Further Studies on the Effects of Corticosterone on Adrenocortical Cells of Hypophysectomized ACTH-Treated Rats

Several lines of investigation seem to indicate that the adrenocortical steroidogenesis can be directly inhibited in vitro by high doses of corticoid hormones[1-3]. Some recent in vitro studies have confirmed these data and have suggested that the mechanism underlying this effect of steroid hormones may consist in the inhibiton of adrenocortical protein synthesis[4-7]. Furthermore, indirect evidence is available indicating that the inhibitory effect on adrenal protein synthesis can occur in vitro with steroid concentrations which may be physiologically obtainable [8-10].

Despite this, it must be noted that there is no general agreement as to the direct effect of steroid hormones on adrenocortical cells in vivo. In fact, SAKIZ[11] and MARTINI et al.[12] were unable to demonstrate any effect of cortisol on adrenal cortex of hypophysectomized rats, whereas earlier studies from our laboratory have indicated that corticosterone may have also in vivo a direct effect upon ultrastructure and protein synthesis of cells of the rat zona fasciculata[13,14]. However, since our data have been obtained using pharmacological doses of hormone, it is doubtful whether this effect of steroid hormones might have any physiological significance. Therefore, it seemed advisable to check our previous reports using paraphysiological doses of corticosterone.

Materials and methods. 30 male rats (Sprague Dawley-derived) weighing about 150 g were hypophysectomized by the parapharyngeal approach and treated with maintenance doses of ACTH (10 IU/kg/die) up to the day before the sacrifice. At the 3rd day after operation, the animals were divided into 3 experimental groups. 2 groups were given i.p. injections of 2 and 4 mg/kg of corticosterone, respectively, for 4 consecutive days. According to ZIMMERMANN et al.[15], these doses of hormone produce a high but still physiological increase in plasma corticosterone concentrations. The control group received only i.p. injections of normal saline. The animals were sacrificed by cervical dislocation. The rats and their respective left adrenals were weighed and the 'relative adrenal weight' was calculated.

Fragments of the right adrenals were fixed in 5% glutaraldehyde, postfixed in 1% OsO4 and embedded in an epoxy resin. Thick sections were made with LKB III ultramicrotome and observed with the light microscope for orientation purposes. Thin sections were cut at the level of the zona fasciculata and observed in a Hitachi HU 11 or HU 12 electron microscope.

300 micrographs from each group, randomly selected, were used for the morphometric assessments. The percent of the cellular volume occupied by various organelles was estimated by the method of 'differential point counting'[16], and the 'surface concentration' by the 'crossing method'[17] on prints at a magnification of 24,000 and 40,000, respectively. The absolute amount of organelles in the individual adrenocortical cell was obtained by determining the average cellular volume on microphotographs at a magnification of 1,250 (200 microphotographs/stock) with the same indirect approach which we have previously described[18]. Student's *t*-test was used for the statistical evaluation of results.

Results and discussion. From Table I, it appears that the corticosterone-induced decrease in the relative weight of hypophysectomized rat adrenal glands is not significant. On the basis of analogous findings, previous authors[11,12] have postulated that steroid hormones do not exert a direct action on the adrenal cortex. In disagreement with this conclusion, we think that, owing to the heterogeneous structure of the adrenal gland (cortex and medulla), the great variability of the blood content of the organ and the general katabolic effect of corticoids, this parameter is too crude to be used for assessing the direct effect of corticoids upon adrenocortical cells.

The morphometric data, shown in Table II, clearly demonstrate that corticosterone directly affects adrenocortical cells. In fact, the volume of cells, nuclei, lipid fraction and the volume (membrane space) and surface of smooth endoplasmic reticulum (SER) decrease significantly in direct relation to the doses of corticosterone administered. These results are in complete agreement with our previous findings after using pharmacological doses of hormone[13]. In fact, the slight differences between the control values found in the two experiments, may be ascribed to the different duration of ACTH-treatment.

In the light of our previous contributions[13,18-20], these ultrastructural quantitative changes may be considered the morphological expression of the corticosterone-induced inhibition of adrenocortical secretion and protein synthesis (synthesis of enzymes and structural proteins). Therefore, it must be assumed that the decrement in SER

Table I. Changes in relative weight of hypophysectomized rat adrenal glands, induced by corticosterone-treatment

Treatment of rats	Relative adrenal weight (mg/100 g body wt.)	Decrease in % (-Δ)	Level of significance (P)
Control	14.61 $\pm$ 0.42		
2 mg/kg $\times$ 4 days	14.12 $\pm$ 0.39	3.5	>0.1 (no significant)
4 mg/kg $\times$ 4 days	13.91 $\pm$ 0.39	4.8	<0.1 (no significant)

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Table II. Changes in morphometric parameters of hypophysectomized rat adrenocortical cells, induced by corticosterone administration

Treatment of rats		Control	2 mg/kg × 4 days	4 mg/kg × 4 days
Volume of cells	μ^3	1603.4 ± 101.3	1353.7 ± 86.3	1195.2 ± 75.9
	-Δ		15.6	25.6
	P		<0.05	<0.05
Volume of nuclei	μ^3	136.9 ± 7.1	120.9 ± 6.4	115.8 ± 4.8
	-Δ		11.7	15.5
	P		<0.05	<0.05
Volume of mitochondria	μ^3	418.6 ± 30.5	397.4 ± 30.0	370.2 ± 24.6
	-Δ		5.1	11.7
	P		no significant	no significant
Surface of mitochondrial cristae	μ^2	2093.0 ± 155.5	2026.7 ± 153.6	1925.1 ± 127.9
	-Δ		3.2	8.1
	P		no significant	no significant
Membrane space	μ^3	877.0 ± 54.1	720.0 ± 44.1	619.1 ± 32.9
	-Δ		17.9	29.5
	P		<0.05	<0.05
Surface of ser	μ^2	9001.5 ± 601.2	7344.1 ± 497.6	6252.9 ± 351.3
	-Δ		18.4	30.6
	P		<0.05	<0.05
Volume of lipids	μ^3	156.0 ± 10.6	104.2 ± 7.0	85.4 ± 5.9
	-Δ		33.2	45.3
	P		<0.05	<0.01

is the effect of the physiological katabolism of the cellular membranes in presence of a deficit in the synthesis of new membranes.

The volume of mitochondrial fraction and the surface of mitochondrial cristae are found to be only slightly decreased after corticosterone administration (Table II). The decrement is not significant: this is consistent with our previous findings[13], and could be explained by assuming that either 1. the trophism of the mitochondrial fraction is exclusively or prevalently controlled by ACTH, or/and 2. the catabolic turnover of adrenocortical mitochondrial fraction is much slower than that of SER membranes. The first hypothesis may be sustained by considering the intrinsic differences in the regulation of the synthesis of mitochondrial and microsomal proteins[21]. Furthermore, ACTH was found to enhance the mitochondrial DNA-dependent protein synthesis[22–24]. However, some indirect evidence tends to support also the second possibility[20]. It must be pointed out that the two hypotheses do not necessarily conflict. To gain further insight into this problem, the effects of corticosteroids on adrenocortical mitochondrial protein synthesis are now being investigated in our laboratory.

In conclusion, our morphometric data, demonstrating a direct effect of paraphysiological doses of corticosterone on adrenocortical cells, lend credence to the view that, in the regulation of adrenocortical function, the peripheral component of the feed-back mechanism involved has a physiological significance[25].

Riassunto. Con metodi morfometrici è stato studiato l'effetto di dosi parafisiologiche di corticosterone sulle cellule corticosurrenaliche (zona fascicolata) di ratti ipofisectomizzati trattati con dosi di mantenimento di ACTH. I dati ottenuti indicano che tali dosi di ormone inibiscono direttamente la funzionalità delle cellule corticosurrenaliche. Questi risultati suggeriscono che la componente periferica dei meccanismi a feed-back che intervengono nella regolazione della funzione corticosurrenalica, giochi un ruolo fisiologico significativo.

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Identification and Biosynthesis of Steroid Hormones in the Gonads of *Ciona intestinalis*

Knowledge of the identification and biosynthesis of steroid hormones in vertebrates and invertebrates has been remarkably enriched in recent years. However, no information is available as regards the prothochordates.

In order to fill this gap, and to understand better the evolutionary factors which brought to light the pattern of steroid secretion in vertebrates, we have studied both the identification and biosynthesis of steroid hormones in the gonads of the urochordate, *Ciona intestinalis*. *Ciona* is a hermaphroditic animal, whose gonads consist of a separate ovary and testis. The pear-shaped ovary lies in the intestinal loop to the left of the stomach; the germinal epithelium is lined internally by a ciliated epithelium, while externally it is surrounded by vascular connective tissue. The testis is a diffuse structure composed of branching tubules spread over the intestine and the posterior part