Investigations on the Turnover of Adrenocortical Mitochondria. VII. Effects of ACTH on the Half-Life of Mitochondria from the Zona reticularis of the Rat Adrenal Cortex

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Summary. The half-life of mitochondria from the zona reticularis of the rat adrenal was calculated by determining the radioactivity decay curves of the mitochondrial compartment of 3H-thymidine-injected animals, using autoradiographic methods. ACTH was found to enhance significantly the half-life of the organelles.

Recently we demonstrated that ACTH controls the growth of the mitochondrial compartments of both zona fasciculata[2] and zona reticularis[3,4] of the rat adrenal cortex, by inducing increase in the volume and number of the organelles. Evidence is now available that the decrease in the rate of mitochondrial degeneration may play an important role in the ACTH-induced increase in the number of zona fasciculata mitochondria[5]. It therefore seemed worth investigating whether ACTH affects the half-life of mitochondria from the zona reticularis, and to this purpose we have studied, by high resolution autoradiography, the effects of a continuous ACTH-treatment on the radioactivity decay in the mitochondrial compartment from the zona reticularis of 3H-thymidine-injected rats.

Materials and methods. 36 male albino rats (Wistar-derived) received an ip. injection of 2.5 μCi/g of 3H-thymidine (specific activity 15 Ci/m*M*) (New England Nuclear Corp.) at 12.00 noon. The animals were divided into 6 experimental groups. The control group (0-time) was sacrificed by cervical dislocation, 2 h after the tracer injection; all the other groups were divided into two subgroups one of which received daily ip. injections of 10 IU/kg of ACTH (Sigma), and were sacrificed after 3, 6, 9, 12 or 15 days.

Sliced pieces of the right adrenal from each rat were processed for the electron microscopy[2]. Thick sections were made with LKB III ultramicrotomes and observed with the light microscope for orientation. Thin sections of silver/gold interference colour were cut at the level of the zona reticularis. The autoradiography was performed according to Caro[6], using Ilford L4 nuclear emulsion. The specimens were observed in a Hitachi HS-9 electron microscope.

For each rat 20 light micrographs at a final magnification of 1250 and 30 electron micrographs at a final magnification of 24,000 were recorded. The number of silver grains superimposed on the mitochondria and the concentration of radioactivity (i.e., the number of silver grains/100 μm^2 of mitochondria) were calculated as described previously[7]. In order to correct the dilution of radioactivity due to the increase in cell volume in ACTH-treated groups, the volume of the zona reticularis cells of 3, 6, 9, 12 and 15 days ACTH-administered rats was determined according to the method of Nussdorfer[8], and the CR of the mitochondrial compartments from ACTH-treated groups was increased proportionally to the percentual increase in the cell volume. The background was expressed as the number of silver grains/100 μm^2 of section area of adrenal tissue from rats not injected with tracer.

The fitting of the curves (CR *versus* time) was performed according to the least squares method and a two-sided *t*-test[9] was run to determine whether the slopes were significantly different from each other. All the

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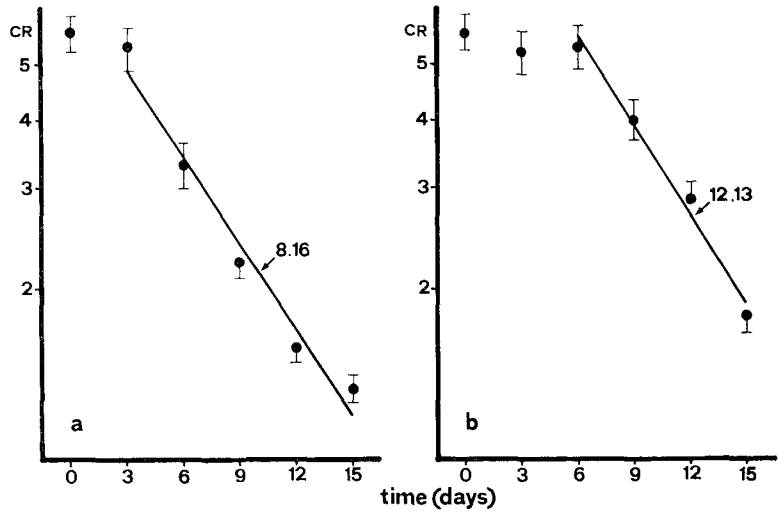

Decay curves of radioactivity in the mitochondrial compartments from the zona reticularis of untreated (a) and ACTH-treated rats (b) after i.p. injection of 3H-thymidine. Each point represents the average of 3 separate determinations ± SE. Half-lifes of mitochondria are specified by the arrows along the slopes.

statistical procedures were performed using an electronic microcomputer (Olivetti No. P-652/ROM 01 – MLU-600).

Results and discussion. In the control group (0-time), 4.5% of the mitochondrial sections appeared to be labelled, indicating that, as in the zona fasciculata[2], also in the zona reticularis of intact rat adrenals about 5% of mitochondria were in 'S' phase. The CR of the mitochondrial compartment in the untreated rats remained unchanged during the first 3 days after the tracer injection, suggesting that in this period each labelled ('S' phase) mitochondrion divides into two organelles of which one is labelled. Thus we can consider that the fate of a pool of 3H-thymidine-labelled newly formed mitochondria was followed in the present experiment.

From the 3rd to the 15th day after the tracer injection, the CR of the mitochondrial compartment of untreated rats decreased in a semilogarithmic manner (figure, a) and the number of days in which the CR is reduced to a half, averaged 8.16 days. Since it is well established that DNA is an extremely stable molecule, which does not display appreciable turnover[10], we can reasonably assume that this parameter can be an estimate of the half-life of mitochondria.

In the ACTH-treated groups, the CR of the mitochondrial compartment did not show any significant change during the first 6 days after 3H-thymidine administration, and thereafter it decreased in a semilogarithmic manner (figure, b). The half-life of zona reticularis mitochondria of ACTH-treated rats was found to be significantly higher than in the untreated animals (12.13 vs 8.16; $p < 0.01$).

These findings suggest that the mechanism underlying the ACTH-elicited stimulation of the growth of the mitochondrial compartment from the rat adrenal zona reticularis involves not only hypertrophy and proliferation of the organelles[4], but also the slowing down of the degeneration rate of mitochondria as 'intact units'. Since the rate of degradation of adrenal mitochondrial proteins[11,12] and phospholipids[13] was demonstrated to be slower in the hypertrophic adrenals of ACTH-treated rats, we hypothesize that metabolic stabilization of the lipoproteic components of the mitochondrial membranes can be involved in the ACTH-induced increase in the half-life of mitochondria from the rat adrenal zona reticularis.

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Ovarian LDH Activity in Gonadotropin-Treated Immature Rats

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Summary. Lactate dehydrogenase (LDH) activity was studied in the ovaries of immature rats treated with pregnant mare serum gonadotropin (PMS). LDH activity increased sharply at 36 h after PMS injection in the ovarian tissue as well as in the blood. It was suggested that the increase of LDH activity in the ovary may be related to its increasing ability to secrete estrogen.

Lactate dehydrogenase (L-lactate: NAD oxidoreductase, E.C. 1.1.1.27) is a key factor of glycolysis and is mainly concerned with the restoration of depleted NAD during glycolysis. It was shown that ovaries exposed to a large dose of PMS had a marked increase in glucose-6-phosphate dehydrogenase[1]. In the immature rat, LH administration caused a marked increase in glucose uptake, lactic acid production and glucose oxidation[2]. The rate of lactic acid production by the ovaries from intact and hypophysectomized immature female rats was found to increase for 8 h after PMS administration[3], and then decline to the initial level by the 16th h after PMS injection. In the present investigation we are providing evidence that LDH activity can be induced in the immature rat ovaries by PMS administration.

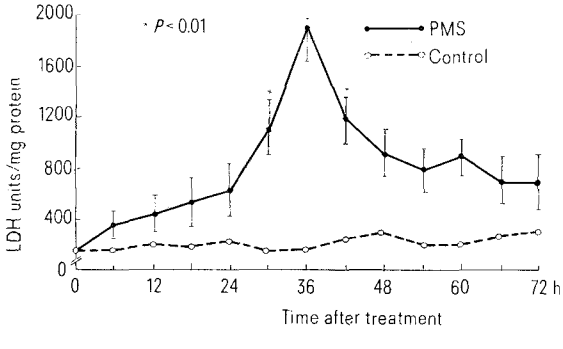

Fig. 1. Effect of 25 IU of PMS injected in the immature female rat (24 days old) on the LDH specific activity (enzyme unit/mg protein). Each point represents 6 animals and the standard errors are shown by the vertical lines.

Methods. Immature Holtzman strain female rats, 24 days old, were used in this experiment. They were kept in controlled light (14LD:10D). Feed and water were available to the animals ad libitum. To induce synchronous ovulation, groups of rats were given 25 IU PMS s.c. (Pregnant Mare Serum gonadotropin, Sigma Chemicals) at 900 h on the 24th day of age. Animals were sacrificed at 6.0-hour intervals and blood from trunk was collected. The occurrence of ovulation was determined by microscopic examination of the oviducts for ova.

After decapitation of the animals, the ovaries were removed immediately, dissected free from the bursa and extraneous tissues, and weighed. Routine assay of lactate dehydrogenase in tissue and blood was conducted as previously reported[4]. Protein concentrations were rou-

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