Effects of ACTH and diabetes on phospholipid metabolism in adrenal mitochondria

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Summary. Incorporation of 32P into adrenal mitochondrial phospholipids (PL) increased in ACTH-treated rats, but it decreased in diabetics, inspite of the fact that these animals showed adrenal overactivity. Since diabetics did not show increased 11 β-hydroxylation, as opposed to ACTH-treated rats, it is suggested that the stimulation of this enzyme activity by exogenous ACTH is related to an increased turnover of PL at the mitochondrial membrane. This process is impaired in diabetics and prevents the stimulation of 11 β-hydroxylation.

ACTH regulates the structure and function of adrenal mitochondria[3-5]. These organelles are known to contain certain specific phospholipid fractions which might be essential for hydroxylation reactions, particularly 11β-hydroxylation[6]. As ACTH is also known to influence the activity of this enzyme[7-9], it seemed probable that the stimulation of 11β-hydroxylation could be associated to changes in the metabolism of phospholipids. To explore this possibility, we studied the incorporation of ^{32}P into mitochondrial phospholipids from rats subjected to exogenous ACTH treatment. In addition, we extended this investigation to diabetic rats, which present hyperadrenocorticism due to high endogenous levels of circulating ACTH[10-13].

Materials and methods. Adult female Wistar rats were used. For studies of adrenal mitochondrial phospholipid metabolism, the animals were sacrificed by stunning 4 or 20 h after the i.p. administration of 200 μCi of ^{32}P. The adrenals were homogenized in 2 ml of 0.32 M sucrose/pair of glands and the homogenate was spun at 900 $\times$ g for 10 min at 4 °C. The supernatant was centrifuged at 5000 $\times$ g in order to sediment the mitochondria[8,9]. The pellet was washed once with sucrose, recentrifuged at the same speed and finally suspended in 0.2 ml of water. Phospholipids were extracted from this suspension by the method of Folch et al.[14] and the extract chromatographed on silica gel G5 $\times$ 20 cm thin-layer plates in the system chloroform–methanol–water (65:25:4) of Villaruel and Castro[15]. After drying, the plates were exposed to iodine vapours and the main spots, corresponding to phosphatidyl-choline (PC; R_f 0.57), phosphatidyl-ethanolamine (PEA; R_f 0.72) and cardiolipin (CL; R_f 0.80), were eluted as described by Brignone et al.[16]. The mobility of these compounds on TLC plates corresponded to that of authentic standards run in parallel with the samples, and were similar to the values shown by Villaruel and Castro[15]. The eluates containing the isolated phospholipids were analyzed for phospholipid phosphorus by the method of Chalvardjian and Rudnicki[17], and also for radioactivity incorporated. Results were expressed as cpm/μg of phosphorus.

11β-hydroxylation of deoxycorticosterone was determined in adrenal mitochondria from normal and diabetic rats by methods already published by our laboratory[8,9]. Animals were treated according to the following procedures: ACTH (Synacthen Depot) was given daily in the amount of 50 μg s.c. for 5 or 12 days. Diabetes was induced by the i.v. injection of 65 mg/kg of Streptozotocin (Upjohn) prepared as described by Junod et al.[18], and the treated animals were used one month after diabetes induction. ^{32}P (disodium ortophosphate) was purchased from the Atomic Energy Commision of Argentina. Pure standards of PC, PEA and CL were obtained from Sigma. All other chemicals were reagent grade.

Results and discussion. Table 1 shows the results of 3 different experiments performed with control and ACTH-treated rats. Animals treated for either 5 or 12 days showed a significant increased incorporation of ^{32}P into adrenal mitochondrial phospholipids, compared to the controls. However, the 3 main fractions of phospholipids were not equally affected: both PC and PEA specific activity was increased, in contrast to CL in which no changes were observed.

Table 2 presents our results of ^{32}P incorporation into normal and diabetic adrenal mitochondria. Diabetic rats showed glycosuria (> 2%) and adrenal hypertrophy at the time of sacrifice. Mitochondria from diabetics showed a decreased incorporation of radioactive phosphate into PC and CL, whereas the decrease in PEA was not statistically significant. Thus opposite effects of ACTH and diabetes were found to occur regarding phospholipid metabolism in adrenal mitochondria.

Table 1. Incorporation of ^{32}P into phosphatidyl-choline (PC), phosphatidyl-ethanolamine (PEA) and cardiolipin (CL) in mitochondria from adrenal cortex of control and ACTH-treated rats

Experiment	Group	Time after ^{32}P injection (h)	n	cpm 32P/μg phosphorus PC	PEA	CL
I	Control	4	6	391 ± 132	71 ± 30	294 ± 111
	ACTH 50 μg/day for 12 days	4	6	1244 ± 70**	219 ± 13**	200 ± 21
II	Control	4	6	137 ± 26	42 ± 13	144 ± 14
	ACTH, 50 μg/day for 5 days	4	6	277 ± 33*	150 ± 13**	214 ± 46
III	Control	20	6	712 ± 86	260 ± 36	433 ± 37
	ACTH, 50 μg/day for 5 days	20	6	1136 ± 83**	503 ± 44**	519 ± 100

*$p < 0.01$; **$p < 0.001$, versus control rats of the same experiment.

Table 2. Incorporation of ^{32}P into phospholipids and 11 β-hydroxylation of deoxycorticosterone in mitochondria from control and diabetic rats adrenals

Group	Adrenal weight (mg/100 g)	^{32}P Incorporation (cpm/μg Pi) PC	PEA	CL	11 β-Hydroxylation (μg cort/mg prot.)
Control	24.4 $\pm$ 1.2	1531 $\pm$ 177	339 $\pm$ 23	596 $\pm$ 64	3.1 $\pm$ 0.3
Diabetic	32.4 $\pm$ 1.7*	697 $\pm$ 135*	277 $\pm$ 48	264 $\pm$ 48**	2.6 $\pm$ 0.4

*$p < 0.01$; **$p < 0.001$, versus control rats. Number of animals studied: 10 controls and 10 diabetics for phospholipid metabolism; 6 controls and 6 diabetics for 11 β-hydroxylation. For abbreviations, see table 1. ^{32}P was injected 20 h before sacrifice.

As mentioned earlier, ACTH treatment increases the activity of the mitochondrial 11β-hydroxylase[6-8]. Since diabetic animals present signs of pituitary-adrenal hyperactivity[10-13], it was of interest to see whether 11β-hydroxylation was also increased in diabetic rats. However, table 2 shows that adrenal mitochondria from diabetic rats incubated with deoxycorticosterone did not metabolize this substrate into corticosterone at a rate different from control mitochondria. Thus, the adrenal hyperfunction described in diabetic animals is not accompanied by an increase in 11β-hydroxylation. ACTH-stimulation of this enzyme activity, therefore, may be the consequence of the increased phospholipid renewal at the mitochondria membranes, and this process is probably impaired in diabetics.

The biosynthesis of mitochondrial phospholipids is a complex process carried out by cytoplasmic and mitochondrial enzymes. Whereas the enzymes that synthesize PC and PEA are microsomal, those conducing to CL formation are mostly mitochondrial[19]. The increment induced by ACTH of radioactive PC and PEA specific activity in our preparations, but not of CL, suggests that the trophic hormone was acting on the cytoplasm, and that the phospholipids preformed in this compartment were subsequently incorporated into the mitochondria. Our previous results showing that ACTH treatment increases the synthesis of cytoplasmic proteins that are secondarily translocated to the mitochondria[20,21], support the view that certain protein and phospholipid components of the mitochondrial membranes originate in the cytoplasm[22], and that this process is under hormonal control. In experimental diabetes, probably both the cytoplasmic and mitochondrial system of enzymes of phospholipid metabolism are equally impaired, as PC and CL specific activity was decreased; this alteration probably prevented the 11β-hydroxylase of adequately responding to the pituitary ACTH hypersecretion present in diabetic rats. Nevertheless, the hormonal control and relationship among mitochondrial proteins, phospholipids and 11β-hydroxylation is still obscure and must await further clarification.

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3 A. Kahri, Acta endocr., Copenh. *52*, 1 (1966).
4 G. G. Nussdorfer, P. Rebuffat, G. Mazzocchi, A. S. Belloni and V. Meneghelli, Cell Tissue Res. *150*, 79 (1974).
5 J. A. Canick and J. L. Purvis, Exp. molec. Path. *16*, 79 (1972).
6 H. P. Wang, D. R. Pfeiffer, T. Kimura and T. T. Tchen, Biochem. biophys. Res. Commun. *57*, 93 (1974).
7 K. Griffiths and D. Glick, J. Endocr. *35*, 1 (1966).
8 A. F. De Nicola, Acta physiol. latinoam. *23*, 178 (1973).
9 A. F. De Nicola and F. Freire, J. Steroid Biochem. *4*, 407 (1973).
10 S. K. Kraus, Proc. Soc. exp. Biol. Med. *143*, 460 (1973).
11 M. L'Age, J. Langholz, W. Fechner and H. Salzmann, Endocrinology *95*, 760 (1974).
12 A. F. De Nicola, O. Fridman, E. J. del Castillo and V. G. Foglia, Horm. Metab. Res. *8*, 388 (1976).
13 A. F. De Nicola, O. Fridman, E. J. Del Castillo and V. G. Foglia, submitted for publication.
14 J. Folch, M. Lees, G. H. Sloane Stanley, J. biol. Chem. *226*, 334 (1961).
15 M. del C. Villaruel and J. A. Castro, Biochem. biophys. Res. Commun. *54*, 108 (1973).
16 J. A. Brignone, C. M. C. de Brignone and A. O. M. Stoppani. Revta Soc. argent. Biol. *46*, 152 (1970).
17 A. Chalvardjian and E. Rudnicki. Analyt. Biochem. *36*, 225 (1970).
18 A. Junod, A. E. Lambert, L. Orci, R. Pictet, A. E. Gonet and A. E. Renold, Proc. Soc. exp. Biol. Med. *126*, 201 (1967).
19 E. A. Dennis and E. P. Kennedy, J. Lipid Res. *13*, 263 (1972).
20 O. Fridman, V. G. Foglia and A. F. De Nicola. Acta physiol. latinoam. *23*, 52 (1975).
21 O. Fridman, V. G. Foglia and A. F. De Nicola, Acta physiol. latinoam. *25*, 141 (1975).
22 S. Ichii, Endocr. jap. *19*, 203 (1972).