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Effect of progesterone and its synthetic analogues on the activity of mitochondrial permeability transition pore in isolated rat liver mitochondria

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ABSTRACT

The influence of progesterone and its synthetic analogues on the induction of the Ca^{2+}-dependent mitochondrial permeability transition pore (MPTP) has been studied. The novel synthetic analogue of progesterone 17a-acetoxy-3b-butanoyloxy-6-methyl-pregna-4,6-diene-20-on (buterol) was compared with progesterone and medroxyprogesterone acetate (MPA). It was found that progesterone and buterol have opposite effects on the induction of MPTP opening by calcium ions. By contrast to progesterone, which decreased the calcium ion concentration necessary for pore opening, and MPA, which also, although at a lesser extent, activated the pore induction, buterol at a concentration of 20–100 μM blocked the pore opening and increased the calcium retention capacity of mitochondria more than twofold. The action of buterol is specific to the pore since it did not affect the respiration, whereas progesterone completely inhibited NAD-dependent respiration. MPA acted similar to progesterone but less effectively. The inhibitory effect of buterol was eliminated in the presence of carboxyatractyloside, which selectively binds the thiol groups of adenylate translocase and prevents the adenine nucleotide binding. These data indicate that buterol interacts with thiol groups, which explains its inhibitory effect not only on the mitochondrial pore but also on the transport system of xenobiotics in tumor cells in which buterol reduces the multidrug resistance.

1. Introduction

Mitochondria are actively involved in the response of cells to steroid hormones. They are a target of the signaling action of many steroid hormones, are implicated in their biosynthesis, and are subjected to the direct effect of some of them. Acting through receptors localized in the cytoplasm, as well as in nuclear, cytoplasmic, and, as it has been shown recently, mitochondrial membranes, steroid hormones affect some mitochondrial functions, such as respiration, oxidative phosphorylation, and production of reactive oxygen species (ROS) [1–4]. Steroid hormones are known to stimulate the biosynthesis of mitochondrial proteins and the components of the mitochondrial respiratory chain by activating the transcription of the corresponding genes [5–8]. Besides, the signaling by steroid hormones triggers the regulation of apoptosis through the expression or phosphorylation of pro- and antiapoptotic mitochondrial proteins involved in the regulation of the nonspecific pore whose opening is the key stage of cell death [9–12].

It is also known that some steroid hormones have a direct effect on mitochondria. These are dehydroepiandrosterone, estradiol and its derivatives, which inhibit complex I of the mitochondrial respiratory chain, as well as testosterone, which activates the ATP-dependent K^+-channel in the mitochondrial membrane [13–16]. The toxic effect of dehydroepiandrosterone used as a precursor of hormones in therapeutic purposes was explained by the inhibition of NAD-dependent respiration. There is evidence that the toxicity of this steroid manifests itself in the activation of lipid peroxidation and the induction of Ca^{2+}-dependent nonspecific pore in the inner mitochondrial membrane [17–19]. Other steroids, such as estradiol and its derivatives 2-methoxyestradiol and estrone, decrease the membrane potential, activate the production of ROS, and induce cell death [20,21]. On the other hand, estradiol can act as a scavenger of ROS, entering the redox cycle to form quinone [22–24]. It was also found that some steroid hormones, such as testosterone and progesterone, have a recoupling effect on

Abbreviations: ANT, adenine nucleotide translocase; BuOOH, *t*-butylhydroperoxide; Buterol, 17a-acetoxy-3b-butanoyloxy-6-methyl-pregna-4,6-diene-20-on; CAT, carboxyatractyloside; MPA, medroxyprogesterone acetate; MPTP, mitochondrial permeability transition pore; NEM, *N*-ethylmaleimide; $\Delta\Psi$, mitochondrial membrane potential.

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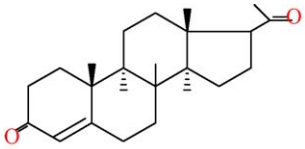
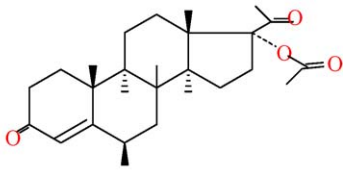
mitochondria uncoupled by low concentrations of protonophores [25,26]. This property is assumed to be due to the modification by these hormones of the structure of mitochondrial membranes. The above-mentioned effects of steroid hormones on mitochondrial functions were shown on isolated mitochondria of the liver, heart, and brain. The effective concentrations of these hormones in different experiments varied from 25 to 150 μM.

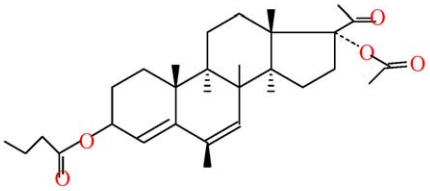
The goal of this study was to compare the effect of the newly synthesized progesterone analogue 17a-acetoxy-3b-butanoyloxy-6-methyl-pregna-4,6-diene-20-on (buterol) with the effects of progesterone and its known synthetic analogue medroxyprogesterone acetate (MPA) on the induction of the Ca^{2+}-dependent cyclosporine-sensitive pore in rat liver mitochondria.



Progesterone



Medroxyprogesterone acetate



Buterol

A distinguishing structural feature of buterol is the absence of the unsaturated ketone group in ring A, which should lead essentially to the pathway of metabolism different from that of progesterone and progesterone analogues with the unchanged ring A. One of the major metabolic transformations in the case of progesterone and MPA is a full or partial hydrogenation of this very group. This structural distinction of buterol also determines the complete absence of the androgenic activity typical for some synthetic progestins [27,28]. Along with hormone replacement therapy, progestins are widely used in oncology and as contraceptives [29–31]. Buterol exhibited a strong antifertile action in combination with ethynylestradiol or its 9α-hydroxy-11β-nitroxy analogue, and in the antitumor activity it was superior to MPA [27,28]. Buterol has a chemosensitizing effect, by increasing the cytotoxic effect of doxorubicin. However, the mechanism of the chemosensitizing effect of buterol is unclear. It is assumed that buterol, like other progestins, has an inhibitory effect on some carrier proteins, such as P glycoprotein and multidrug resistance-associated protein (MRP), which are overexpressed in multidrug resistance (MDR). The effect of these steroids on nonspecific membrane permeability has not been studied earlier. Here we found that buterol selectively inhibits the Ca^{2+}-dependent cyclosporin-sensitive pore in mitochondria, interacting with the ADP/carboxyatractyloside binding site of adenylate translocase, an essential component of the mitochondrial pore. The contribution of thiol groups to the inhibitory effect of buterol indicates some common features in the regulation of the mitochondrial pore and MDR system.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma–Aldrich (United States) and were of the highest purity available. Buterol was synthesized as described (Patent 2004119586, Russia).

2.2. Preparation of rat liver mitochondria

Mitochondria were isolated from adult male Wistar rats according to a standard differential centrifugation procedure [32]. Rats were killed by decapitation, and the liver was rapidly removed and homogenized in ice-cold isolation buffer containing 220 mM mannitol, 70 mM sucrose, 1 mM EGTA, and 10 mM HEPES–Tris (pH 7.4). The homogenate was centrifuged at 600 × *g* for 7 min at 4 °C, and the supernatant fraction was then centrifuged at 9000 × *g* for 10 min to obtain mitochondria. The mitochondria were washed twice in the above medium without EGTA and BSA. The final mitochondrial pellet was suspended in the washing medium to yield 60–80 mg protein/ml and kept on ice for analysis. The protein content was measured by the Biuret method with bovine serum albumin as a standard [33].

2.3. Measurements of the oxygen consumption rate and mitochondrial membrane potential in isolated mitochondria

The oxygen consumption by isolated rat liver mitochondria (RLM) was measured polarographically using a Clark oxygen electrode linked to a Record 4 computerized recording system (Russia) in a closed 1-ml chamber at 25 °C with continuous stirring. The effects of progesterone and its analogues on mitochondrial respiration were estimated by measuring the respiration rate in the presence of 2 mM ADP, which was added to initiate oxidative phosphorylation (V_3), or 1 μM FCCP (uncoupled respiration). The membrane potential on the inner mitochondrial membrane ($\Delta\Psi_M$) was determined from the distribution of the lipophilic cation tetraphenylphosphonium (TPP^+) whose concentration in incubation medium $[TPP^+]_{out}$ was recorded by a TPP^+-selective electrode [34]. RLM were incubated in a medium containing 120 mM KCl, 1.5 mM KH_2PO_4, 10 mM HEPES (pH 7.25), 4 mM substrate of oxidation, and 1 μM TPP^+. All measurements were carried out in a thermostated 1-ml cuvette at 25 °C under continuous stirring. The effects of steroids were examined in mitochondria oxidizing either pyruvate *plus* malate or glutamate *plus* malate (complex I substrates of the respiratory chain) or succinate in the presence of rotenone (complex II substrate of the respiratory chain). In some experiments, the mitochondrial respiration rates and changes in $\Delta\Psi$ were recorded simultaneously using a Record 4 computerized recording system (Russia).

2.4. Determination of permeability transition pore activity

The effects of progesterone and its analogues on the induction of mitochondrial permeability transition pore (MPTP) by calcium ions were determined by measuring the Ca^{2+} retention capacity or by monitoring the calcium-induced mitochondrial swelling as described previously [35,36]. The calcium retention capacity test measures the threshold Ca^{2+} concentration required to open the MPTP in a population of mitochondria in suspension [35,37] which makes it possible to quantitatively compare the effects of steroids and other reagents with control. The Ca^{2+} retention capacity was estimated from the Ca^{2+} concentration necessary for the irreversible decrease of the membrane potential in the course of successive additions of calcium ions at concentrations of 20–50 μM. Mitochondria were incubated in a medium containing 120 mM KCl, 10 mM HEPES, and 1.5 mM phosphate, pH 7.25. The concentration of TPP^+ in the cuvette was 1 μM, and the concentration of the mitochondrial protein was 1.2 mg/ml. All measurements were carried out in a thermostated 1-ml cuvette at 26 °C under continuous stirring. The influence of the steroids on calcium-induced mitochondrial swelling was estimated from changes in the rate and magnitude of swelling, which accompany the pore induction. Mitochondrial swelling was recorded as a decrease in absorbance at 540 nm (A_{540}) using a computer-controlled Specord UV–vis spectrophotometer.