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THE EFFECT OF ACTH ON THE PLASMA CONCENTRATIONS OF THE "HYPERTENSINOGENIC" STEROIDS, 17α-HYDROXYPROGESTERONE AND 17α,20α-DIHYDROXY-4-PREGNEN-3-ONE IN SHEEP

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Summary—The plasma concentrations of 17α-hydroxyprogesterone (17αOHP) and 17a'20α-dihydroxy-4-pregnen-3-one (17α20αOHP) have been measured in sheep during 5 days of ACTH administration at 20 μg/kg/day a rate of infusion known to produce hypertension. Five days of ACTH administration produced a progressive increase in plasma 17OHP from 0.45 ± 0.12 to 128.9 ± 28.4 nmol/l and in 17α20αOHP from 0.54 ± 0.15 to 73.1 ± 7.2 nmol/l. Calculation of the blood production rate of both steroids during ACTH treatment confirms that the rates of infusion of 17OHP (3.0 μmol/h) and 17α20αOHP (1.5 μmol/h) used to produce hypertension, when infused together with the other major ovine adrenocortical steroids, produced plasma concentrations in the range as found following administration at a rate to increase blood pressure.

INTRODUCTION

Following the demonstration of the hypertensive effects of ACTH administration in conscious sheep [1] a number of studies were carried out in an attempt to identify the adrenocortical steroids responsible for the increase in blood pressure. It was shown that individual steroids—cortisol, corticosterone, 11-deoxycortisol, deoxycorticosterone and aldosterone had no metabolic or pressor effects when infused at rates to reproduce the blood concentrations seen with ACTH [2]. Infused together, the steroids reproduced the metabolic effects of ACTH such as hypokalemia and increased urine output but the effects on blood pressure were significantly less than those observed with ACTH [2].

Characterization of the steroid profile of adrenal venous blood from the ACTH treated animal enabled us to identify a number of other steroids [3]. Of these 17α-hydroxyprogesterone (17α-hydroxy-4-pregnen-3,20-dione, 17αOHP) and 17α,20α-dihydroxy-4-pregnen-3-one (17α20αOHP) were shown to reproduce the effects of ACTH on blood pressure when infused together with the other major adrenocortical steroids [4]. Studies of the mode of action of 17αOHP and 17α20αOHP led us to propose that these steroids may be members of a new class of steroid hormone action—the "hypertensinogenic" steroids [5]. It has also been shown that 17α20αOHP can be derived in blood from secreted 17OHP by an enzyme in ovine red cells [6].

The aim of the present study is to report the blood concentrations of these two steroids in response to ACTH administration at rates known to raise blood pressure in sheep.

EXPERIMENTAL

Experimental protocol

ACTH (20 μg/kg/day, Synacthen, ACTH 1–24) was administered to 6 conscious sheep (adult oophorectomised ewes) for 5 days using protocols previously described for this model of experimental hypertension [1]. Blood samples (10 ml) were taken by direct carotid arterial puncture between 0900–1000 h each day. The heparinized blood samples were taken in ice and immediately spun to obtain plasma. Plasma was stored at −20°C until analysis. Samples were taken for the 2 days prior to commencement of ACTH treatment, daily throughout ACTH treatment and then on the third day after cessation of ACTH administration.

Steroid measurements

17OHP was measured by radioimmunoassay using an antiserum raised in sheep against 17OHP-6-carboxymethylthioether coupled to thyroglobulin [9]. [^{3}H]17OHP (New England Nuclear Corporation) sp. act. 60 Ci/mmol was used as the tracer.

17α20αOHP was measured by radioimmunoassay using an antiserum (kindly donated by Dr A. Flint,

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Oxford, U.K.) raised in sheep against 17α20α-dihydroxypregn-4-en-3-carboxymethyloxime BSA [9]. Variable volumes of plasma (0.2–1.0 ml according to experimental conditions) were adjusted to 1.0 ml with distilled water and extracted with 15 vol of purified dichloromethane, washed once with distilled water and dried down at 37°C under an air stream. The extract was resuspended in 1 ml 100 mM phosphate buffer pH 7.2 containing w/v 0.9% NaCl, 0.1% gelatin and 0.1% sodium azide. The radioimmunoassay procedure used is a modification of that of Flint and colleagues [7]. The separation of free and bound steroid was achieved by the use of cold saturated ammonium sulphate rather than charcoal and the sensitivity of the assay was increased for low steroid concentrations by increasing the final dilution of antibody to 1:20000. [^{3}H]17α20αOHP was prepared from [^{3}H]17OHP using rat ovarian tissue [8]. Plasma cortisol was measured by radioimmunoassay (Diagnostic Products) and was measured in 5 animals on the first and fifth day of ACTH treatment.

Results are expressed as mean ± SEM for each of the 8 days blood samples were taken.

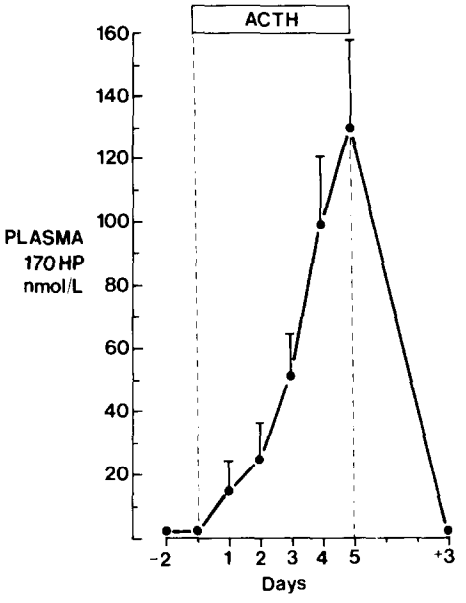

Fig. 2. The effect of ACTH (20 μg/kg/day) for 5 days on plasma 17OHP concentration in 6 sheep. Results mean ± SEM.

RESULTS

ACTH treatment produced a progressive increase in plasma 17α20αOHP concentrations from 0.54 ± 0.15 nmol/l to a maximum of 73.1 ± 7.2 nmol/l on the fifth day (Fig. 1). Three days after cessation of ACTH administration plasma 17α20αOHP had returned to the pre-ACTH level.

The plasma concentration of 17OHP rose progressively with ACTH treatment from 0.45 ± 0.12 nmol/l to a maximum of 128.9 ± 28.4 nmol/l on the fifth day (Fig. 2). On the third day post ACTH the concentration had fallen to 1.66 ± 1.03 nmol/l.

Unlike the other two steroids plasma cortisol concentration rose from 28 ± 5.6 to 389 ± 44.8 nmol/l at 24 h, a value that was similar to that found on the fifth day of ACTH treatment (336 ± 53.2 nmol/l).

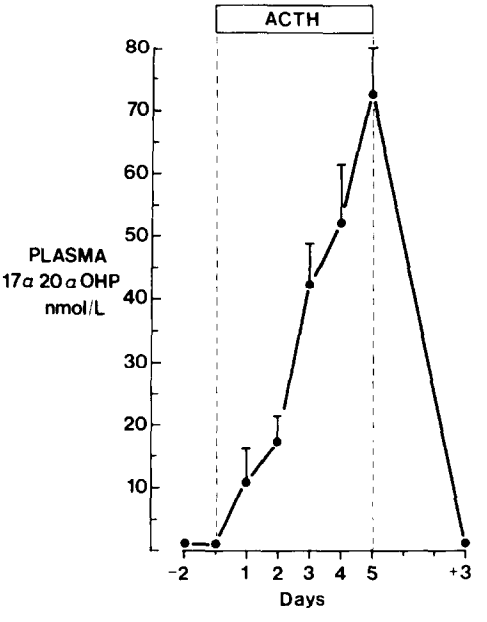

Fig. 1. The effect of ACTH (20 μg/kg/day) for 5 days on plasma 17α20αOHP concentration in 6 sheep. Results mean ± SEM.

DISCUSSION

From the plasma concentration of 17OHP and 17α20αOHP measured in this study and the previously reported clearance rates for the two steroids [6] it is possible to calculate their production rate. These calculations for both steroids under basal conditions and after 5 days ACTH administration are shown on Table 1. These calculations have made the assumption that the 17OHP and 17α20αOHP are distributed evenly between plasma and red cell as occurs for other adrenocortical steroids in the sheep. ACTH results in large increases in the calculated production rate of both steroids.

Table 1. The effect of 5 days ACTH administration on the plasma concentration, clearance rate and production rate of 17OHP and 17α20αOHP in sheep ($n = 6$)

	Plasma concentration (nmol/l)	Clearance* rate (l/h)	Production rate (μmol/h)
Basal	0.45 ± 0.12	150 ± 10	0.051 ± 0.014
17OHP	***	NS	***
ACTH	128.9 ± 28.4	174 ± 15	22.43 ± 5.30
Basal	0.54 ± 0.15	94 ± 9	0.070 ± 0.020
17α20αOHP	***	NS	***
ACTH	73.1 ± 7.2	94 ± 5	6.87 ± 0.76

*Data obtained from [6]. ***$P < 0.001$.

In these experiments the 17OHP measured in peripheral plasma is presumably of adrenal origin since the sheep were oophorectomised ewes. In contrast 17α20αOHP is derived from both adrenocortical secretion and by conversion of 17OHP to 17α20αOHP in blood [6]. The blood to blood conversion of 17OHP to 17α20αOHP [σ] has been calculated to be 0.30 both under basal conditions and after 5 days ACTH administration. *In vitro* studies indicate that this interconversion is due to an enzyme in ovine red cells [6].

The estimated secretion rates of 17OHP and 17α20αOHP during ACTH administration were 3.0 and 1.5 μmol/h respectively and these were the rates of intravenous infusion which together with the other major corticosteroids produced hypertension in sheep [3, 4, 5]. By using the previously derived clearance rate for these two steroids [6] it can be calculated that these rates of infusion will give plasma concentrations of 17.2 and 15.9 ng/ml for 17OHP and 17α20αOHP respectively. These plasma concentrations were obtained on the second day of ACTH administration in the present study. If as seems likely 17OHP exerts its "hypertensinogenic" action by conversion to 17α20αOHP then the plasma 17α20αOHP produced by the infusion of the two steroids is probably similar to that found after 48–72 h of ACTH administration. However, by the end of 5 days the rates of steroid infusion were substantially less than the calculated production rates after a similar period of ACTH administration.

The increases in plasma concentration of 17OHP and 17α20αOHP are progressive over the 5 days. In contrast plasma cortisol levels are as high after the first 24 h as they are after 5 days, thus raising the possibility of a rate limiting step between 17OHP and cortisol on the biosynthetic pathway. Other studies have shown similar increases in plasma concentrations of both 17OHP and 17α20αOHP with ACTH administration in the human [9]. In man ACTH produced a more rapid increase in 17OHP and 17α20αOHP than that observed in sheep. However, although 17α20αOHP is under the control of ACTH in man it is not derived in blood from 17OHP [9].

The present study reports for the first time the plasma concentrations of the steroids thought to be involved in the production of ACTH induced hypertension in the sheep. The mechanism of action of this "hypertensinogenic" class of steroid action has still to be elucidated.

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