EFFECT OF STEROID HORMONES ON ELECTRON TRANSPORT IN MITOCHONDRIA

R. D. Seifulla, Yu. P. Denisov, P. V. Sergeev, and Yu. A. Vladimirov

UDC 615.357.453.015.42:[612.35.014.31:612.26

Steroid hormones inhibit respiration of mitochondria and uncouple oxidative phosphorylation. Estrogens are the most active. The effect of these hormones is evidently connected with the presence of a free hydroxyl group in the C_3-position of the aromatic ring. Interaction between steroids and the enzymes of the electron transport are hydrophobic in character.

The object of this investigation was to make a comparative study of the action of ethinylestradiol and testosterone propionate on respiration and oxidative phosphorylation in rat liver mitochondria.

EXPERIMENTAL METHOD

Experiments were carried out with a suspension of liver mitochondria from male rats (weighing 100-150 g). The mitochondria were isolated in medium containing 0.3 M sucrose, 0.03 M Tris-HCl buffer, and 0.2 mM EDTA. The composition of the incubation medium (in millimoles/5 ml) was: sucrose 200, Tris-HCl 30, $MgCl_2$ 0.5, KCl 20, KH_2PO_4 2, EDTA 0.2; protein 10 mg; pH 7.4. Parallel measurements were made of the level of reduction of endogenous NAD (fluorimetrically) and the oxygen consumption of the mitochondria (polarographically) at 20°C. Steroid hormones (Calbiochem, USA), dissolved in ethanol, were introduced into a cuvette for preincubation for 3 min in a volume of 0.1 ml. The results were analyzed as follows: the intensity of fluorescence of NAD · H_2 in states I, III, and IV after Chance was measured from fluorescence curves and converted into a ratio of the intensity of fluorescence in state II (100%). The fluorescence in state IV was then compared in the experimental and control series and the value of $\Delta I/I = (I_c^{IV} - I_e^{IV})/I_c^{IV}$ was calculated, where I_e^{IV} and I_c^{IV} represent the intensity of fluorescence in state IV in the experimental and control series respectively. Ability of the pyridine-nucleotides to undergo oxidation on the addition of ADP was estimated by the equation

$$\frac{I_c^{I} - I_c^{II}}{I_e^{I} - I_e^{II}} \cdot 100\%,$$

The rate of oxygen consumption in states III and IV, i.e., the value of ODC = V_{III}/V_{IV}, was calculated from the polarographic curve.

EXPERIMENTAL RESULTS AND DISCUSSION

Analysis of the results (Table 1) shows that administration of ethinylestradiol (10^{-5} M) gave a mean value of $\Delta I/I$ of 15.6% and the response to addition of ADP was reduced by 24%. The rate of respiration in state III was reduced. A decrease in the rate of respiration was observed when glutamate was used as the oxidation substrate. During the oxidation of succinate, this concentration of the hormone caused no change in the parameters of oxidative phosphorylation. In a concentration of 10^{-4} M ethinylestradiol significantly

Department of Molecular Pharmacology and Radiobiology; and Department of Biophysics, Medico-Biological Faculty, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 77, No. 1, pp. 40-42, January, 1974. Original article submitted January 17, 1973.

TABLE 1. Effect of Hormones on Intensity of Fluorescence of NAD · H_2 and Respiration of Mitochondria

Expt. No.	Oxidation substrate	Concentration of hormone (in M)	Intensity of fluorescence					Rate of oxygen consumption		
			I_e	I in state II	I in state VI	I in state III after second addition of ADP	$\frac{\Delta I}{I}$ (in%)	state III	state IV	ODC
			Ethinyl estradiol							
	Succinate	Control	181±4	100	172±5	100±3	—	2,0±0,2	0,36±0,02	5,6
1	»	10^{-5}	134±3	100	145±4	120±3	15,6	1,40±0,2	0,55±0,02	2,5
	»	Control	147±4	100	151±2	118±3	—	1,72±0,1	0,43±0,1	4,0
2	»	10^{-4}	109±4	100	119±2	119±5	21,2	0,38±0,04	0,41±0,1	0,9
	Succinate	Control	143±3	100	172±4	148±2	—	1,91±0,2	0,60±0,03	3,1
3	»	10^{-4}	106±6	100	133±4	133±2	22	1,11±0,1	0,90±0,03	1,2
			Testosterone propionate							
	Glutamate	Control	148±6	100	128±3	119±4	—	2,67±0,11	0,71±0,06	3,8
4	»	10^{-4}	131±4	100	135±3	118±3	5,5	1,93±0,06	0,60±0,02	3,2
	Succinate	Control	156±4	100	192±5	153±3	—	3,00±0,07	1,42±0,02	2,1
5	»	10^{-4}	117±2	100	165±5	132±4	14,1	2,67±0,07	1,45±0,02	1,9
6	»	10^{-3}	125±5	100	166±4	148±4	13,5	3,0±0,14	2,14±0,09	1,4

inhibited respiration and uncoupled phosphorylation (stimulation of respiration in state IV during oxidation of succinate). The high degree of reduction of NAD · H_2 and the absence of inhibition of electron transport in the respiratory chain during the oxidation of succinate suggest that the zone of inhibition lies in the flavoprotein region of the electron chain. This hypothesis is supported by data in the literature [3]. Since the estrogen inhibits respiration in lower concentrations than those which uncouple oxidative phosphorylation, it can be concluded that ethinylestradiol differs significantly in the mechanism of its action from classical uncouplers (of the dinitrophenol type), which stimulate respiration. The fact that there was little or no increase in the rate of respiration in state IV during the action of ethinylestradiol (with glutamate as the substrate) can be explained by the masking effect of inhibition of electron transport: no increase in the rate of respiration is possible under those conditions. Testosterone propionate was much less active. In a concentration of 10^{-5} M, for instance, it had virtually no effect on respiration or phosphorylation, and only within the concentration range of 10^{-3} to 10^{-4} M was this hormone able to uncouple oxidative phosphorylation and to cause slight inhibition of respiration. Inhibition of respiration by ethinylestradiol can be explained by its hydrophobic interaction with the respiratory chain enzymes (for example, with NAD · H_2-dehydrogenase), as has been demonstrated for phenols with a low dissociation constant [1]. Estrogens (pK > 9) are practically undissociated at neutral pH values and their interaction with enzyme cannot therefore be electrostatic in character. Since hydrophobic interactions take place in the nonaqueous phase [2] the degree of dissociation of the hormone molecule in water plays no important role in the activity of the steroids. However, the polar groups evidently are of some importance in the specific activity of the estrogen and, in particular, for the manifestation of its uncoupling action. These groups may arrange the lipid moiety of the steroid along the partition boundary between the phases so as to create the most favorable position for interaction with the enzyme in the mitochondrial membrane.

LITERATURE CITED

1. L. A. Ratnikova, L. S. Yaguzhinskii, and V. P. Skulachev, Biokhimiya, 36, 376 (1971).
2. L. A. Ratnikova and L. S. Yaguzhinskii, in: Mitochondria. Molecular Mechanisms of Enzymic Reactions [in Russian], Moscow (1972), p. 77.
3. A. A. Boveris and A. O. M. Stoppani, Rev. Soc. Argent. Biol., 45, 64 (1969).