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PRELIMINARY NOTE

THE APPARENT LACK OF STIMULATION OF RABBIT ADRENAL 21-HYDROXYLASE ACTIVITY BY ACTH

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SUMMARY

The stimulation of rabbits twice daily for three days with 25 U of porcine ACTH, a treatment previously demonstrated to increase the specific activity of microsomal 17α-hydroxylase, did not increase the specific activity of microsomal 21-hydroxylase.

INTRODUCTION

Steroid 17α-hydroxylase activity in rabbit adrenal tissue is increased by chronic ACTH-treatment [1, 2]. This enzyme activity has been shown to be located predominantly in the microsomal fraction, corresponding to the smooth-surfaced endoplasmic reticulum (SER) [3]. The question arose whether this increase in activity was due to a non-specific stimulation of the proliferation of the SER as has been described after ACTH stimulation of adrenal tissue [4] and stimulation of liver cells by various drugs [5], or whether the ACTH was specifically stimulating 17α-hydroxylation. Yudaev and Morozova[6] reported that ACTH treatment inhibited rabbit adrenal 21-hydroxylase activity suggesting a reciprocal effect on the 17α- and 21-hydroxylases. Since the SER is also the location of steroid 21-hydroxylase activity [7], a very direct answer to the question of stimulatory specificity could be obtained by determining whether or not chronic ACTH treatment affects the rate of 21-hydroxylation.

METHODS

Sixteen male New Zealand white rabbits were divided into two groups of eight animals each. One group was injected intramuscularly twice daily for three days with 25 U ACTH and the other with the beeswax–peanut oil vehicle alone, as previously described [1]. They were anesthetized with carbon dioxide on the morning of the fourth day, exsanguinated and their adrenal glands removed. The glands from the eight ACTH-injected and the eight vehicle injected (control) animals were pooled separately and a microsomal fraction prepared from each tissular pool as earlier described [3]. The fraction used in the present studies was that sedimenting between 17,500 *g* for 30 min and 105,000 *g* for 60 min. This microsomal pellet was resuspended in 0.1 M sodium phosphate buffer, pH 6.8, containing 0.15 M KCl and 0.001 M dithiothreitol and recentrifuged two times. This final "washed" microsomal pellet was resuspended in Krebs–Ringer phosphate buffer, pH 7.0. Duplicate aliquots of each microsome preparation equivalent to 0.49 (control) and 0.40 (ACTH) mg of protein were incubated with 25 and 50 μM (2 μCi per μmol) concentrations of 17α-hydroxy-[4-^{14}C]-progesterone (New England Nuclear) for zero, 1, 2, and 4 min in a total vol. of 2.0 ml Krebs–Ringer phosphate buffer, pH 7.35, containing: glucose, 1.0 mg/ml; glucose-6-phosphate, 1.0 mg/ml; NADPH, 0.5 mM; and glucose-6-phosphate dehydrogenase (Sigma, Type IV), 2 U. NADPH and an NADPH regenerating system were both included to be as sure as possible that this reduced cofactor could not become limiting during the assay. The gas phase was air. The incubation media were extracted twice with 10 ml of dichloromethane. After evaporation of the solvent the sole incubation product (11-deoxycortisol) was separated from unconverted substrate (17α-hydroxyprogesterone) by paper chromatography in two systems. The chromatograms were initially developed in a heptane–benzene (1:1, v/v)/formamide system for 1 h after the mobile phase had reached the end of the strips, followed by development in a chloroform–benzene (1:1, v/v)/formamide system [8]. The amount of product formed from the added substrate was quantified by liquid scintillation spectrometry and corrected for losses by determining the recovery of 0.05 μCi ^{3}H-labelled 11-deoxycortisol (New England Nuclear, 10 Ci/mmol) added to the incubation media prior to extraction. Microsomal protein determinations were performed by the method of Lowry *et al.*[9].

RESULTS AND DISCUSSION

Adrenal stimulation by the ACTH injections was evident from the increased average combined adrenal weight from 184 mg to 255 mg. The average body weights were 2.4 and 2.1 kilograms for the control and ACTH injected rabbits, respectively.

Figure 1 depicts the results of the kinetic analyses at the 25 μM substrate concentration. Rates appeared to be fairly linear up to 120 s, but started to decrease between the one and two min interval, or slightly before. Rate values were subsequently calculated by linear regression analysis using the zero, 60, and 120 s values, and are tabulated in Table 1. Correlation coefficients of greater than 0.99 were obtained for both substrate concentrations and with both microsomal preparations. It is obvious that although the microsomes from ACTH stimulated tissue had lower average 21-hydroxylase activity on a per mg of protein basis, there was no significant difference between the ACTH and control-microsome rates at either substrate concentration. Since a small part of the ACTH microsome preparation was lost due to the failure of a centrifuge tube, no direct comparison of total 21-hydroxylase activity can be made. However, the cytochrome P-450 content per milligram of protein of the ACTH-stimulated microsome preparation (1.00 nmol/mg) was also somewhat lower than that of the control preparation (1.48 nmol/mg) as has been

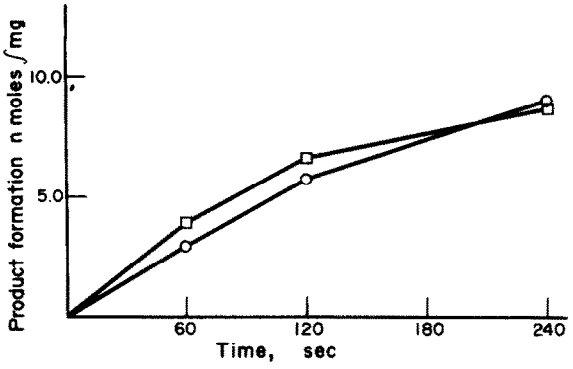

Fig. 1. Formation of 17,21-hydroxy-[4-^{14}C]-progesterone from 17α-hydroxy-[4-^{14}C]-progesterone at a 25 μM concentration by two different rabbit adrenal microsome preparations: ⊡——⊡ control rabbits; ⊙——⊙ ACTH-stimulated rabbits. Other assay conditions are described in the text.

Table 1. Comparison of the rate of 21-hydroxylation of [4-^{14}C]-17α-hydroxyprogesterone by microsome preparations from control and ACTH-stimulated rabbits

Substrate concentration, μM	Rate (nmol/min/mg)	
	Control	ACTH-stimulated
25	3.33 ± 0.74*	2.89 ± 0.22
50	3.92 ± 0.95	3.12 ± 0.10

* 95% Confidence limits, 1.96 σ.

reported previously [3] for similar preparations. These results suggest that total protein synthesis was stimulated to a greater extent than microsomal cytochrome P-450 synthesis, and to the same extent or greater than the 21-hydroxylase activity. This is in contrast to the stimulation of microsomal 17α-hydroxylase activity which has been shown to increase on a milligram of microsomal protein basis with similar ACTH stimulation [10].

These preliminary results suggest that ACTH does not stimulate rabbit adrenal microsomal 21-hydroxylase activity, under conditions previously shown [3, 10] to effect an increased 17α-hydroxylation. Studies in progress are designed to elaborate on this observation.

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