Effects of Some Steroid Hormones on Ca^{2+} Transport and Oxidative Metabolism of Isolated Mitochondria

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Effects of prednisolone, estradiol, and testosterone on the transport of Ca^{2+} and the respiration induced by it in the heart and liver mitochondria of rats were studied. Prednisolone and testosterone were found to reduce the Ca-accumulating capacity of the mitochondria, the rates of ion entry and exit, and the rate of Ca^{2+}-induced respiration. Estradiol, while inhibiting Ca^{2+} transport across mitochondrial membrane, did not influence the respiration in the phase of Ca^{2+} absorption, but accelerated it in the phase of ion exit. These data suggest that due to their lipophilic properties, the steroids become incorporated in the mitochondrial membrane, thereby changing its viscosity and permeability and limiting the mobility of transmitter proteins.

Key Words: *Ca^{2+} transport; mitochondria; oxidative metabolism; steroid hormones*

The transport of Ca^{2+} ions in a mitochondrion is a function extremely important for the vital activity of cells, as it is a component of their regulatory mechanisms controlling their energy-synthesizing function. It is well known that Ca^{2+} ions modulate the activity of three matrix dehydrogenases regulating the Krebs' cycle [5]. The Ca-accumulating capacity of the mitochondria is known to be realized through two different mechanisms: 1) active electrophoretic absorption of Ca^{2+} owing to the electric component of the proton-moving force and 2) electroneutral exit of Ca^{2+} via the Na/Ca transmitter, which is particularly active in the heart and excitable tissues [5]. The energy-dependent route of Ca^{2+} absorption and metabolism through the Na/Ca transmitter form a biphasic Ca cycle [6] whose disturbance may interfere with the mitochondrial energy-producing function and the mitochondrial-cytosol interactions. It was demonstrated previously that some steroid hormones, by transforming the state of mitochondrial membranes and inhibiting the Ca-dependent oxidation of NAD-dependent substrates, suppress the active transport of Ca^{2+} in the mitochondria [4] and reduce the intensity of oxidative phosphorylation [2]. The present work aimed to explore further the mechanisms of steroid hormone effects on Ca^{2+} transport in the mitochondria and, specifically, to investigate their direct effect on both components of the Ca cycle.

MATERIALS AND METHODS

Experiments were carried out with outbred male albino rats weighing 180 to 220 g. Cardiac and hepatic mitochondria were isolated as described previously [7,8]. The isolation medium contained 250 mM sucrose, 10 mM Tris-HCl (pH 7.4), and 1 mM EDTA. The incubation medium contained 250 mM sucrose, 10 mM Tris-HCl (pH 7.4), 120 mM KCl, and 20 mM KH_2PO_4. The protein concentration in the mitochondria was measured by

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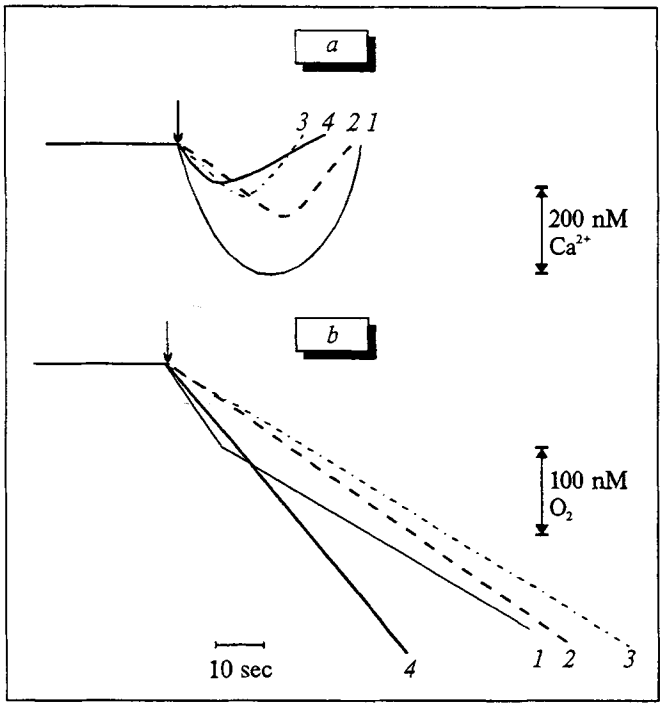

Fig. 1. Effects of steroid hormones on Ca^{2+} ion transport (*a*) and associated respiration (*b*) in rat heart mitochond-ria (MC). *1*) control; *2*) MC+prednisolone (25 μM); *3*) MC+ +testosterone (25 μM); *4*) MC+estradiol (25 μM). Arrows show the addition of MC (5 mg MC protein), cell volume 2 ml.

the microbiuret method. Ca flows were measured with an ion-selective Ca electrode and mitochondrial respiration was measured polarographically using Clarke's electrode. Mitochondria (2-3 mg/ml mitochondrial protein), $CaCl_2$ (100 μM for liver mitochondria and 200 μM for heart mitochondria), and sodium succinate (5 mM) were placed in the gage cell with incubation medium. In the experimental series, mitochondria were preincubated with steroid hormones (prednisolone, estradiol, testosterone) in a dose of 5 mM/mg mitochondrial protein for 10 min.

RESULTS

Heart and liver mitochondria responded to Ca^{2+} by cyclic changes reflecting its active absorption and subsequent release from the mitochondria (Fig. 1, *a*). The rate of Ca^{2+} release from the mitochondria did not reliably differ from the rate of its accumulation in them. Oxygen uptake by the mitochondria was increased during the first phase and reduced twofold during the second phase, this confirming, respectively, their energy-dependence and electroneutrality (Fig. 1, *b*; Table 1). No reliable differences were detected between the tested parameters in the heart and liver mitochondria, although the rate of Ca^{2+} absorption, its total accumulation, and, specifically, its release from the mitochondria were somewhat higher in the heart. An increase of the Ca^{2+} concentration to 500 μM led to a dissociative effect. This was paralleled by an appreciable increase of the rate of Ca^{2+} absorption and conjugated respiration, as well as the absence of a Ca^{2+} release phase.

A ten-minute incubation of mitochondria with the steroid hormones studied led to a reduction of Ca^{2+} transport, the magnitude of which depended on the substance.

Prednisolone suppressed all the tested parameters during both phases, but most of all it depressed the rate of Ca^{2+} accumulation (twofold) and the total absorption of Ca^{2+}. To a somewhat lesser degree it reduced the rate of oxygen uptake in this period (Table 1, Fig. 1, *b*), this leading to an increase of the amount of oxygen needed for Ca^{2+} transfer, that is, to a reduction of the energy efficacy of the process. Hence, prednisolone suppressed the electron-transporting activity of the respiratory chain, which was able to bring about a reduction of the electrochemical potential and, eventually, suppression of Ca^{2+} accumulation in the mitochondria. The rate of Ca^{2+} release from the mitochondria was similarly suppressed in the absence of an effect of prednisolone on the rate of oxygen uptake in this period (Table 1), this indicating a suppressed electroneutral function of the Na/Ca transmitter. Nonetheless, the time frame of the cycle was unchanged (Fig. 1).

In contrast to prednisolone, estradiol suppressed the rate of Ca^{2+} absorption in the mitochondria, though to a lesser degree, and at the same time shortened the period of its accumulation, this resulting in a drastic decrease of the total absorption of Ca^{2+}. Note that estradiol had practically no influence on the mitochondrial respiration in the first phase (Table 1). Estradiol suppressed the rate of Ca^{2+} release from the mitochondria, i.e., the electroneutral Na/Ca transfer, more intensively than prednisolone. However, this was not associated with a reduction of oxygen uptake in comparison with the second phase, as it was in control, this leading to a relative increase of its values (by 1.6 to 1.8 times) and indicating a weak dissociating effect of estradiol (Table 1; Fig. 1).

Testosterone showed virtually the same inhibitory effect on the rate and total absorption of Ca^{2+} as estradiol and reduced the duration of the entire cycle twofold (Table 1; Fig. 1). Like prednisolone, testosterone negligibly reduced the rate of oxygen uptake in heart mitochondria for Ca^{2+} release, but had virtually no effect on this parameter in liver mitochondria. Testosterone just slightly reduced the

TABLE 1. Effects of Steroid Hormones on Ca^{2+} Transport and Associated Oxygen Uptake in Heart Mitochondria (MC)

Experimental conditions	Total Ca^{2+} absorption, nmol/mg MC protein	Rate of Ca^{2+} absorption, nmol/sec/mg MC protein	Rate of Ca^{2+} release, nmol/sec/mg MC protein	Respiration rate during Ca^{2+} absorption, nmol O_2/sec/mg MC protein	Ca^{2+}/O_2 molar ratio during Ca^{2+} release	Respiration rate during Ca^{2+} release, nmol O_2/sec/mg MC protein
Control	115.4±21.2 (100)	9.54±2.41 (100)	8.90±1.23 (100)	2.41±0.32 (100)	3.96 (100)	1.24±0.21 (100)
Prednisolone	59.32±13.14 (60)	4.51±1.04 (47)	6.14±1.01 (69)	1.75±0.13 (73)	2.57 (65)	1.30±0.07 (105)
Estradiol	44.01±14.32 (38)	6.34±1.42 (65)	3.43±0.91 (38)	2.21±0.12 (91)	2.86 (72)	1.96±0.30 (158)
Testosterone	53.13±15.24 (46)	6.51±1.33 (68)	7.22±1.14 (81)	1.74±0.39 (72)	3.74 (94)	1.09±0.22 (88)

Note. Here and in Table 2: percentage changes are given in parentheses.

rate of Ca^{2+} release from heart mitochondria and their respiration and did not affect these two parameters in liver mitochondria (Table 1). Hence, testosterone poorly suppressed Na/Ca-dependent transfer and had virtually no effect on the electron-transporting function of the respiratory chain. These data indicate that all the studied steroid hormones suppressed the Na/Ca-dependent release of Ca^{2+} from the mitochondria, though with a various intensity. This proves their direct inhibitory effect on this transmitter, which was the most potent with estradiol, less potent with prednisolone, and the least expressed with testosterone. At the same time, these hormones suppressed the active transport of Ca^{2+} in the mitochondria, and in this case their effects were reduced, ranking in terms of intensity as follows: prednisolone > estradiol > testosterone.

Since no data are available on the presence of receptor structures for different steroids in the mitochondria [3], we may assume that the effects of the three substances observed in our experiments are associated with their effect on the mitochondrial membrane. Due to their lipophilic properties, steroids may become incorporated in the membrane, thus changing its microviscosity, permeability, and protein-lipid interactions and limiting the mobility of transmitter proteins. This very property may be the principal cause of the observed suppression of the electroneutral phase of Ca^{2+} transport by the Na/Ca transmitter. The same steroid properties may be responsible for their direct interactions with the hydrophobic sites of the respiratory chain, disrupting electron transfer, reducing the proton-moving force, and eventually suppressing the mitochondrial capacity to accumulate Ca^{2+}. Such an explanation is the most likely for prednisolone. On the other hand, the effects of estradiol and testosterone seem to be to a greater degree related to their direct membranotropic action, only indirectly disturbing the function of the respiratory chain and the Ca-accumulating capacity of the mitochondria.

Such a limiting effect of steroids on Ca^{2+} transport in the mitochondria may result in an increased pool of cytoplasmic Ca^{2+} in cardiomyocytes, this increase enhancing their contractility. It is in such terms that the positive effects of prednisolone and estradiol on the heart [1] and

TABLE 2. Effects of Steroid Hormones on Ca^{2+} Transport and Associated Oxygen Uptake in Liver Mitochondria (MC)

Experimental conditions	Total Ca^{2+} absorption, nmol/mg MC protein	Rate of Ca^{2+} absorption, nmol/sec/mg MC protein	Rate of Ca^{2+} release, nmol/sec/mg MC protein	Respiration rate during Ca^{2+} absorption, nmol O_2/sec/mg MC protein	Ca^{2+}/O_2 molar ratio during Ca^{2+} release	Respiration rate during Ca^{2+} release, nmol O_2/sec/mg MC protein
Control	93.44±19.01 (100)	8.11±1.32 (100)	7.32±1.22 (100)	2.07±0.23 (100)	3.91 (100)	1.09±0.21 (100)
Prednisolone	57.04±11.21 (60)	4.04±1.13 (49)	5.33±0.82 (72)	1.82±0.37 (88)	2.21 (57)	1.16±0.18 (106)
Estradiol	42.22±12.01 (45)	6.03±2.14 (74)	3.02±0.73 (40)	2.04±0.33 (99)	2.96 (77)	1.94±0.04 (177)
Testosterone	53.12±13.31 (57)	6.32±1.74 (79)	6.81±1.04 (93)	1.98±0.21 (95)	3.19 (82)	1.28±0.11 (117)

the therapeutic effect of prednisolone in myopathies [9] are assessed. Moreover, these properties of steroids may promote diminished energy dissemination and increased energy accumulation in the mitochondria due to ATP accumulation. A rise of the ATP content after prednisolone administration was demonstrated previously [1].

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