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STRUCTURAL REQUIREMENTS OF STEROIDS FOR THEIR ACTION AS INHIBITORS OF OXIDATIVE PHOSPHORYLATION. EFFECT OF α-TOCOPHEROL ON THE INHIBITION OF OXIDATIVE PHOSPHORYLATION BY PROGESTERONE

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SUMMARY

1. Inhibition by progesterone of succinate (+rotenone) and of β-hydroxybutyrate oxidation in intact rat liver mitochondria in metabolic state 3 was reversed by α-tocopherol. The percentage of reversal of the inhibition was a function of the α-tocopherol concentration. Inhibition of succinate oxidation by progesterone was reversed more efficiently than the inhibition of β-hydroxybutyrate at the same concentration of α-tocopherol. Tocopherol acetate reversed the inhibitory effect of progesterone on succinate oxidation much more weakly than α-tocopherol. Inhibition of succinate oxidation in metabolic state 3 was studied with various steroids bearing structural resemblance to progesterone. It was found that C-20,C-21(CH_3—C(=O)—) group of the steroids examined plays a decisive role in the inhibition of succinate oxidation.

2. Progesterone inhibited mitochondrial ATP synthesis with succinate (+rotenone) as substrate. This inhibition was also reversed by α-tocopherol.

3. Inhibition by progesterone of NADH oxidation was also reversed by α-tocopherol in sonic particles.

4. Inhibition of NAD-linked substrates oxidation in intact mitochondria by: deoxycorticosterone, corticosterone, cortisol, oestradiol and testosterone was not reversed by α-tocopherol at concentrations which reversed the inhibitory effect of progesterone in metabolic state 3.

5. α-Tocopherol was without effect on succinate oxidation inhibited by protamine, phenethylbiguanide (DBI) and oligomycin.

6. Inhibition of β-hydroxybutyrate oxidation by rotenone in intact mitochondria was not reversed by α-tocopherol, neither was succinate oxidation inhibited by stilboestrol or antimycin A.

7. Binding of progesterone to rat liver mitochondria was slightly increased by α-tocopherol.

8. A hypothesis involving the possible formation of a chemical complex between progesterone and α-tocopherol is offered to explain the mechanism of α-tocopherol action on oxidative phosphorylation inhibited by progesterone.

INTRODUCTION

Progesterone and other hormonal steroids have been shown to inhibit oxidative phosphorylation by acting directly on the respiratory chain or on the energy conserving processes [1–7]. These compounds inhibit respiratory chain electron transport at two different sites, the more sensitive one being located in the NADH-flavoprotein region [2, 3, 6], the second in the cytochrome b and cytochrome c region [6]. Stoppani *et al.*[7] showed (using heart muscle sarcosomal fragments) that inhibition of electron transport by steroids depends on the structural characteristics of the steroid molecule, particularly its shape and size, and presence of properly oriented polar substituents at critical sites. Yielding and Tomkins[11] reported that inhibition by progesterone of NADH oxidation in microsomes was reversed competitively by α-tocopherol.

Progesterone is supposed to affect the energy conserving mechanism at the second phosphorylation site according to Vallejos and Stoppani[5], or at the first phosphorylation site as claimed by Chance *et al.*[4]. Our recently published results [8–10] lead us to propose a new model aimed at explaining the inhibition of oxidative phosphorylation by progesterone. It is implicit from this model that progesterone interacts with the mitochondrial membrane causing inhibition of proton transport into the mitochondrion.

The above investigations inspired the present study, the aim of which is to obtain information: (a) concerning the relationship between the structure of the steroids and their inhibitory effect on mitochondrial

Trivial names and abbreviations used: Corticosterone, 11β,21-dihydroxy-4-pregnene-3,20-dione; Cortisol, 11β,17,21-trihydroxy-4-pregnene-3,20-dione; Deoxycorticosterone, 21-hydroxy-4-pregnene-3,20-dione; 17-Hydroxyprogesterone, 17-hydroxy-4-pregnene-3,20-dione; Oestradiol, oestra-1,3,5-triene-3,17β-diol; Pregnanolone, 3α-hydroxy-pregnan-20-one; Progesterone, 4-pregnene-3,20-dione; Testosterone, 17β-hydroxy-4-androsten-3-one; NEA (19-nor-ethynyltestosterone acetate) 4-estren-17α-ethynyl-3-one-17yl-acetate; Stilboestrol, trans-4,4′-dihydroxy-αβ-diethylstilbene; DBI, phenethylbiguanide-HCl; BSA, bovine serum albumin; Hexokinasae. ATP: D-hexose 6-phosphotransferase (EC 2.7.1.1).

respiration with succinate (+rotenone) as substrate (which is mainly caused by inhibition of oxidative phosphorylation); (b) whether α-tocopherol reverses the inhibitory effect of progesterone on energy conserving mechanisms and on the electron transport chain of rat liver mitochondria.

We have shown that: (a) the $CH_3—C(=O)$ function of progesterone is directly responsible for inhibition of oxidative phosphorylation; (b) inhibition by progesterone of the energy conserving mechanisms and electron transport was reversed by α-tocopherol. The mechanism of α-tocopherol action on the inhibition by progesterone oxidative phosphorylation is discussed.

MATERIALS AND METHODS

Mitochondria and sonic particles

Rat liver mitochondria were prepared as described previously [9]. The mitochondria thus obtained (from about 15 g of livers) were suspended in 40 ml of 25 mM Tris–HCl buffer (pH 7·3), washed and centrifuged at 15,000 *g* for 10 min. The pellet was suspended in 20 ml of 25 mM Tris–HCl buffer (pH 7·3) and sonicated at 0°C at 16 kHz for 2 × 15 s with a 1 min break using a MSE 100 W Ultrasonic Disintegrator. After sonication the disrupted mitochondria were centrifuged at 15,000 *g* for 10 min, and the supernatant obtained was centrifuged at 105,000 *g* for 30 min. The final pellet was suspended in 25 mM Tris–HCl (pH 7·3) and was used as sonic particles. The protein concentration of the mitochondrial and sonic particle suspension was determined by the biuret method [12].

Measurement of mitochondrial respiration

Respiration was measured with a Clark electrode at 25°C in a medium containing: 15 mM KCl, 50 mM Tris–HCl (pH 7·3), 5 mM $MgSO_4$, 5 mM potassium phosphate, 1 mM ADP and either 5 mM succinate (+rotenone), or 5 mM β-hydroxybutyrate, or other NAD-linked substrates. Reaction was started by addition of 0·1 ml mitochondrial suspension. Other additions are indicated in the legends to the figures.

Measurement of NADH oxidation

NADH oxidation was measured by following the decrease of absorbancy at 340 nm using a Unicam SP-800 recording spectrophotometer. The incubation medium (final volume 3 ml) contained: 15 mM KCl, 50 mM Tris–HCl (pH 7·3), 0·5 mM EDTA and 0·2 mM NADH. The reaction was started by addition of 0·2 mg sonic particle protein. Other additions are indicated on the figure.

Measurement of oxidative phosphorylation

Oxidative phosphorylation was estimated according to Skulachev *et al.*[13] by measuring H^+ consumption with a combined glass calomel electrode GR 232 Radiometer and N 512 pH-meter Elpo. Oxygen consumption was measured in the same vessel with a Clark oxygen electrode. Both oxygen and pH were registered with two synchronised recorders type I 37/N from Zip. The incubation medium (final volume 2·6 ml) contained: 200 mM sucrose, 15 mM KCl, 1 mM $MgSO_4$, 1 mM ADP, 1 mM potassium phosphate, 3 mM Tris–HCl (pH 7·3), 5 mM potassium succinate (+rotenone) and 8 mg mitochondrial protein. The reaction was started by addition of ADP. Measurements were carried out at 25°C.

Measurement of [^{3}H]-progesterone binding to mitochondria

Mitochondrial suspension (5 mg) was incubated in 1 ml medium containing: 15 mM KCl, 50 mM Tris–HCl (pH 7·3) 5 mM potassium phosphate, 5 mM $MgSO_4$, 1 mM ADP, 5 mM potassium succinate, 4 μg rotenone and [^{3}H]-progesterone (specific radioactivity 0·13 μCi/μmol) at increasing concentrations as indicated on the figure, with or without 100 μM α-tocopherol. Incubation was carried out at 25°C for 5 min. After the incubation, the sample was transferred to a 0°C bath and centrifuged at 17,000 *g* for 5 min. The pellet obtained was immediately washed three times at 0°C with 2 ml of medium containing: 15 mM KCl and 50 mM Tris–HCl (pH 7·3). The last pellet was placed in a glass counting vial and dissolved in 0·2 ml of 1 M methanolic solution of hyamine. The radioactivity was determined in a spectrometer for liquid scintilation Nuclear Chicago Mark 1, after 10 ml of scintillator solution (which contained 4 g PPO and 0·2 g POPOP in 1 l. toluene) had been added.

Steroids, α-tocopherol and α-tocopherol acetate were dissolved in ethanol and added in a 10 μl volume. The same volume of solvent was added to the control samples.

Reagents

All steroids examined, α-tocopherol, succinate, β-hydroxybutyrate, oligomycin, rotenone, antimycin A, NADH, stilboesterol, 2,4-dinitrophenol and EDTA were purchased from Sigma Chem. Co., ADP from AM Łódź-Poland, protamine and hyamine from Koch–Light, DBI from Polfa Poland, tocopherol acetate from Merck.

RESULTS

Effect of α-tocopherol on the inhibition by progesterone of succinate (+rotenone) or β-hydroxybutyrate oxidation in metabolic state 3

In metabolic state 3 progesterone inhibited succinate (+rotenone) oxidation. Fig. 1a shows that this inhibition was almost completely reversed by α-tocopherol. When α-tocopherol was added prior to progesterone, the latter only slightly inhibited succinate oxidation in metabolic state 3 (Fig. 1b). The experiments presented in Figs. 1a and 1b suggest that α-tocopherol acts on progesterone inhibition of succinate oxidation in metabolic state 3 like a classical

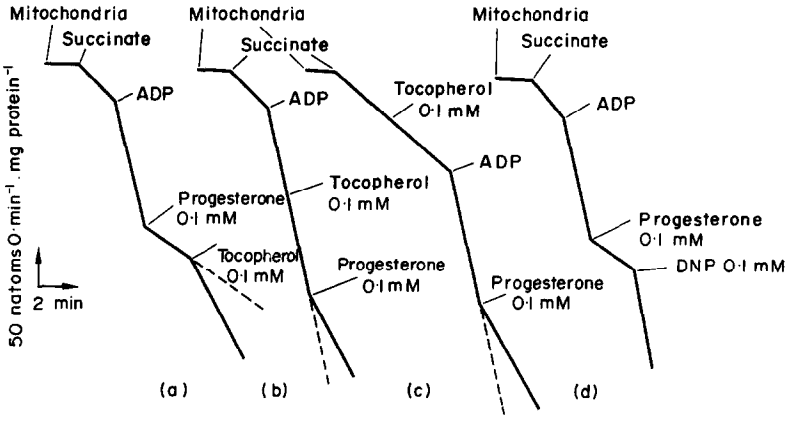

Fig. 1. Effect of α-tocopherol on inhibition of rat liver mitochondrial succinate oxidation by progesterone. Experimental conditions as described in Materials and Methods.

uncoupling agent—2,4-dinitrophenol (Fig. 1d). It led us to examine the effect of α-tocopherol on succinate oxidation in metabolic state 4. Tocopherol in these conditions did not affect succinate oxidation (Fig. 1c). This indicated that α-tocopherol does not act on mitochondria as classical uncoupling agents do. It has been shown previously that inhibition of succinate oxidation by progesterone is a consequence of the action of this steroid on energy conserving mechanisms [9]. Progesterone inhibited oxidation of NAD-linked substrates by (i) direct action on the respiratory chain and (ii) by action on energy conserving mechanisms. As indicated on Fig. 2, α-tocopherol partially reversed progesterone inhibition of β-hydroxybutyrate oxidation. Addition of α-tocopherol prior to progesterone decreased the inhibitory effect of progesterone on β-hydroxybutyrate oxidation in metabolic state 3. The differences between the effect of α-tocopherol on inhibition by progesterone of succinate (+ rotenone) oxidation or β-hydroxybutyrate oxidation in metabolic state 3 are shown on Fig. 3. This figure shows the effect of different concentrations of α-tocopherol on inhibition by progesterone of succinate oxidation or β-hydroxybutyrate oxidation in intact rat liver mitochondria. As indicated the percentage of reversal of inhibition is a function of α-tocopherol concentration. The experiments revealed also that at the same concentration of α-tocopherol the progesterone inhibition of succinate oxidation was reversed to a larger extent than the oxidation of β-hydroxybutarate.

Effect of α-tocopherol on progesterone inhibition of oxidative phosphorylation

It has been shown recently [9] that some anions reverse not only inhibition of succinate oxidation by progesterone but also progesterone inhibited oxidative phosphorylation. In contrast, uncoupling agents reversed only inhibition of succinate oxidation but not progesterone inhibited oxidative phosphorylation with succinate as substrate. Therefore it was very interesting to examine the effect of α-tocopherol on progesterone inhibition of oxidative phosphorylation. Using the pH-meter technique for measurement of oxidative phosphorylation, we have shown that progesterone inhibited oxidative phosphorylation and that this inhibition could be released by α-tocopherol (similar to the case with succinate oxidatin) (Fig. 4). The release by α-tocopherol of the progesterone inhibition of oxidative phosphorylation has been demonstrated also by measuring P_i uptake in the presence of hexokinase and glucose (not shown here). By using

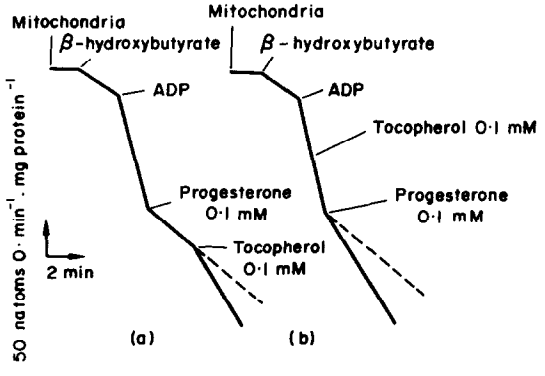

Fig. 2. Effect of α-tocopherol on inhibition of rat liver mitochondrial β-hydroxybutyrate oxidation by progesterone. Experimental conditions as described in Materials and Methods.

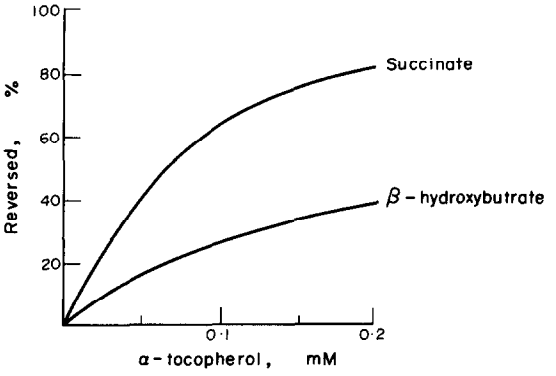

Fig. 3. Effect of different concentration of α-tocopherol on inhibition of succinate or β-hydroxybutyrate oxidation by progesterone. Experimental conditions as described in Materials and Methods.

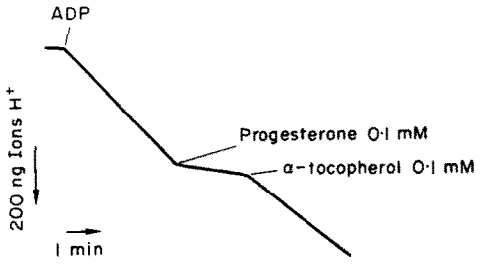

Fig. 4. Effect of α-tocopherol on inhibition of oxidative phosphorylation by progesterone. Experimental conditions as described in Materials and Methods.

this method of measurement, we have found that progesterone at a concentration of 0·1 mM, inhibited ATP synthesis only by about 30%, whereas at the same concentration of progesterone succinate oxidation was inhibited by about 70–80%. This, as we have shown, was caused by the binding of progesterone to the hexokinase added.

Relationship between inhibition of respiration by steroids and their chemical structure. The effect of α-tocopherol

Vallejos *et al.*[5] observed that deoxycorticosterone acetate acts on succinate oxidation in metabolic state 3 similar to progesterone. This observation is in accordance with our previously published results [9]. However, deoxycorticosterone was without effect on succinate oxidation. In this study we have examined 15 steroids for their ability to inhibit succinate (+ rotenone) oxidation in metabolic state 3. Table 1 lists the relevance of the structures of some of the more important ones to the inhibitory activity. Pregnanolone, lacking the 3-keto-4-ene structure of progesterone, had a similar effect on succinate oxidation. Pregnandiol in which the 20-keto group is reduced to a hydroxyl group was without effect on succinate oxidation. These results indicate that the 20-keto group of progesterone is directly responsible for inhibition of energy conserving mechanisms. This is strongly supported by the data of experiments with androstenedione and testosterone, 3-keto-4-ene steroids which have 17-keto or 17-hydroxy group and were without effect on succinate oxidation in metabolic state 3. On the other hand, testosterone acetate which has a $CH_3—C(=O)$ system affected mitochondrial respiration in a way similar to progesterone. 17α-hydroxy and 21-hydroxy substituents in a 3-keto-4-ene steroids (17α-hydroxyprogesterone and deoxycorticosterone respectively) showed no inhibitory effect. Deoxycorticosterone acetate acted like progesterone. Other steroids examined: corticosterone, cortisone, androsterone, aldosterone, oestradiol and oestriol were without effect (not shown in table). The above observation led us to conclude that the $CH_3—C(=O)$ system of progesterone is directly responsible for the inhibition of oxidative phosphorylation with succinate as substrate. The remaining hydrophobic part of the progesterone molecule reacting with the mitochondrial membrane provides condition in which the $CH_3—C(=O)$ group can display its inhibitory action. The inhibitory effect of steroids presented in Table 1 was reversed by α-tocopherol (not shown).

Table 1. Structural requirements of steroids for their action as inhibitors of succinate oxidation in metabolic state 3

Additions	Rate of respiration
None	142
progesterone	40
pregnanolone	40
pregnandiol	142
androstendione	142
testosterone	142
testosterone acetate	40
17α-hydroxyprogesterone	142
deoxycorticosterone	142
deoxycorticosterone acetate	40

Experimental conditions were as described in Fig. 1. All tested steroids were used at 100 μM concentration.

Rate of respiration is expressed as natoms 0/min/mg of mitochondrial protein.

Table 2. Comparison of the effects α-tocopherol and α-tocopherol acetate on inhibition of succinate oxidation in metabolic state 3

Additions	Rate of respiration
None	360
α-tocopherol, 100 μM	360
progesterone, 70 μM	160
α-tocopherol, 100 μM + progesterone, 70 μM	260
α-tocopherol acetate, 100 μM	360
α-tocopherol acetate, 100 μM + progesterone, 70 μM	200

Rate of respiration is expressed as natoms 0/min/3·1 mg of mitochondrial protein. Experimental conditions were as described in Fig. 1.

A comparison of the effect of α-tocopherol acetate and α-tocopherol on progesterone inhibition of succinate oxidation in metabolic state 3

Experiments were done to determine whether α-tocopherol acetate affects the inhibition of succinate oxidation in metabolic state by progesterone similarly to α-tocopherol. As may be seen from the data presented in Table 2, α-tocopherol acetate had a smaller effect on progesterone inhibition of succinate oxidation than α-tocopherol at the same concentration.

Lack of the effect of α-tocopherol on inhibition of succinate oxidation by protamine, DBI and oligomycin

In an attempt to check whether α-tocopherol is effective in reversing inhibition of oxidative phosphorylation caused by other compounds, we tested the effect of α-tocopherol on inhibition of succinate oxidation by protamine, DBI or oligomycin. Protamine and DBI have been chosen because there are many analogies between the action of these compounds and progesterone on succinate oxidation in metabolic state 3 [ref. 9, 14–17], oligomycin has been used as

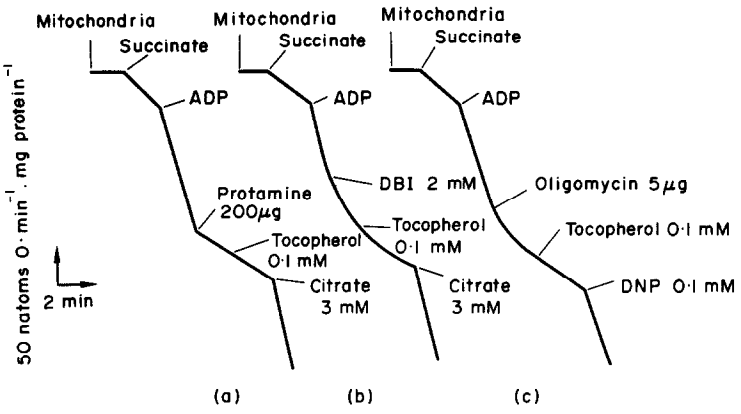

Fig. 5. Effect of α-tocopherol on inhibition of succinate oxidation by protamine, DBI or oligomycin. Experimental conditions as described in Materials and Methods.

a classical inhibitor of oxidative phosphorylation. Figure 5 shows that α-tocopherol at the concentration in which it reversed inhibition by progesterone of succinate oxidation in metabolic state 3, was without effect on inhibition of succinate oxidation by protamine or DBI. Citrate at the same concentration in which it reverses the inhibitory effect of progesterone [9] released completely the inhibitory effect of protamine and DBI (Fig. 5). α-Tocopherol did not reverse the oligomycin inhibition of succinate oxidation. The results presented above suggest that the action of α-tocopherol on inhibition of succinate oxidation by other compounds is not as effective as on the inhibition caused by progesterone and the steroids of related chemical structure.

Binding of progesterone to rat liver mitochondria. The effect of α-tocopherol

It may be seen from Fig. 6 that α-tocopherol slightly increased the binding capacity of progesterone to mitochondria under conditions in which progesterone inhibition of succinate oxidation was released by α-tocopherol. The binding of progesterone to rat liver mitochondria increased with the increasing concentration of this steroid in the incubation medium. It may be seen that in the concentration range of 25–100 μM progesterone binding to rat liver mitochondria was linear both in the absence and presence of 100 μM α-tocopherol.

Effect of α-tocopherol on progesterone inhibition of NADH oxidation by sonic particles

As indicated above, progesterone may affect oxidative phosphorylation by acting both on the respiratory chain and on the energy conserving mechanism. When succinate is used as substrate, inhibition of its oxidation is caused mainly by the action of progesterone on energy conserving processes. On the other hand, when β-hydroxybutyrate is the oxidized substrate inhibition of its oxidation by progesterone is caused both by the action of progesterone on the electron transport chain and on the energy conserving mechanisms. As shown on Fig. 3, inhibition of succinate oxidation by progesterone is reversed more efficiently than the inhibition of β-hydroxybutyrate at the same concentration of α-tocopherol. This is probably caused by a dual mechanism of progesterone action: (a) on the respiratory chain and (b) on the energy conserving processes. The effect of progesterone on the respiratory chain alone was studied by using nonphosphorylating sonic particles prepared from rat liver mitochondria. Figure 7 shows that progesterone inhibited NADH oxidation in sonic particles and the inhibition was released by α-tocopherol.

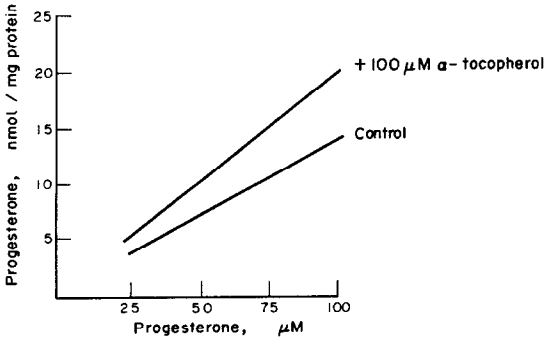

Fig. 6. Progesterone binding to rat liver mitochondria in the presence or absence of α-tocopherol. For experimental conditions see text.

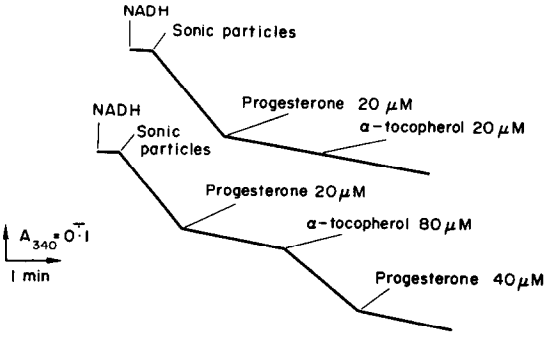

Fig. 7. Effect of α-tocopherol on progesterone inhibition of NADH oxidation in sonic particles. Experimental conditions as described in Materials and Methods.

Table 3. Effect of α-tocopherol on inhibition of β-hydroxybutyrate or succinate oxidation in rat liver mitochondria by different steroids, stilboestrol and typical inhibitors of the respiratory chain

Additions	β-hydroxybutyrate	succinate
progesterone, pregnanolone, testosterone acetate, deoxycorticosterone acetate	+	+
deoxycorticosterone, corticosterone cortisol, testosterone, oestradiol	−	
stilboestrol	−	−
rotenone	−	
antimycin A	−	−

Experimental conditions as described in Materials and Methods. All steroids tested were used at 100 μM concentration. (−) without effect, (+) inhibition reversed.

However, there are differences between the reversing effect of α-tocopherol on progesterone-inhibited substrate oxidation in intact rat liver mitochondria and NADH oxidation in sonic particles. Inhibition of substrate oxidation by progesterone in intact mitochondria was reversed by α-tocopherol at a concentration ratio progesterone: α-tocopherol of 1:1, at these condition inhibition of NADH oxidation by progesterone was not reversed by α-tocopherol in sonic particles. A higher concentration of α-tocopherol had to be used in order to achieve the release of inhibition (Fig. 7).

The effect of α-tocopherol on succinate or β-hydroxybutyrate oxidation inhibition by various steroids, stilboestrol and inhibitors of the respiratory chain

It has been shown previously that the oxidation of NAD-linked substrates may be inhibited by several steroids and related substances [1–7]. On the other hand, oxidative phosphorylation with succinate as substrate may be inhibited only by progesterone and deoxycorticosterone acetate as shown by Vallejos *et al.*[5] and Aleksandrowicz *et al.*[9]. In this paper we have demonstrated that pregnanolone and testosterone acetate affect mitochondrial respiration in metabolic state 3 with succinate as substrate in a way similar to progesterone. Table 3 shows the effect of α-tocopherol on the inhibition of succinate or β-hydroxybutyrate oxidation in metabolic state 3 by some steroids, stilboestrol and typical inhibitors of respiratory chain. It was found that α-tocopherol reversed the inhibitory effect of progesterone, pregnanolone, deoxycorticosterone acetate and testosterone acetate but not the inhibitory effect of other steroids examined, stilboestrol and typical inhibitors of the respiratory chain.

DISCUSSION

The results of the present investigation indicate that α-tocopherol reverses the inhibitory effect of progesterone both on the energy conserving mechanism and on the electron transport chain. The experiments described previously suggested that inhibition of oxidative phosphorylation by progesterone in rat liver mitochondria was exerted by interference with the uptake of protons into the mitochondrion [8–10]. This inhibition may be reversed by compounds specific for H^+ translocation into the mitochondrion *e.g.* classical uncoupling agents or some anions [8, 9]. As indicated above, α-tocopherol did not affect succinate oxidation in metabolic state 4. These experiments suggest that this vitamin does not act on mitochondrial respiration as an uncoupling agent. The results of experiments presented on Fig. 5 show that α-tocopherol did not affect inhibition of succinate oxidation in metabolic state 3 by protamine and DBI. Therefore, it may be assumed that α-tocopherol does not act on oxidative phosphorylation as a compound specific for H^+ translocation in the presence of progesterone or other inhibitors of H^+ movements across the mitochondrial membrane. It has been shown previously that inhibition of mitochondrial respiration by NEA [18] and progesterone [19] is reversed by bovine serum albumin. It is probably due to binding of steroids with albumin and in consequence to a decrease of the concentration of steroids in the mitochondrial membrane. The results of experiments presented on Fig. 6 show that α-tocopherol slightly increases binding of progesterone to rat liver mitochondria. These data suggest that α-tocopherol does not act on inhibition of oxidative phosphorylation by progesterone in a manner similar to albumin. From the comparisons on Table 1, it appears that the $CH_3—C(=O)$ system of progesterone is directly responsible for the inhibition of energy conserving mechanisms. The remaining hydrophobic part of progesterone molecule reacting with the mitochondrial membrane provides conditions in which the $CH_3—C(=O)$ group can display its inhibitory action. According to the above, it is a reasonable assumption that the substances which: (a) change the interaction between the hydrophobic part of progesterone molecule with mitochondrial membrane and (b) block the $CH_3—C(=O)$ system, would give a release of the inhibition of oxidative phosphorylation.

The chemical structures of progesterone and α-tocopherol provide possibilities for intermolecular binding of the following types: (a) hydrogen bonding between hydroxyl group of α-tocopherol and carbonyl groups of progesterone; (b) π-bonding between the aromatic ring of α-tocopherol and the αβ-unsaturated carbonyl system of progesterone; (c) hydrophobic bonding between the hydrocarbon nucleus of each of the two compounds. We have shown previously that an interaction takes place between progesterone and α-tocopherol in nonaqueous solution and that hydrogen bonding between the hydroxyl group of α-tocopherol and carbonyl groups of progesterone plays an important role in this interaction [20]. It is possible that hydrogen bonding between the hydroxyl group of α-tocopherol and carbonyl groups of progesterone in the mitochondrial membrane is formed. Therefore we proposed that the formation of a complex between progesterone and α-tocopherol is taking place in the mitochondrial membrane. One can suppose that oxi-

dative phosphorylation is less inhibited by a progesterone–tocopherol complex than by progesterone alone. Many features of the proposed mechanism are clearly speculative and refined physical methods will be needed to test the possibility of the complex formation between α-tocopherol and progesterone. Nevertheless, this model has proved useful in explaining why only the steroids which have the $CH_3—\overset{|}{C}=O$ system are able to inhibit oxidative phosphorylation by a direct action on energy conserving mechanisms, and why steroids which have a $CH_3—\overset{|}{C}=O$ system and a hydroxyl group near this system are without effect. It has been shown (Table 1) that 17α-hydroxyprogesterone, which differs from progesterone by the presence of a hydroxyl group at the C_{17} position, is without effect on oxidative phosphorylation with succinate as substrate. This fact can be explained by the assumption that between the hydroxyl group at the C_{17} position of 17α-hydroxyprogesterone and the carbonyl group at C_{20} position of this steroid intramolecular hydrogen bonding is formed.

Why deoxycorticosterone did not affect succinate oxidation in metabolic state 3 and deoxycorticosterone acetate inhibited it can be explained similarly. In the molecule of deoxycorticosterone, intramolecular hydrogen bonding between the carbonyl group at C_{20} position and hydroxyl group at C_{21} position can be formed. The proposed mechanism proved useful also in explaining why α-tocopherol reversed the inhibitory effect of progesterone and other steroids which act in a manner similar to progesterone on oxidative phosphorylation more strongly than α-tocopherol acetate (Table 2). These results suggest that a free hydroxyl group is essential for α-tocopherol action on mitochondrial respiration inhibited by progesterone. According to the proposed mechanism, the free hydroxyl group is required for hydrogen bonding formation with the carbonyl group of progesterone. Taking into account that tocopherol acetate also reversed the inhibitory effect of progesterone on succinate oxidation (significantly less, however, than tocopherol), we assume, independently of the above proposed mechanism, that tocopherol displays a partial reversal of the inhibitory effect of progesterone by changing the interaction between the hydrophobic part of the progesterone molecule and the arrangement of the mitochondrial membrane. As indicated above, α-tocopherol reversed also the inhibitory effect of progesterone on respiratory chain (Fig. 7). Taking into account that many steroids and related substances which have no $CH_3—\overset{|}{C}=O$ system are able to inhibit the electron transport, we assume that the mechanism of progesterone action at this level is different from progesterone action on energy conserving mechanisms. Probably the reversing mechanism of α-tocopherol is dependent on the change of the interaction between α-tocopherol, progesterone and the mitochondrial membrane.

Irrespective of the exact nature of the intermolecular interaction, the fact that the presence of α-tocopherol greatly weakens the effect of progesterone on oxidative phosphorylation, may well be important in the biological function of these compounds. The results presented do raise the interesting possibility that a direct molecular interaction between progesterone and α-tocopherol may determine at least in part their mode of physiological action.

Taking into account that very large concentrations of progesterone have been used in our experiments our results do not allow a firm conclusion that inhibition of electron transport and oxidative phosphorylation plays a primary role in the physiological action of progesterone. The high concentrations of steroids exhibit an undoubted effect on oxidative phosphorylation and electron transport chain; however we observed [9] that progesterone at concentration as low as 6 μM exerts also inhibitory effect on oxidative phosphorylation in mitochondria isolated from rat liver. The physiological concentration of hormonal steroids in tissues are not known precisely. Nevertheless one may suspect that in steroidogenic tissues *e.g.* in placenta where progesterone synthesis is taking place, the concentration of this hormone is sufficiently high to inhibit electron transport chain and oxidative phosphorylation. It is known that mitochondria isolated from full term placenta in the absence of BSA oxidize substrates more slowly and have lower phosphorylating ability than mitochondria isolated in the same manner in the presence of BSA [21]. It has been shown [18, 19] that BSA whose steroid binding properties are well known [22] releases also the inhibitory effect of steroids on oxidative phosphorylation in rat liver mitochondria. We suggest that rat liver mitochondria treated with progesterone are a good model of mitochondria from steroidogenic tissue. The results presented may explain partially the differences between the liver and steroidogenic tissue mitochondria as far as their respiration and oxidative phosphorylation is concerned.

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