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ADVISORY PANEL 1968

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Prof. G. ASBOE-HANSEN	Rigshospitalet, 2100 Copenhagen Ø
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Dr. ERLING ØSTERGAARD	Frederiksberg Hospital, 2000 Copenhagen F

II. Medizinische Klinik der Universitaet Muenchen, Germany
(Direktor: Prof. Dr. Dr. G. Bodechtel)

DISSOCIATION OF STIMULATORY EFFECTS
OF CORTICOTROPHIN ON ADRENAL CORTICOSTERONE
AND PROTEIN SYNTHESIS FOLLOWING
HYPOPHYSECTOMY

By

P. C. Scriba and F. Kluge

ABSTRACT

At various intervals after hypophysectomy some dissociation of stimulation by corticotrophin (ACTH) treatment of the activity of a factor in adrenal homogenates, previously shown to be rate limiting for *in vitro* ^{14}C -glycine incorporation into adrenal proteins, from stimulation by ACTH of the *in vivo* secretion of corticosterone, was found.

Studies of protein synthesis in a cell free adrenal system were performed in order to obtain further information on the controversial question, namely whether the ACTH effect on corticosteroid production is related to stimulation of protein synthesis (*Ferguson* 1962, 1963; *Bransome & Reddy* 1963; *Davidson & Hofmann* 1956; *Halkerstone et al.* 1964, 1965; *Koritz et al.* 1957; *Garren et al.* 1965).

Incorporation of amino acids by $15\,000 \times g$ supernatants (SN) of adrenal homogenates is increased by previous treatment of the rats (*Farese & Reddy* 1963). This increase is due to stimulation of microsomal activity (*Farese* 1964, 1965 *a, b*, 1966) and to a protein in the soluble cell fraction ($105\,000 \times g$ SN), which may be an aminoacyltransferase (*Scriba & Reddy* 1965; *Scriba & Fries* 1967). Increased activity of adrenal $105\,000 \times g$ SN has been observed as early as 2 and 4 hours (*Farese* 1966; *Scriba & Reddy* 1965) after ACTH administration. Since rat adrenal $105\,000 \times g$ SN contains the rate limiting factor

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(s) for ^{14}C -glycine incorporation by pig adrenal $15\,000 \times g$ SN, the effect of hypophysectomy and ACTH treatment on the activity of rat adrenal $105\,000 \times g$ SN was studied.

RESULTS

Thirty hours after hypophysectomy in rats, stimulation of ^{14}C -glycine incorporation by pig adrenal $15\,000 \times g$ SN on addition of rat adrenal $105\,000 \times g$ SN, was almost as high as seen after ACTH treatment of normal rats for 3 days (*Scriba & Reddy 1965*) (Table 1). At this time, amino acid incorporation

Table 1.

Effect of hypophysectomy and ACTH treatment on the stimulating activity of rat adrenal $105\,000 \times g$ SN for glycine incorporation by pig adrenal $15\,000 \times g$ SN.

Test Solutions		^{14}C -Glycine Incorporated (cpm)
I	Buffer (Baseline)	207 \pm 24.9
II	Control Rats	631 \pm 60.7
III	ACTH Rats	1010 \pm 87.0
IV	30 h Hypox. Rats (Control)	891 \pm 28.0
V	30 h Hypox. Rats (ACTH)	826 \pm 14.0
VI	14 Days Hypox. Rats (Control)	460 \pm 13.7
VII	14 Days Hypox. Rats (ACTH)	681 \pm 25.2

Incubation mixtures contained in a total volume of 1.05 ml: 1 μc ^{14}C -glycine (0.014 μmoles), 10 μmoles phosphoenol pyruvate, 1 μmole ATP, 0.25 μmoles GTP, 60 μg pyruvate kinase, 80 mg-equivalent of $15\,000 \times g$ SN of pig adrenals homogenized at 200 mg per ml medium A (0.25 M sucrose, 0.025 M KCl, 0.005 M MgCl_2 , 0.05 M Tris-HCl buffer, pH 7.5) as described (*Scriba & Reddy 1965*) and test solutions (40 mg-equivalents of rat adrenal $105\,000 \times g$ SN) as indicated. After incubation for 60 min at 37°C , ^{14}C -glycine incorporation into protein was determined (*Scriba & Reddy 1965*). The results reported as means \pm S. D. are from 9 experiments on rats treated (III) for 3 days with $2 \times$ daily i. m. injections of 20 U ACTH-gel (Upjohn Co.), or 5 U synthetic β^{1-24} corticotrophin (Ciba, 36716 Ba.). The means (II and III) are significantly different ($P < 0.001$). Means of two incubations are given for IV to VII (\pm S. E.). Rats were treated 27 h after hypophysectomy (IV and V) by infusion of 1.7 U oxycel-ACTH or of control solution over 210 min. This experiment (IV and V) has been confirmed in further incubations 34 h after hypophysectomy with two i. m. injections of 5 U β^{1-24} corticotrophin 10 and 2 h before sacrifice. Finally rats (VII) were treated 14 days after hypophysectomy with ACTH-infusions (2 U over 4 h). The difference of the means is of low significance ($0.02 > P > 0.01$).

could not be further raised by previous ACTH infusion or intramuscular treatment of hypophysectomized rats (cf. Table 1). Thus 30 h after hypophysectomy, protein synthesis in adrenal homogenates still occurs at an increased rate, presumably due to the stress of hypophysectomy, whereas corticosterone secretion rapidly decreases to baseline levels after hypophysectomy (*Liddle et al.* 1962). Moreover, maximal stimulation by ACTH of corticosterone secretion into the adrenal vein appears to be already reduced 30 h after hypophysectomy (cf. Table 2) as compared with values obtained after intramuscularly ACTH administration or intravenous ACTH (*Scriba et al.* 1966) 1 to 4 h after hypophysectomy.

Ten days after hypophysectomy in rats, *Stahelin et al.* (1965) observed no increase in corticosterone secretion after intravenous injection of 3 U ACTH per kg. Table 2 shows that 8 days after hypophysectomy slight stimulation of corticosterone secretion was still obtained by two intramuscular injections of 5 U ACTH. However, the stimulation was less than one tenth of the increase observed 4 h after hypophysectomy. In contrast, 14 days after hypophysectomy, following ACTH infusion for 4 h, rat adrenal $105\,000 \times g$ SN produced approximately twice the stimulation of ^{14}C -glycine incorporation by $15\,000 \times g$ SN of pig adrenals as compared with the controls (Table 1). Additional experiments with intramuscular injections of 5 U β^{1-24} corticotrophin given 10 and 2 h before sacrifice were performed 10 days after hypophysectomy and showed the same results. Thus, at these intervals after hypophysectomy, a

Table 2.
Effect of hypophysectomy and ACTH on corticosterone secretion.

Time after hypophysectomy		ng Corticosterone per 4 min \pm S. E.	
		Control	ACTH
I	4 h	29.1 \pm 8.0 (n = 20)	1805.7 \pm 201.0 (n = 3)
II	34 h	20.3 \pm 2.4 (n = 4)	895.0 \pm 197.2 (n = 4)
III	8 days	11.8 \pm 6.2 (n = 4)	150.8 \pm 7.4 (n = 4)

Hypophysectomy was performed by the parapharyngeal route and the adrenal vein was cannulated via the femoral vein (*Scriba et al.* 1966). Assay of corticosterone in the total plasma collected was performed by the acid fluorescence method (*Zenker & Bernstein* 1958). Treated rats of group I received i. m. 5 U ACTH (β^{1-24} corticotrophin) two hours before adrenal cannulation, whereas groups II and III had i. m. injections 10 and 2 h before the cannulation.

doubling of the activity of the rat adrenal $105\,000 \times g$ SN was brought about by the *in vivo* administration of ACTH, whereas corticosterone synthesis appeared to be refractory to ACTH.

DISCUSSION

The results indicate that stimulation by ACTH of the activity of the rate limiting factor for *in vitro* adrenal protein synthesis (*Scriba & Reddy* 1965; *Scriba & Fries* 1967) and the stimulation of *in vivo* corticosterone secretion can be dissociated. This supports the interpretation, that ACTH can stimulate adrenal steroidogenesis independently of protein synthesis (^{14}C -glycine incorporation), although, of course, stimulation of synthesis of a specific enzyme, rate limiting for corticosterone synthesis, can not be excluded on the basis of this study.

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