

Inhibition of Dexamethasone Suppression of ACTH Secretion *in vivo* By Actinomycin D.* (32606)

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Although it is widely accepted that glucocorticoids inhibit ACTH secretion in man and experimental animals(1,2), the mechanism responsible for this effect is not known. In recent years it has been established that glucocorticoids, in certain situations, are capable of influencing specific protein synthesis by stimulating RNA synthesis(3). Actinomycin D inhibits RNA synthesis and, therefore, will prevent a glucocorticoid induced stimulation of RNA synthesis(4). Since Actinomycin D is capable of inhibiting this biochemical action of glucocorticoids, we have studied *in vivo* the effect of Actinomycin D on a biological action of glucocorticoids, namely suppression of ACTH secretion. The results of these studies indicate that Actinomycin D inhibits the effect of glucocorticoids on ACTH secretion.

Materials and methods. Male rats of the Cheek-Jones strain, 180-200 g, were adrenalectomized in groups of approximately 30 and maintained for two weeks on Purina lab chow and 0.9% NaCl. On each experimental day either normal or adrenalectomized rats were randomly divided into multiple groups and treated with intraperitoneal injections as follows: Control treatment (saline), 1 mg dexamethasone 21-PO₄, 1 mg Actinomycin D and various combinations of the above drugs as outlined in Table II and Fig. 2. The index of ACTH secretion in normal rats was the corticosterone content in a three-minute sample of the left adrenal vein effluent obtained promptly following laparotomy. These rats were anesthetized with 30 mg/kg nembutal 10 minutes prior to sampling. Corticosterone concentration in plasma was measured by the method of Silber *et al*(5). The index of ACTH secretion in adrenalectomized

TABLE I. Incorporation of ³H Uridine into Brain and Pituitary RNA in Control and Actinomycin D Treated Rats. The numbers represent dpm's per 100 mg wet tissue incorporated. The studies of brain were performed on three separate occasions. "N" represents the number of rats utilized.

	N	Control	Actinomy- cin D	% Inhibition of RNA synthesis
Pitui- tary	12	2746	866	69
Brain	6	363	262	28
	6	546	404	26
	6	559	364	35

rats was the plasma ACTH concentration measured as follows: Unanesthetized adrenalectomized rats were rapidly decapitated and trunk blood was collected into heparinized containers, promptly centrifuged and the plasma stored frozen. The plasma from 3-5 adrenalectomized rats composing a specific group was pooled to allow sufficient volume for multiple dose 4 point bioassays. Plasma ACTH concentration was measured by the method of Lipscomb and Nelson(6) as modified by Liddle *et al*(7) utilizing rats hypophysectomized 2 hours previously.

The site of glucocorticoid inhibition of ACTH secretion is generally assumed to be either the brain and/or pituitary. To determine the effect of Actinomycin D on RNA synthesis in these tissues, rats pretreated for 30 minutes with either saline or 1 mg Actinomycin D were given 50 μ c of ³H-uridine intravenously. The anterior pituitaries and the entire brains were removed 45 minutes later, and incorporation of ³H-uridine into RNA was determined according to the method of Ney *et al*(8).

Results. Table I shows the effect of Actinomycin D on the incorporation of ³H-uridine into RNA in pituitary and brain tissue. Inhibition of RNA synthesis was more striking in the pituitary than in brain but an effect was noted in both tissues.

ACTH secretion was monitored in two

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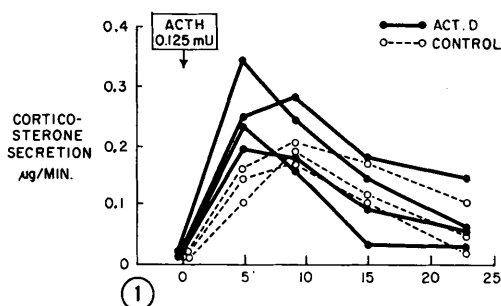


FIG. 1. Effect of Actinomycin D on adrenal responsiveness to ACTH. 200 g hypophysectomized rats were treated with either saline or 1 mg Actinomycin D and 150 minutes later they received 0.125 mU ACTH I.V. The left adrenal vein effluent was monitored at intervals for its content of corticosterone.

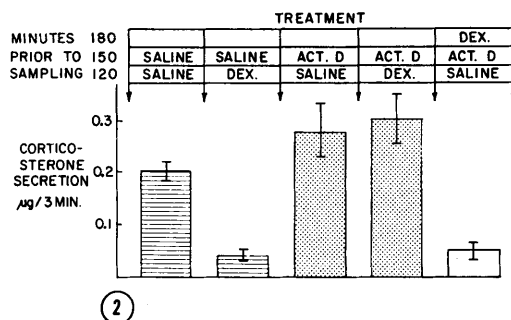


FIG. 2. Corticosterone secretion in randomly divided groups of normal rats. The chart above the bars indicates time in minutes at which treatments were given prior to sampling the left adrenal vein effluent. The lined bars demonstrate corticosterone secretion in control and dexamethasone treated rats. The stippled bars represent the same experiment as the lined bars but each rat received Actinomycin D 30 minutes before either saline or dexamethasone injection. The clear bar represents corticosterone secretion in rats given dexamethasone 30 minutes prior to Actinomycin D. Data was subjected to the analysis of variance(13). Corticosterone secretion in animals receiving saline and then dexamethasone and in animals receiving dexamethasone and then Actinomycin D were significantly different from the other groups.

ways: (1) the plasma ACTH concentration in adrenalectomized rats and (2) the adrenal secretion of corticosterone in normal rats. In order to use the latter method as an index of ACTH secretion, it was necessary to demonstrate that in the presence of Actinomycin D, adrenal responsiveness to ACTH was not altered over the time course of these experiments. Such studies are shown in Fig. 1. In general, steroidogenesis in response to ACTH occurred in a similar fashion in both control and Actinomycin D treated animals. However, corticosterone secretion was slightly increased initially in the Actinomycin D treated animals. 5 minutes following injection of ACTH there was a significantly higher corticosterone secretion rate ($p < .01$) in Actinomycin D treated rats than in saline treated animals. Thus in rats treated acutely with Actinomycin D, the secretion of corticosterone appears to grossly reflect ACTH secretion. However, Actinomycin D may slightly potentiate the action of ACTH and thereby corticosterone secretion as an index of ACTH secretion may modestly overestimate ACTH secretion in Actinomycin D treated rats.

The treatment protocol and results of studies designed to delineate what effect Actinomycin D has on ACTH secretion and dexamethasone inhibition of ACTH secretion in

normal rats are shown in Fig. 2. The administration of dexamethasone inhibited ACTH secretion as indicated by a fall in the adrenal secretion of corticosterone in normal rats from $0.2 \mu\text{g}/3 \text{ min}$ to $0.04 \mu\text{g}/3 \text{ min}$. Actinomycin D treatment did not significantly alter corticosterone secretion but dexamethasone administration 30 minutes following the administration of Actinomycin D was ineffective in suppressing ACTH secretion. Adrenal secretion of corticosterone in animals treated with Actinomycin D and then dexamethasone was not significantly different from that of either control animals or animals receiving Actinomycin D and saline.

It was important to demonstrate that this apparent inhibition of dexamethasone suppression of ACTH secretion by Actinomycin D was not the result of a direct stimulatory effect of Actinomycin D on ACTH secretion. The administration of dexamethasone 30 minutes prior to Actinomycin D treatment resulted in suppression of corticosterone secretion analogous to that seen in animals receiving saline and dexamethasone.

In order to monitor more directly ACTH secretion, rats were adrenalectomized. After 2 weeks ACTH levels in plasma were sufficiently high to permit direct measurement of plasma ACTH. Results of these studies

are shown in Table II. The mean plasma ACTH levels in adrenalectomized rats receiving control injections (Group 1) was 10.4 mU/100 ml. 60 minutes following dexamethasone treatment the mean ACTH level was 1.4 mU/100 ml (Group 2). The administration of Actinomycin D (Group 3) did not alter plasma ACTH levels. However, suppression of ACTH secretion by dexamethasone did not occur in rats which received Actinomycin D 30 minutes prior to dexamethasone administration (Group 4). In contrast, the administration of dexamethasone 30 minutes prior to Actinomycin D treatment (Group 5) resulted in suppression of ACTH secretion analogous to that seen in Group 2.

Discussion. In this study when Actinomycin D was administered in doses sufficient to decrease RNA synthesis in the pituitary and brain, dexamethasone was ineffective in suppressing ACTH secretion. This result did not appear to be secondary to a nonepetic stimulation of ACTH secretion by Actinomycin D because Actinomycin D treated rats did not have significantly higher ACTH secretion than control rats, and when dexamethasone was administered 30 minutes prior to Actinomycin D treatment, suppression of ACTH secretion occurred in a fashion analogous to that seen when dexamethasone alone was given.

The lack of unique specificity(9,10) in terms of the pharmacologic action of Actinomycin D and the imponderables inherent in an *in vivo* system prohibit a definitive interpretation of these data. Since the major reported effect of Actinomycin D is to inhibit RNA synthesis, we consider that the results of the present study are consistent with the hypothesis that dexamethasone inhibits ACTH secretion by a mechanism that involves RNA synthesis. The findings in this study are analogous to the findings that the mineralocorticoid, aldosterone, exerts its effect on the toad bladder through a mechanism that is inhibited by Actinomycin D(11), and that the suppression of pituitary TSH secretion by thyroxine is inhibited by Actinomycin D(12).

Summary. Actinomycin D inhibited the capacity of dexamethasone to suppress pituitary ACTH secretion *in vivo* in normal and

TABLE II. Plasma ACTH in Adrenalectomized Rats. At the top of this chart the timing and order of various treatments is indicated. All rats were bled 60 minutes following the last injection. Note that groups 4 and 5 received both dexamethasone and Actinomycin D but the order of administration was reversed. The analysis of variance(13) indicated an F value of .01 and the "Test of Duncan"(14) comparing each group with the first group (control) indicated that groups 3 and 4 were not significantly different and that groups 2 and 5 were significantly different at the .01 level.

Group	1		2		3		4		5		Experiment No.
	90	60	Saline	Saline	Actinomycin D 1 mg	Saline	Actinomycin D 1 mg	Dexamethasone 1 mg	Actinomycin D 1 mg	Dexamethasone 1 mg	
Plasma ACTH, mU/100 ml (95% confidence limits)	12.8 (10.2-13.8)	1.1 (0.8-1.3)	1.1 (0.8-1.3)	16.2 (14.0-18.5)	15 (13.0-17.0)	15 (13.0-17.0)	15 (13.0-17.0)	15 (13.0-17.0)	17 (1.6-1.8)	17 (1.6-1.8)	1
	4.6 (3.9-5.4)	1.0 (0.9-1.1)	1.0 (0.9-1.1)	5.6 (4.0-7.5)	4.5 (3.2-6.2)	4.5 (3.2-6.2)	4.5 (3.2-6.2)	4.5 (3.2-6.2)	0.9 (0.8-1.2)	0.9 (0.8-1.2)	2
	13.4 (9.7-18)	1.1 (0.9-1.3)	1.1 (0.9-1.3)	10.6 (5.6-15.7)	19.7 (14.5-27.8)	19.7 (14.5-27.8)	19.7 (14.5-27.8)	19.7 (14.5-27.8)	1.1 (1.0-1.2)	1.1 (1.0-1.2)	3
	10.8 (9.4-12.3)	1.0 (0.9-1.1)	1.0 (0.9-1.1)	4.8 (3.5-6.2)	8.0 (6.6-9.4)	8.0 (6.6-9.4)	8.0 (6.6-9.4)	8.0 (6.6-9.4)	1.1 (0.9-1.3)	1.1 (0.9-1.3)	4
	8.7 (7.0-11.0)	1.3 (0.9-1.9)	1.3 (0.9-1.9)	2.8 (1.9-3.7)							
	12.0 (7.0-20.0)	2.8 (1.9-3.7)	2.8 (1.9-3.7)								
	10.4 ± 1.3	1.4 ± 0.3	1.4 ± 0.3	9.3 ± 2.6	11.8 ± 3.4	11.8 ± 3.4	11.8 ± 3.4	11.8 ± 3.4	1.2 ± 0.2	1.2 ± 0.2	Avg ± SEM

adrenalectomized rats. While a definitive explanation for such results awaits further elucidation, the results are consistent with the hypothesis that dexamethasone inhibits ACTH secretion by a mechanism that involves RNA synthesis.

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