Investigations into the effects of ACTH on the half-life of mitochondrial proteins in the rat adrenal cortex

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Summary. ACTH elongates the half-life of the mitochondrial proteins from the rat adrenal cortex, and chloramphenicol inhibits this effect of ACTH. The hypothesis is advanced that the ACTH-provoked stabilization of the adrenocortical mitochondrial proteins requires continuous mitochondrial DNA-dependent protein synthesis.

Previous investigations show that ACTH decreases the rate of mitochondrial protein degradation in the rat adrenal cortex[1,2]. To gain further insight into this problem, we studied the effects of chloramphenicol (CAP), a specific inhibitor of mitochondrial protein synthesis[3,4], on the ACTH-induced increase in the half-life of adrenocortical mitochondrial proteins.

Materials and methods. 152 male adult albino Wistar rats of about 200 g received an i.p. injection of 1.5 μCi/g of 3H-leucine (specific activity, 33 Ci/mM, New England Nuclear Corporation, Frankfurt/M, BRD) at 10.00 h and were divided into 4 experimental groups, one of which served as a control. Starting 2 h after the tracer injection, the other 3 groups received every 12 h injections of ACTH (Acthar gel, Armour-Erba, Milan, Italy), CAP (Synthomycetin, Lepetit, Milan, Italy) or ACTH plus CAP. 1 day after the label injection, all the animals received daily i.p. injections of 30 mg of unlabelled L-leucine (Sigma Chemical Company, St. Louis, USA). 6 animals from each group were sacrificed 2 h (0-time), 3, 6, 9, 12 or 15 days after the tracer injection.

The adrenal glands, cleaned of all connecting fat, were sliced in half and the *zona medullaris* removed under the dissecting microscope. Mitochondrial proteins were prepared by differential centrifugation[1,5]. The amount of 3H present in the mitochondrial fraction was determined by the filter-disc technique[6], using a Beckman L-100 liquid scintillation spectrometer. The mean half-lives ($t^1/_2$) of mitochondrial proteins were calculated by analytical methods from the semilogarithmic plots of cpm vs time. A 2-sided t-test was run to determine whether the slopes were significantly different from each other.

Results and discussion. CAP-administration induces no significant decrease in the half-life of adrenocortical mitochondrial proteins ($t^1/_2 = 7.66$ vs 8.42 days, $p > 0.05$), while ACTH-treatment provokes a significant increase ($t^1/_2 = 14.38$ days, $p < 0.01$). CAP-administration decreases this parameter in ACTH-treated animals to the values found in normal and CAP-treated rats ($t^1/_2 = 7.06$ days) (figure). It is well known that ACTH stimulates both microsomal and mitochondrial protein synthesis[7-9].

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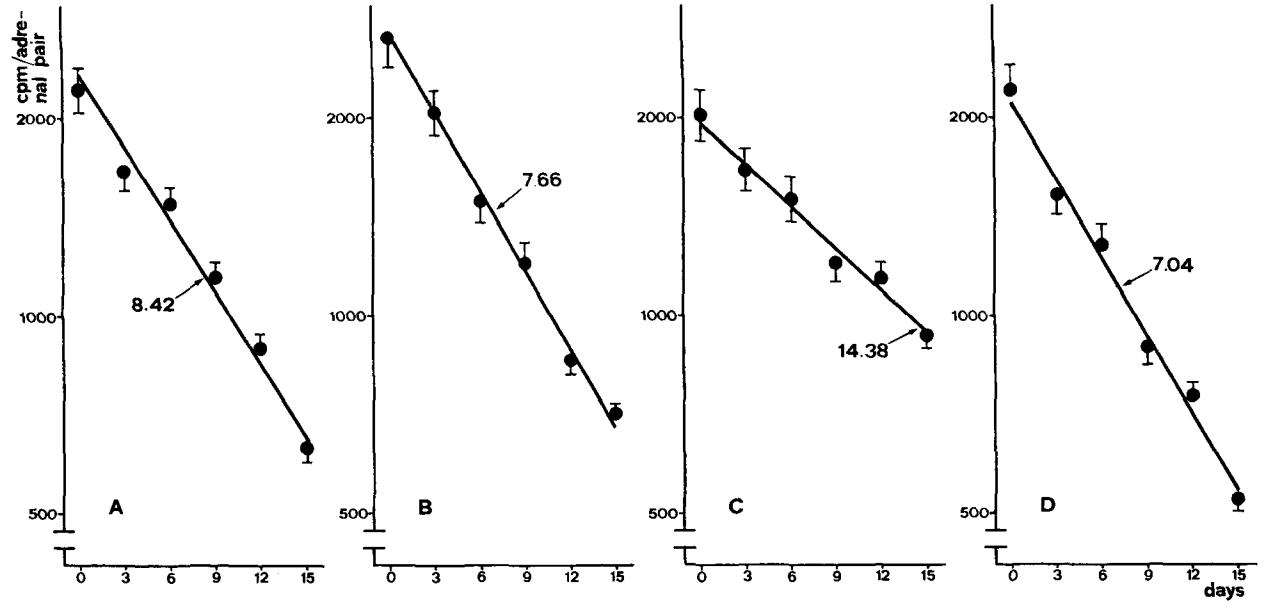

Decay curves of radioactivity of adrenal mitochondrial proteins labelled with 3H-leucine. *A* control rats; *B* rats treated with 50 mg/kg of CAP i.p. twice a day; *C* rats treated with 20 IU/kg of ACTH gel s.c. twice a day; *D* rats treated with CAP and ACTH at the same doses than in *B* and *C*. The radioactivity was expressed as cpm per adrenal pair, since the treatments with ACTH and CAP induce significant variations in the adrenal weight. Each point represents the average of 3 separate determinations ± SE, 2 pooled adrenal pairs being used for each determination. Half-lives are specified by the arrows along the slopes.

Therefore, the ACTH-induced increase in the half-life of adrenocortical mitochondrial proteins could be explained by assuming that this hormone elicits a higher rate of neutralization of the label. However, this possibility can be disregarded, since this effect of ACTH is not inhibited by injecting the animals with daily doses of L-leucine, which presumably would decrease the tracer concentration in the intracellular aminoacidic pool.

On this ground, the CAP-induced inhibition of the ACTH-enhanced half-life of adrenocortical mitochondrial proteins indicates that this effect of the hormone requires the continuous synthesis (possibly stimulated by ACTH) of protein(s) translated by the CAP-sensitive mitochondrial enzymatic machinery. We advance the hypothesis that such protein(s) might be involved in the attachment to the mitochondrial membranes (and, therefore, in the stabilization) of the enzymatic proteins synthesized at the microsomal level.

Stereological evidence indicates that a chronic treatment with ACTH induces, in the rat adrenal cortex, a significant increase in the surface of the mitochondrial cristae[10], which can be interpreted as a morphological counterpart of the ACTH-enhanced growth and steroidogenic capacity of adrenocortical cells; in fact, numerous enzymes of the steroid-synthesis are inserted on the mitochondrial cristae[11]. The present data appear to indicate that the mechanism underlying this trophic effect of ACTH involves not only the stimulation of nuclear and mitochondrial DNA-dependent protein synthesis[9], but also the stabilization of the mitochondrial enzymatic and structural proteins.

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Dynamics of estrogen binding by uterine cells in vivo[1]

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Summary. The dynamics of the in vivo binding and release of tritiated estradiol in different uterine cell types are described. The very early binding of estrogens by the cytosol-nuclear and the eosinophil receptor systems is in accordance with the hypothesis that some estrogenic effects are mediated by these receptor systems.

The current hypothesis on the mechanisms of estrogen action assumes the binding of the steroid to the specific receptors on target cells[2,3]. This binding triggers a series of events, which constitute the response to estrogens[2,4,5]. According to this hypothesis, the binding of estrogens to their receptors must occur before any change in any parameter of estrogen stimulation. The kinetics of the events that occur in the uterus after estrogen stimulation have been studied in detail[5]. Early increases in uterine cyclic AMP[6] and in the number of uterine eosinophils[5] have been found in vivo min after estrogen administration. There is no information, however, on the kinetics of in vivo estrogen binding to the receptors in different uterine cells. The present report describes the dynamics of in vivo estrogen binding and release by various rat uterine cell types.

Material and methods. Female Sprague-Dawley rats, weighing 200 g, in estrus and on the 1st day of diestrus, were used. Tritiated estradiol-17β (500 μCi, corresponding to 1.4 μg) was injected into the jugular vein and the animals were killed at various times after the estrogen injection. The uteri were excised and processed by a dry radioautographic technique for soluble compounds designed by Gersh[7–10] and modified by Stumpf[11]. The radioautograms were developed after 3 months of exposure and stained with hematoxylin-eosin or methyl green-pyronine.

For each experimental condition and for each cell type, radioautographic granules over 40 cells (and/or nuclei) were counted. The radioactivity of the 'extracellular space' was estimated by counting 40 areas of a size comparable to an average eosinophil, marked by a circle in the ocular piece of the microscope in areas chosen at random between cells located in the deep stroma.

Results. The figure shows the dynamics of estrogen binding and release by the different uterine cell types. Glandular, stromal and muscular cells appear to follow a common pattern of uptake of radioactive estradiol. Very little concentration of radioactivity over the nuclei of the connective tissue cells is observed within 1 min after the injection. However, in the vicinity of the blood vessels, there is a clear tendency for localization of radioactivity in the nuclei of stromal and glandular epithelial cells. 3 min after the injection of the labelled steroid, there is already a clear localization on the nuclei of glandular, stromal and muscular cells. The amount of radioactivity increases with time, and reaches its maxi-

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