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INHIBITORY EFFECT OF MORPHINE ON METABOLISM OF ADRENAL AND TESTICULAR STEROIDS

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Abstract—Adrenal slices from morphinized rats produce steroid hormones to a minor extent respecting controls, when incubated *in vitro* under proper conditions. After morphine additions of ACTH did not produce such an increase of the hormone production as realized in controls. Morphine when added *in vitro* to adrenal and testicle homogenates from normal rats, appeared to act as an inhibitor of 3-beta-ol-dehydrogenase system. The rate of conjugation of DOC by liver slices excised from morphinized rats was decreased.

INTRODUCTION

IN A previous work[1] we have shown that in morphine-treated rats the urinary excretion of adrenal and testicular hormones displays a characteristic pattern according to the stage of treatment. When "stabilizing" doses were injected for a sufficient period, the amounts of hydroxysteroids, 17-ketosteroids and aldosterone in the urine decreased to a great extent. ACTH administration induced a lesser increase than normal of steroid excretion as target glands were unresponsive to proper hypophyseal stimulation. Moreover under morphine the conjugated forms of steroids appeared to be decreased in urine.

In the present work attention was focused on the possible existence of a direct effect of morphine on steroid metabolism by adrenal and testicular tissue, as well as on the occurrence of an inhibitory effect of the drug on liver corticosteroid conjugation.

In the present work the following situations have been studied:

(1) *In vitro*, hormone production by adrenal slices prepared from glands of morphinized rats.

(2) Influence of morphine on the stimulatory effect of added ACTH on corticosteroid hormone production by adrenal cortical slices.

(3) Effects of morphine on the conversion of Δ^4-3 hydroxysteroids into Δ^4-3 keto compounds by whole homogenates of adrenals and testicles.

(4) The rate of disappearance of Δ^4-3 keto grouping from corticosteroids by liver slices excised from morphinized rats.

Investigation on 3-beta-ol-dehydrogenase system was suggested by the emphasis that the increase of Sudan stained drops in adrenal cortex, as observed after chronic morphine by various authors and by us[2, 3] may depict the failure of the gland to produce hormonal steroids from precursors.

METHODS

Twenty male rats of the Sprague–Dawley strain weighing 150–180 g and maintained in the same ambient conditions as previously described[1] were given morphine intraperitoneally at the dose of 20 mg/kg for 5, 15 and 30 days.

Corticosteroid hormone production by adrenal slices was investigated according to Saffran *et al.*[4] ACTH was added to adrenal slices at a concentration of 1 I.U./g tissue. The enzymic action of 3-beta-ol-dehydrogenase was investigated according to Samuels *et al.*[5] in whole homogenates of adrenals and testicles excised from normal Sprague–Dawley rats. Morphine was added to the medium at concentrations of 50 and 100 μg/g tissue. Liver conjugation of DOC was estimated according to Louchart and Jailer[6] in normal and tolerant rats.

RESULTS

(1) Table 1 shows an inhibitory effect of the previous treatment with morphine on hormone production by adrenal slices under incubation conditions previously described by Saffran *et al.*[4] As may be observed, a short treatment corresponds to a slight

TABLE 1. CORTICOSTEROID PRODUCTION BY ADRENAL SLICES FROM MORPHINIZED RATS, UNDER BASAL CONDITIONS AND AFTER INCUBATION WITH ACTH

			α : β-Unsaturated ketone†			
Morphine (mg/kg)	Days of treatment	No. of adrenals	Preincubation samples*	(μg/g per 3 hr)	ACTH, 1 I.U./g samples*	(μg/g per 3 hr)
—	—	14	2	73·7 ±1 79	2	211·0±17·08
20	5	12	2	80·0 ±2·82	2	201·0± 4·94
20	15	14	2	50·5 ±3·53	2	71·0± 8·48
20	30	14	2	26·0 ±1·41	2	47·5± 6·40

* One sample = 40–45 mg of bifected adrenals.
† As cortisone.

effect, if ever there is one, on the performance of the glands to synthetize hormonal steroids. Moreover a striking decrease of steroid production is apparently displayed by adrenals of longer treated rats.

(2) When ACTH was added to adrenal slices obtained from glands of morphinized rats the stimulatory effects of the hormone on corticosteroid production appeared to be lessened. As a matter of fact we have detected in morphinized rats a failure of the ACTH injection to produce the expected increase of urinary steroids.

(3) Table 2 shows that morphine inhibits the 3-beta-ol-dehydrogenase activity of whole adrenal and testicle homogenates when added to the medium at concentration of 50–100 μg/g tissue.

TABLE 2. EFFECT OF MORPHINE ON ACTIVITY OF 3-β-OL DEHYDROGENASE OF RAT ADRENALS AND TESTICLES

Gland	Weight tissue g	Morphine μg/g	α : β-Unsat. ketone (μmoles/g per hr) (average)	% Decrement under morphine
adrenal	0·05	—	22·0	
id.	id.	50	8·0	63·6
id	id.	100	4·8	78·2
testes	2	—	3·5	
id.	id.	50	2·0	42·1
id.	id.	100	1·3	62·9

(4) Table 3 depicts the liver performance to conjugate corticosteroids when hepatic slices excised from normal and morphinized rats are incubated in presence of DOC. The rate of DOC conjugation by liver slices from morphinized rats appears to be reduced when compared to controls.

TABLE 3. INHIBITORY EFFECT OF MORPHINE ON *in vitro* CONJUGATION OF DOC BY LIVER SLICES FROM TOLERANT RATS (MORPHINE 20 MG/KG DAILY FOR 30 DAYS)

No of samples	Rate of conjugation (μmoles/hr per g)	% of inhibition by morphine
Controls, 10	75	—
Tolerant rats, 10	12	84

DISCUSSION

In a previous report[1] it was shown that repetitive treatment with stabilizing doses of morphine induces in rats a decrease of urinary excretion of hydroxysteroids, aldosterone and 17-ketosteroids. It has been also referred that ACTH injection and exposure to cold resulted in a remarkable lesser adrenal response.

Apart from the possibility that the impairement of liver conjugation presented here may result in a decrease of urinary excretion of less soluble steroids, the present report demonstrates that morphine may display a direct influence on the hormonal metabolism of the adrenocortical tissue. Indeed, adrenal slices obtained from glands of properly morphinized rats produce corticosteroids at a very much lower degree than the controls. According to our previous observations on guinea pigs,[7] morphine exerted an inhibitory effect on steroid production even when added *in vitro* at various concentrations to rat adrenal and testicle homogenates.

Moreover ACTH added to incubation medium fails to increase the synthesis of hormones by adrenal slices obtained from morphinized rats. This observation attracts attention to the question of the steroid metabolic pathway undergoing morphine inhibition in adrenals.

Actually Samuels preparation for 3-β-ol-dehydrogenase system appears to be inhibited by morphine; it is not known, however, if this inhibition occurs in living animals, or if the drug may act on other enzymatic chains in adrenal and testicle.

Present results indicate that morphine may affect the endocrine functions much more than is expected. The question of the effect on these secretions by morphine, as due to a nonspecific mechanism, appears largely to be reviewed. The assessment of the pharmacological value of the property of morphine to influence steroid metabolism requires further investigations.

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