CORTICOSTERONE SUPPRESSION OF ACTH SECRETION: ACTINOMYCIN D SENSITIVE AND INSENSITIVE COMPONENTS OF THE RESPONSE[1]

by

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SUMMARY. Corticosterone suppresses ACTH secretion by isolated pituitary cells. Actinomycin D (Act D) added simultaneously with, or slightly prior to, the steroid prevents the suppressive action on cells from adrenalectomized animals but not on cells from intact animals. A working hypothesis encompassing these findings is proposed: corticosterone induces the formation of a cellular factor with which it subsequently interacts to suppress ACTH secretion. The formation of the factor is sensitive to Act D, the subsequent interaction with corticosterone is not. The factor is present in the pituitary cells of intact animals owing to their previous in vivo exposure to endogenous steroid; it is absent from the pituitary cells of adrenalectomized animals.

Corticosterone suppresses the secretion of ACTH by the adenohypophysis. Kinetic analysis of the inhibitory action reveals a rapid component, manifest a few minutes after administration of the steroid (1, 2) and a delayed component, which requires several hours for maximum effect to develop (2, 3, 4). Both the rapid and delayed components are displayed by isolated pituitary cells in suspension and hence are mediated, at least in part, by mechanisms intrinsic to the anterior pituitary (5; Sayers and Portanova, submitted for publication).

Fleischer and Battarbee (6) and Arimura et al. (7) reported that actinomycin D (Act D) prevented the suppressive action of the potent synthetic glucocorticoid, dexamethasone, on ACTH secretion, a finding interpreted to mean that the suppressive action of the steroid is mediated by mechanisms involving RNA formation. However, in these experiments the blocking effect of Act D was with respect to suppression of ACTH secretion subsequent to

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dexamethasone pretreatment for one or more hours, a protocol which would not reveal effects on the rapid component of the response. We decided to investigate the interaction, if any, between Act D and corticosterone during short term exposure of isolated pituitary cells. Furthermore, since the previous history of the animal, with regard to steroid exposure, might influence the relationships observed, the results obtained using cells prepared from intact and adrenalectomized animals were compared.

Materials and Methods. The experimental techniques used in these studies have been previously described in detail (8,9). In brief, pituitary cells were dispersed from anterior lobes of male Sprague-Dawley rats by trypsin and mechanical agitation. Intact animals or animals adrenalectomized 14 to 28 days previously and maintained on 0.9% saline drinking solution were donors of pituitary tissue. The dispersed cells were collected by centrifugation and resuspended in Krebs-Ringer bicarbonate buffer containing 0.2% glucose, 0.5% bovine serum albumin, and 0.2% lima bean trypsin inhibitor. Aliquots (0.9 ml) of cell suspension were incubated together with additions of hypothalamic median eminence extract (HME-CRF), corticosterone, and/or Act D as described below. After incubation, the cells were removed by centrifugation and the separated medium was bioassayed for ACTH as described by Sayers et al. (10); results are expressed as pg of ACTH secreted, using synthetic $ACTH_{1-24}$ (Cortrosyn, Organon) as standard. To eliminate possible artifacts due to direct effects of substances tested for an action on ACTH secretion, on the subsequent bioassay for ACTH, all additions made prior to or during incubation were "crossed" at its termination, i.e., after incubation, substances tested for an action on ACTH secretion were added to controls and the appropriate vehicles to experimentals.

HME-CRF was prepared by homogenizing freshly excised ventral hypothalamic-median eminence tissue from intact male Sprague-Dawley rats in 0.2 M acetic acid. Insoluble material was removed by centrifugation and twice re-extracted with 0.2 M acetic acid. The extracts were adjusted to

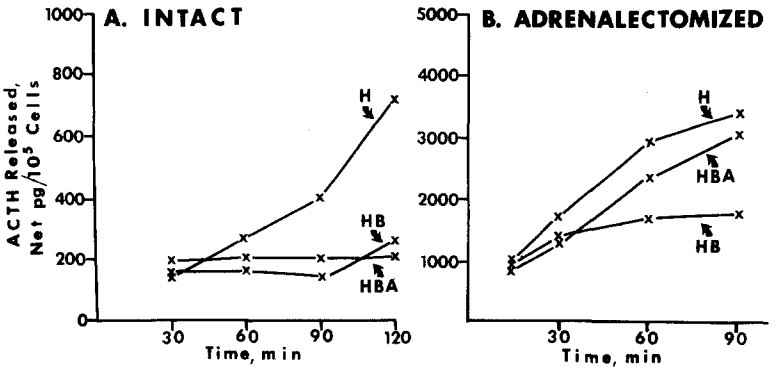

Figure 1. Isolated pituitary cells prepared from intact (A) or adrenalectomized (B) rats were incubated for the indicated times in the presence of HME-CRF (H); HME-CRF + corticosterone (HB), or HME-CRF + corticosterone + Act D (HBA); doses used were 0.08 HME-CRF, 0.1 μg corticosterone and 0.5 μg Act D; all additions were made at zero-time; data are expressed as net pg ACTH secreted with respect to zero-time controls.

pH 7.0, appropriately diluted, and added to the incubates in a volume of 0.1 ml. Doses of HME-CRF are expressed as fractions of a hypothalamic-median eminence tissue unit which in these experiments had a wet weight of approximately 7 mg. Corticosterone (Sigma), 10 μg/ml in 2.5% aqueous methanol was added to the incubates in a volume of 10 μl. Act D (Sigma) was dissolved in a small volume of propylene glycol, diluted with water to 50 to 125 μg/ml and added to the incubates in a volume of 10 μl.

Results and Discussion. Our findings indicate that the effect of Act D on the corticosteroid suppressibility of ACTH secretion is determined by the previous history of the animal with regard to steroid exposure, i.e., whether the cells were prepared from intact or adrenalectomized animals.

Figure 1 shows the results of experiments in which isolated pituitary cells prepared from intact (Fig. 1A) or adrenalectomized (Fig. 1B) animals were incubated for various times in the presence of HME-CRF (H), HME-CRF + corticosterone (HB), or HME-CRF + corticosterone + Act D (HBA). All additions were made at zero-time. Both types of cells (intact and adrenalectomized) secrete ACTH in the presence of HME-CRF and this response is inhibited by corticosterone. In the case of cells prepared from adrenal-

Table I. The Effect of Act D on Corticosterone Suppression of ACTH Secretion by Isolated Pituitary Cells Prepared from Intact and Adrenalectomized Rats.

Incubate No.	Preincubation Additions, Time Prior to HME, min.		ACTH Release, Net pg/10^5 Cells Mean ± SEM	
	20	10	INTACT	ADRENALECTOMIZED
1	-	-	730 ± 10	4670 ± 120
2	-	Corticosterone	200 ± 30	2550 ± 150
3	Act D .5	-	570 ± 60	3630 ± 140
4	Act D 1.25	-	450 ± 50	3580 ± 90
5	Act D .5	Corticosterone	210 ± 50	3650 ± 150
6	Act D 1.25	Corticosterone	270 ± 40	3760 ± 180
7	Corticosterone	Act D .5	140 ± 30	2470 ± 100
8	Corticosterone	Act D 1.25	220 ± 50	2380 ± 70

Pituitary cells were preincubated for 20 min; 0.2 HME-CRF (Intact) or 0.1 HME-CRF (Adrenalectomized) was added and the incubation continued for 45 min.

Act D was present at a dose of 0.5 μg/incubate (Act D .5) or 1.25 μg/incubate (Act D 1.25); Corticosterone was present at a dose of 0.1 μg/incubate.

ectomized animals, the inhibitory action of corticosterone is almost completely blocked by the simultaneous addition of Act D. In contrast, Act D does not block the inhibitory action of corticosterone on cells prepared from intact animals.

The data in Table I were derived from experiments in which the temporal sequence of addition of Act D and corticosterone was altered. Isolated pituitary cells were preincubated for 20 min; HME-CRF was added and the incubation was continued for 45 min. During the preincubation, corticosterone, Act D, or the combination corticosterone + Act D was added to the cells. In some cases (as indicated) corticosterone was added 10 min prior to Act D while in other cases Act D was added 10 min prior to corticosterone.

As expected, corticosterone added 10 min prior to HME-CRF inhibits the secretory response of cells prepared from intact and adrenalectomized animals (Table I, No. 2 vs No. 1, intact and adrenalectomized, respectively); Act D added 20 min prior to HME-CRF inhibits ACTH secretion to some extent (No. 3 and 4 vs No. 1, intact and adrenalectomized, respectively). In the case of cells prepared from intact animals, Act D failed to block the inhibitory action of corticosterone whether it was added 10 min before (No. 5 and 6 vs No. 2, intact) or 10 min after the steroid (No. 7 and 8 vs No. 2, intact). In the case of cells prepared from adrenalectomized animals, Act D added 10 min prior to corticosterone blocked the suppressive action of the steroid (No. 5 and 6 vs No. 3 and 4, adrenalectomized). However, Act D does not block the inhibitory action of corticosterone when added 10 min after the steroid (No. 7 and 8 vs No. 2, adrenalectomized). This latter observation is highly significant in that it demonstrates that the effect of Act D is dependent upon its time of addition relative to corticosterone, a finding not to be expected if the action of the drug were simply due to a non-specific, toxic, interaction with the cell.

If it is assumed that the effects of Act D described above are due to the action primarily ascribed to the drug, i.e., the inhibition of DNA-dependent RNA formation (11) then our findings may be explained as follows. Corticosterone induces the formation of a cellular factor(s) with which it subsequently interacts to suppress ACTH secretion. The formation of the putative factor is dependent upon RNA synthesis whereas the interaction of the factor with corticosterone is not. The factor is present in the pituitary cells of intact animals as a result of their previous, in vivo, exposure to corticosterone, and thus, short-term exposure to Act D does not prevent the suppressive action of the steroid. On the other hand, the steroid deprivation of adrenalectomy results in the decline and ultimate absence of the factor from the pituitary cells of these animals. Induction of the factor is a prerequisite for steroid suppression and hence the

10 min prior, or even simultaneous, presentation of Act D prevents the suppressive action of corticosterone, whereas exposure to Act D 10 min after corticosterone does not.

We are, of course, aware of the pitfalls involved in predicating molecular models on phenomena induced by drugs such as Act D. The above interpretation of the experimental findings is therefore presented only as a tentative model for the purpose of designing new experiments to gain further insight into the mechanisms by which corticosteroids inhibit ACTH secretion. In this connection the use of inhibitors of protein synthesis such as puromycin and/or cycloheximide might provide information regarding the chemical nature of the putative factor (RNA itself or protein subsequent to RNA formation).

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