Penetration of Intact Mitochondria by Dehydroepiandrosterone and its Conjugates or Derivatives

The permeability of biological membranes is known to represent a major factor in the in-vivo metabolism of any compound. Previous investigations, therefore, were concerned with the penetration of intact human erythrocytes by steroid or steroid conjugates and the subsequent inhibition of intracellular glucose-6-phosphate dehydrogenase[1]. In continuation of such experiments, the penetration of intact mitochondria by dehydroepiandrosterone (3β-hydroxy-5-androsten-17-one, DHEA) and its conjugates or derivatives was studied.

Liver tissue from male guinea-pigs (Pirbright white) was homogenized in 5 ml 0.25 *M* sucrose per g of wet tissue, employing a Potter-Elvehjem glas homogenizer. Following a 10 min centrifugation at 900 *g*, the supernatant was removed and centrifuged for 20 min at 13,000 *g*. Precipitated mitochondria were washed twice with 0.15 *M* phosphate buffer of pH 7.4, the suspension being centrifuged for 20 min at 15,000 *g*. The final precipitate was resuspended in 1 ml of 0.2 *M Tris* buffer of pH 7.4 per g of original weight of wet tissue. To 0.5 ml of this suspension 2.5 ml of the *Tris* buffer and 0.02 ml dioxane with steroid or steroid derivative were added. The final concentration of steroidal compounds corresponded to a 10^{-4} *M* solution. The various ^{3}H-labelled substrates with 44,600 to 899,000 cpm ^{3}H are listed in Table I. After incubation for 30 min at 37°C under continuous shaking, the mitochondria were separated again by centrifugation for 20 min at 15,000 *g* and washed twice, using 3.0 ml 0.15 *M* phosphate buffer of pH 7.4 per washing. The washed mitochondria were suspended in water and homogenized in the Ultra-Turrax (JANKE and KUNKEL, Staufen). In the homogenates ^{3}H-labelled compounds were determined by standard procedures[2].

As can be derived from Table I, substantial amounts of free DHEA and DHEA sulfatide had entered the mitochondria, whereas little more than 8% of ^{3}H-activity were detected in mitochondria following the incubation with DHEA sulfate. Since in the latter experiment practically all ^{3}H-activity was associated with the fraction of lipophile steroid sulfatide, it may be assumed that a partial conversion of the hydrophile steroid sulfate to the lipophile steroid sulfatide[3] was responsible for the penetration of incubated mitochondria labelled compounds. Such a conclusion is supported by the failure of 2 other water-soluble conjugates, namely DHEA phosphate and DHEA glucuronoside, to penetrate the mitochondrial membrane. Furthermore, after incubation of intact human erythrocytes with ^{3}H-labelled DHEA sulfate, phosphate or glucuronoside no ^{3}H-activity had been found in the hemolysates[1]. The permeability of biological membranes for steroids or steroid conjugates obviously depends on the lipophile properties of the particular compound. This may be deduced from the remarkable penetration of mitochondria by DHEA alkylates, the percentage of ^{3}H-activity in their homogenates increasing with the chain length of the esterifying alkanoic acid.

Table I. Penetration of intact mitochondria from guinea-pig liver by 7α-^{3}H-DHEA, its conjugates or derivatives

Compound ($10^{-4}M$)	^{3}H-activity in mitochondria (%)
7α-^{3}H-DHEA	20.6
-sulfate	8.1
-phosphate	0.1
-glucuronoside	<0.1
-sulfatide	53.2
-acetate	20.1
-caproate	28.4
-laurate	34.7
-stearate	40.2

Table II. Metabolism of 7α-^{3}H-DHEA, 7α-^{3}H-DHEA sulfate and 7α-^{3}H-DHEA sulfatide in intact mitochondria

Steroid	nMoles/mitochondria equiv. to 0.5 g wet tissue after incubation in 3.0 ml of a $10^{-4}M$ solution of		
	DHEA	DHEA sulfate	DHEA sulfatide
Dehydroepiandrosterone	43.5	17.8	110.8
Androstenediol	6.9	2.1	17.4
16-OH-dehydroepiandrosterone	2.4	1.2	6.0
Androstenetriol	3.3	1.6	18.3
Androstenedione	1.2	1	3.9
Androsterone	1	1	1.5
Etiocholanolone	1	1	1.8

Concerning the metabolism of some of the substrates, minor but significant amounts of well-known metabolites were isolated from the homogenates of incubated mitochondria. Androstenediol (5-androstene-3β,17β-diol), androstenetriol (5-androstene-3β,16α,17β-triol), 16-OH–DHEA (3β,16α-dihydroxy-5-androsten-17-one) and androstenedione (4-androstene-3,17-dione) turned out to be the predominant metabolites of DHEA and DHEA sulfatide and accounted for 11.9% and 16.1% resp. of intramitochondrial, ^{3}H-labelled steroids. Their identification was based upon the chromatographic purification of free compounds and suitable derivatives to constant specific activity. Of the various enzyme systems involved in the formation of these metabolites so far only the 17β-hydroxy steroid oxido-reductase has been demonstrated in mitochondria from guinea-pig liver[4], the other enzymes usually being allocated to the microsomal fraction[5].

Zusammenfassung. Nach Bebrütung intakter Mitochondrien aus Meerschweinchenleber in 10^{-4} *M* Lösung von 7α-^{3}H-DHEA bzw. seinen Konjugaten oder Derivaten zeigte es sich, dass lediglich lipophile Verbindungen wie das freie Steroid, besonders aber DHEA-sulfatid, durch die Membran eingedrungen und metabolisiert worden waren.

P. BENES, G. HERZ and G. W. OERTEL

Abteilung für Experimentelle Endokrinologie, Universitäts-Frauenklinik, Langenbeckstrasse 1, D-65 Mainz (Germany), 21 September 1971.

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