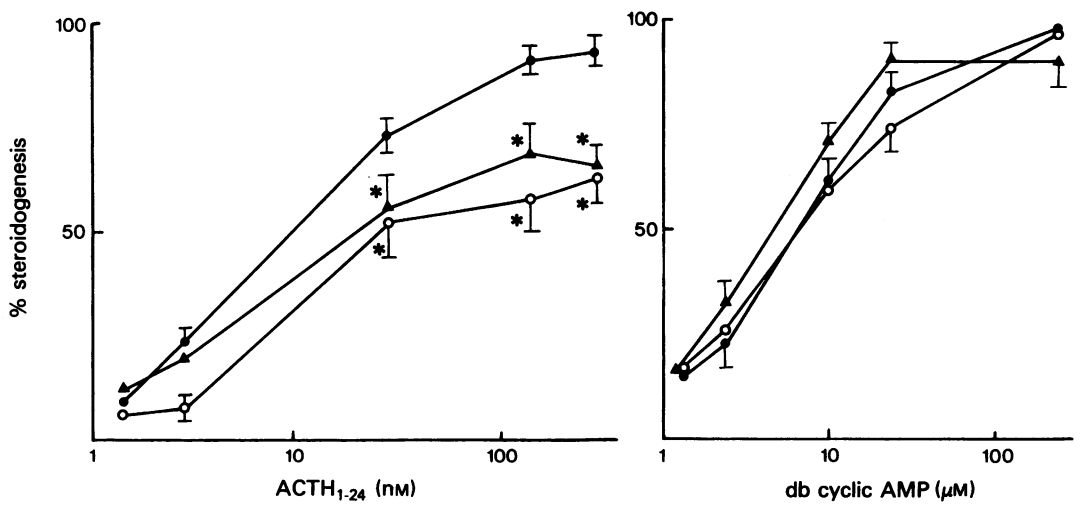

Figure 2 Dose-response curves of the steroidogenic response of rat adrenal cells suspensions to $ACTH_{1-24}$ or db cyclic AMP, acting alone (●) or in the presence of 100 μg/ml naltrexone (▲) or 100 μg/ml naloxone (○). Each point is the mean of at least 6 observations; vertical lines show s.e. mean. * $P < 0.05$, Student's t test.

at 37°C for 2 h the corticosteroid content of the suspension was estimated spectrophotofluorimetrically (Zenker & Bernstein, 1958). Drugs were added to the suspensions in the appropriate concentration before the 2 h incubation period. For the dose-response curves, steroidogenesis is expressed as a percentage of the maximum increase in corticosteroid production caused by either $ACTH_{1-24}$ or db cyclic AMP under control conditions on each experimental day. This procedure reduces the daily variation in results which occurs in this system and allows comparison of results from different experiments. Materials used included: $ACTH_{1-24}$ (Synacthen, CIBA); bovine serum albumin (Sigma); dibutyryl adenosine 3',5' cyclic monophos-

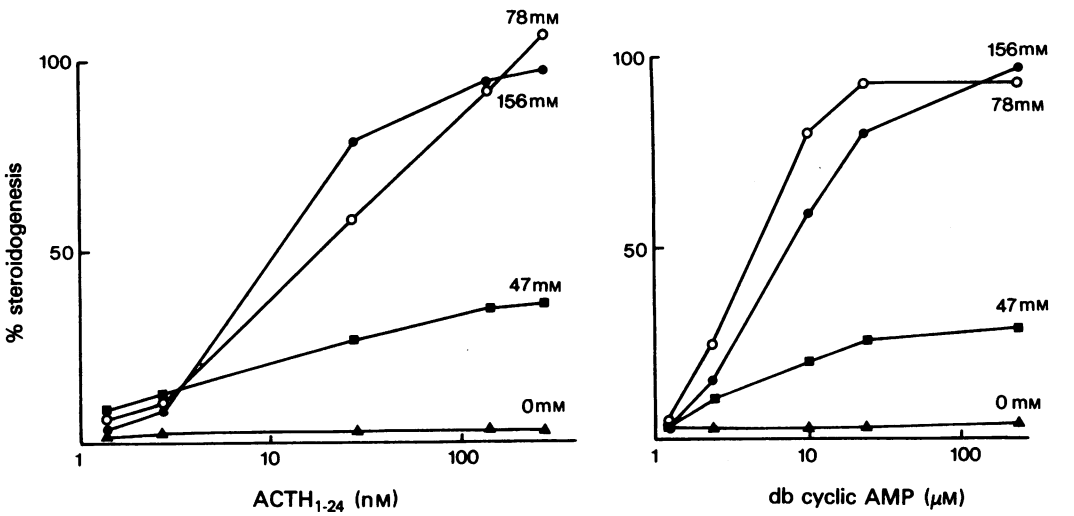

Figure 3 Dose-response curves of steroidogenic response of rat adrenal cell suspensions to $ACTH_{1-24}$ and db cyclic AMP in Hanks medium containing various Na^+ concentrations (shown beside the appropriate graph). When Na^+ was reduced below normal (156 mM) choline was used as substitute. Each point is the mean of at least 6 observations. Results are expressed as a percentage of the maximum response obtained in 156 mM Na^+ medium.

phate (Sigma); etorphine (Reckitt and Coleman); Hanks medium (Gibco Biocult); methionine enkephalin (Calbiochem); naloxone (Endo Laboratories); naltrexone (Endo Laboratories); bovine pancreas trypsin (Sigma); lima bean trypsin inhibitor (Sigma).

Results

Steroidogenesis in Hanks medium containing normal (156 mM) Na^+ concentrations

$ACTH_{1-24}$ produced a dose-dependent stimulation of steroidogenesis in the adrenal cell suspension (Figure 1). Etorphine and methionine-enkephalin (both 100 μg/ml) did not stimulate corticosteroid production by themselves and were without effect on $ACTH_{1-24}$- or db cyclic AMP-induced steroidogenesis (Figure 1). The opioid antagonists, naloxone and naltrexone, were also inactive by themselves but in high concentrations (100 μg/ml) they reduced the steroidogenic activity of $ACTH_{1-24}$ (Figure 2). However, the opioid antagonists did not reduce steroidogenesis induced by db cyclic AMP (Figure 2).

Steroidogenesis in Hanks medium containing reduced Na^+ concentrations

The effect of lowering the Na^+ content of the Hanks medium from normal (156 mM), on the steroidogenic activity of $ACTH_{1-24}$ and of db cyclic AMP is shown in Figure 3. In both cases a 50% reduction in Na^+ concentration was without effect, while further reductions produced an inhibition of responses to both $ACTH_{1-24}$ and to db cyclic AMP.

Since steroidogenesis was normal in Hanks solution containing 78 mM Na^+ this medium was used to determine the effect of lowered Na^+ levels on the actions of opioid drugs (Figure 4). Etorphine (100 μg/ml) which had been inactive in normal Na^+ medium produced a 52% inhibition of $ACTH_{1-24}$-induced steroidogenesis in medium containing 78 mM Na^+, while the inhibitory effect of naloxone was unchanged (Figure 4). However, methionine-enkephalin remained inactive against steroidogenesis even in medium containing only 78 mM Na^+.

In the above experiments the missing Na^+ was replaced by choline. However, in a number of experiments lithium was used as a Na^+ substitute and in these, a different pattern of results was obtained (Figure 5). While replacement of 50% of the Na^+ by choline had no effect on steroidogenesis, 50% replacement with lithium produced a marked inhibition. A similar degree of inhibition was observed even when only small amounts (1.2 to 7.2 mM) of lithium were added to medium containing normal Na^+ concentrations. This inhibitory effect of lithium on steroidogenesis appeared to be selective against $ACTH_{1-24}$ since the responses of the adrenal cells to db cyclic AMP was unaltered by lithium (Figure 6).

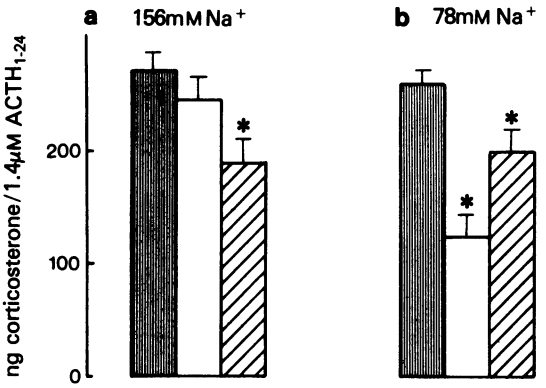

Figure 4 Histogram of the steroidogenic response of rat adrenal cells to 1.4 μM $ACTH_{1-24}$ in Hanks medium containing (a) 156 mM Na^+ and (b) 78 mM Na^+ acting alone (vertically lined columns) or in the presence of 100 μg/ml etorphine (open columns) or 100 μg/ml naloxone (hatched columns). The 78 mM Na^+ medium also contained 78 mM choline as a replacement. Each column represents the mean of at least 6 observations; vertical lines show s.e. mean. * $P < 0.05$, Student's t test.

Discussion

The results obtained in the present study confirm that the adrenal cortex may be a site at which opioid drugs can interfere with the HPA system. However, the doses required were high and it is unlikely that the adrenal cortex is the primary site on which the opioids act to induce modifications of corticosteroid production (Sloan, 1971), a much more likely candidate being the hypothalamus (George, 1971; Gibson, Ginsburg, Hall, Hart & Kitchen, 1978).

The inhibitory effects of the opioids on $ACTH_{1-24}$-induced steroidogenesis would appear to be an action exerted on the ACTH rather than the opioid receptor, since both agonists and antagonists produced the same effect and since the steroidogenic activity of db cyclic AMP was unaffected. Thus our original hypothesis that there may be some similarity between the opioid and ACTH receptors may be justified, although the possibility that the drugs are acting on some site beyond the ACTH receptor cannot entirely be dismissed.

In physiological Na^+ concentrations, opioid antagonists are more effective inhibitors of steroidogenesis

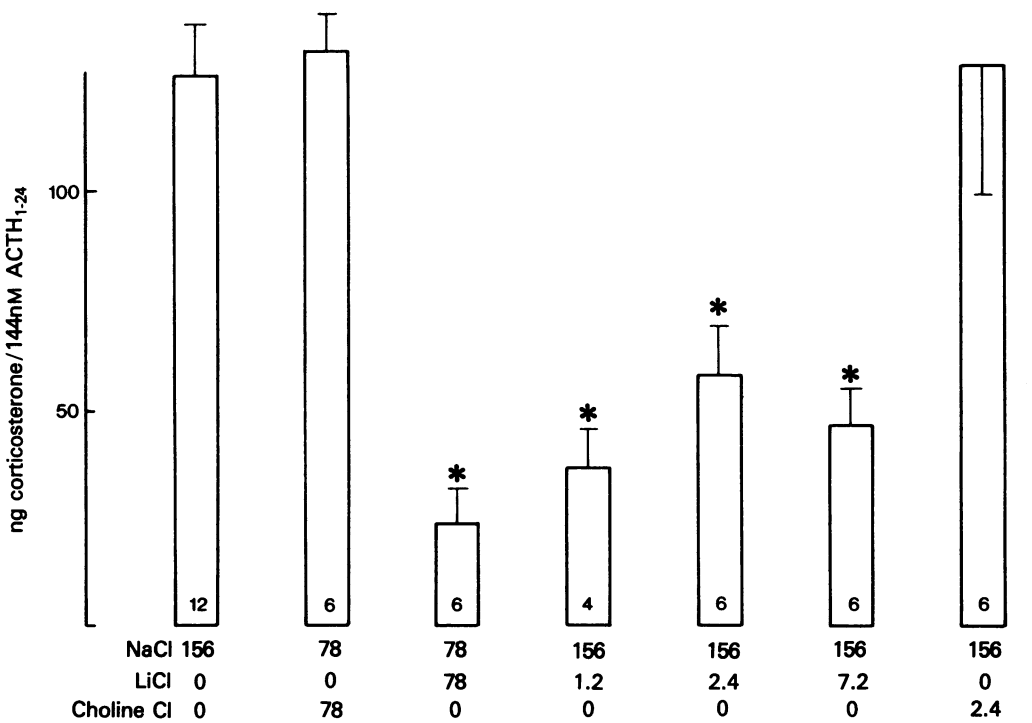

Figure 5 Histogram of the steroidogenic response of rat adrenal cells to 144 nM $ACTH_{1-24}$ in Hanks medium containing normal Na^+ (156 mM) or reduced Na^+ which was replaced with choline or lithium. The relevant concentrations are shown under the appropriate columns. The numbers within the column represents no. of observations. * $P < 0.05$, Student's t test.

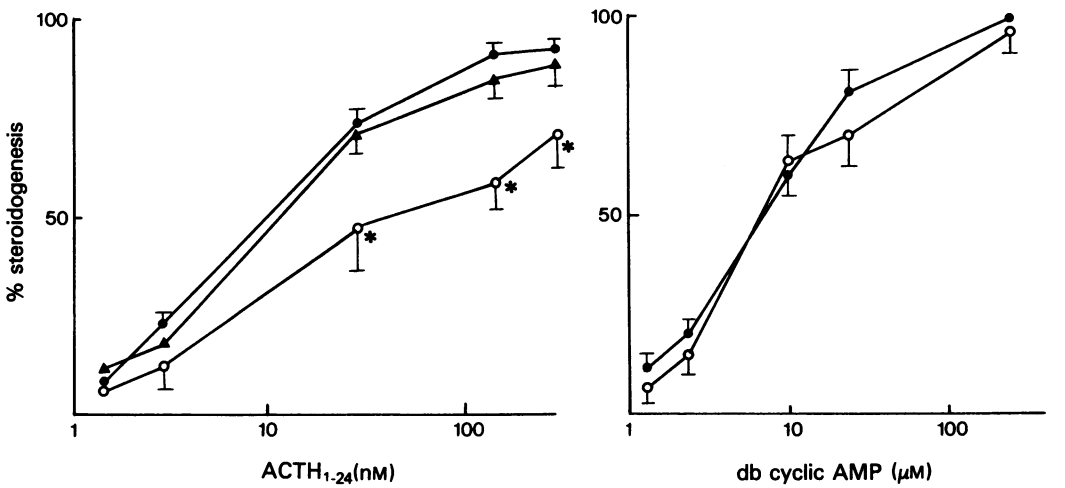

Figure 6 Dose-response curve of the steroidogenic response of rat adrenal cell suspensions to $ACTH_{1-24}$ or db cyclic AMP acting alone (●) or in the presence of 2.4 mM lithium (○) or 2.4 mM choline (▲). Each point is the mean of at least 6 observations; vertical lines show s.e. mean. * $P < 0.05$, Student's t test.

than opioid agonists, and it is possible that a direct effect on the adrenal may be of importance in the actions of opioid drugs with a low agonist:antagonist potency ratio (Hughes, Kosterlitz & Leslie, 1975). The lack of effect of enkephalin may be explained by the rapid breakdown of this compound when exposed to tissues (Meek, Yang & Costa, 1977). The effect of β-endorphin on steroidogenesis was not examined in this study, and although the lack of effect of opioids in low concentrations suggests that β-endorphin is unlikely to be active on the adrenal cortex, such an effect cannot be ruled out since β-endorphin is released along with ACTH in acute stress (Rossier, French, Rivier, Ling, Guillemin & Bloom, 1977) and the characteristics of opioid receptors in different tissues is not uniform (Lord, Waterfield, Hughes & Kosterlitz, 1977).

Matthews & Saffran (1973) reported that replacement of 82% of the NaCl of the medium by choline chloride had no effect on steroidogenesis, although in the present study steroidogenesis induced by $ACTH_{1-24}$ and by db cyclic AMP was reduced by replacement of 70% of the NaCl by choline chloride. However, there were differences in the methods used since Matthews & Saffran (1973) superfused adrenals from neonatal rabbits.

Perhaps the most interesting results obtained in this study concern the ability of lithium in low concentrations to inhibit $ACTH_{1-24}$-induced steroidogenesis. The effect of lithium on steroidogenesis appears to be exerted on the ACTH-adenyl cyclase system since the effects of db cyclic AMP were unaltered. For this reason it does not appear to be due merely to a competition between lithium and Na^+ since Na^+ lack reduced the steroidogenic potency of both $ACTH_{1-24}$ and of db cyclic AMP. In many other systems the effect of lithium is believed to be due to prevention of increased production of cyclic AMP by hormones and neurotransmitters (Schou, 1976), and the effect of lithium described here would fall into this general pattern. It is a matter for speculation how far the impairment of ACTH actions by lithium may contribute to the therapeutic effects of the ion in affective disorders. Since these disorders are associated with abnormality in control of the HPA system (Carroll, 1969), lowering of corticosteroid production by impaired ACTH response of the adrenal cortex may be of significance. Alternatively, the inhibition of ACTH by lithium may not be limited to the adrenal cortex but may extend to brain and to the behavioural effects of the peptide (de Wied, 1969), some of which have been shown to resemble anxiety states (File & Vellucci, 1978).

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