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NORMAL VA QISQA MUDDATLI STRESS CHAQIRILGAN MITOXONDRIYANING NAFAS OLISH TEZLIGI VA OKSIDLANISHLI FOSFORLANISH JARAYONIGA ZONGORIN ALKALOIDINING TA'SIRI

Annotatsiya

Stress sharoitlari normal fiziologik funksiyalarga salbiy ta'sir ko'rsatishi tufayli hujayra disfunksiyalarining kelib chiqishiga va undagi molekular mexanizmlarning o'zgarishiga sabab bo'lmoqda. Hujayralarning ko'p funksiyali organoidi hisoblanuvchi mitoxondriyalar ATP sintezlashdan tashqari, hujayra fiziologiyasining turli sohalarida muhim rol o'ynaydi.

Biofaol moddalardan zongorin diterpenoid alkaloidi *in vitro* sharoitida normal va stress chaqirilgan mitoxondriyalarning nafas olish tezligi va oksidlanishli fosforlanish jarayonlariga faollashtiruvchi ta'sir ko'rsatdi.

Kalit so'zlar: zongorin, jigar mitoxondriyasi, nafas olish tezligi, oksidlanishli fosforlanish.

ВЛИЯНИЕ АЛКАЛОИДА ЗОНГОРИНА НА СКОРОСТЬ МИТОХОНДРИАЛЬНОГО ДЫХАНИЯ И ПРОЦЕССА ОКИСЛИТЕЛЬНОГО ФОСФОРИЛИРОВАНИЯ НОРМАЛЬНОГО И КРАТКОВРЕМЕННОГО СТРЕССА

Аннотация

В связи с тем, что стрессовые состояния отрицательно влияют на нормальные физиологические функции, они вызывают клеточные дисфункции и изменения молекулярных механизмов. Митохондрии, считающиеся многофункциональными органеллами клетки, играют важную роль в различных областях физиологии клетки, помимо синтеза АТФ.

Среди биологически активных веществ дитерпеноидный алкалоид зонгорин оказывает активирующее действие на скорость дыхания и на процессы окислительного фосфорилирования нормальных и стресс-индуцированных митохондрий *in vitro*.

Ключевые слова: зонгорин, митохондрии печени, частота дыхания, окислительное фосфорилирование.

THE EFFECT OF ALKALOID SONGORINE ON MITOCHONDRIAL RESPIRATORY RATE AND OXIDATIVE PHOSPHORYLATION PROCESS UNDER NORMAL AND SHORT-TERM STRESS

Annotation

Stress conditions adversely affect normal physiological functions, resulting in cellular dysfunctions and alterations in molecular mechanisms. Mitochondria, multifunctional organelles within cells, play crucial roles across various aspects of cell physiology, beyond their role in ATP synthesis.

Among the biologically active substances, the diterpene alkaloid songorine has an activating effect on the rate of respiration and on the processes of oxidative phosphorylation of normal and stress-induced mitochondria *in vitro*.

Key words: songorine, liver mitochondria, respiratory rate, oxidative phosphorylation.

Introduction. The acceleration of technological development in the 21st century and sudden changes in the environment, influenced by human activities, are causing several negative consequences, such as air pollution, global warming, and noise. These factors contribute to stress, which has become an integral part of people's lives. Chronic stress conditions can lead to various diseases due to their negative impact on normal physiological functions. Nowadays, cardiovascular diseases, diabetes, and neurodegenerative disorders, which are the main causes of disability and death globally, are attributed to the adverse effects of chronic stress. These conditions arise from the disruption of normal physiological processes, including various physicochemical and biological processes in cells. The origin of cell dysfunctions is directly linked to changes in their molecular mechanisms. Particularly, mitochondria, known as the "energy generators" of cells, play a crucial role in cell physiology, including the transport of electrons generated in the Krebs cycle through the respiratory chain and the synthesis of ATP via oxidative phosphorylation (OP).

Currently, scientific research is underway to identify biologically active substances from indigenous plants and to develop effective medicines based on them, while elucidating their mechanisms of action. Diterpene alkaloids are gaining importance as one of the classes of promising substances for creating new pharmacological drugs. Various physiological activities of diterpenoid alkaloids have been identified in the studies conducted to date, including antiarrhythmic, antispasmodic, regenerative, antimetastatic, anti-inflammatory, antihypoxic, antidepressant, antipyretic, and antioxidant properties [2-7].

The aim of the study is to determine the effect of the songorine diterpenoid alkaloid on oxidative phosphorylation processes in rat liver mitochondria in vitro.

Material and methods

The diterpenoid alkaloid songorine ($C_{22}H_{33}O_3$) extracted from the *Aconitum monticola* plant, which belongs to the *Aconitum L*, was used in the research (Fig. 1) [8]. The structural formulas of alkaloid was drawn by the ChemOffice 2002, Chem Draw Ultra 7.0 software (Fig. 1).

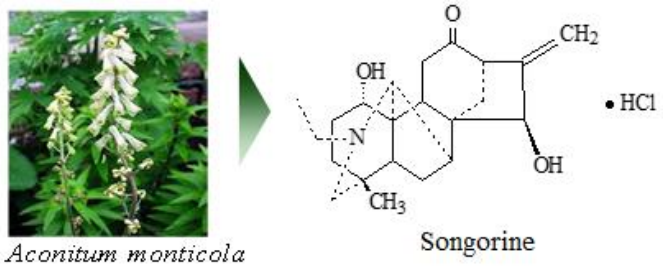

Figure 1. Structural formulas of songorine

The mitochondrial respiration rate and oxidative phosphorylation (OP) process were determined using a Clark-type MitoCell MT200 respirometer (Strathkelvin Instruments, Scotland).

The medium contained (in mM): KCl - 120, Tris-HCl - 10, KH_2PO_4 - 1, EGTA - 1, along with respiratory substrates (for complex I) glutamate - 5, malate - 5, with a pH of 7.1. The oxygen content in 0.5 ml of the incubation medium was 250 ng atoms. All experiments were conducted at 25°C with constant agitation, and mitochondria were added at a concentration of 1 mg/ml protein. Mitochondrial protein was determined using the Peterson modification of Lowry's method [9]. The obtained results were statistically processed using Origin 8.6 computer software (USA). In this, $p < 0.05$, $p < 0.01$, and $p < 0.001$ values represent statistical reliability.

Results. To determine the effect of diterpene alkaloids on respiration and oxidative phosphorylation processes in mitochondria, glutamate-malate, the complex I substrates of the respiratory chain, were used. At the initial stage of the experiments, the effect of the alkaloid songorine on the respiration rate of mitochondria and the oxidative phosphorylation process was studied. Case V_2 was taken as 100.0% as the control (Figure 2). Concentrations of the alkaloid songorine at 50, 75, and 100 μM reliably increased the rate of mitochondrial respiration in the V_2 state by up to 10%. In the V_3 metabolic state, activated by the addition of 200 μM ADP to the solution, the respiratory rate V_2 was increased approximately fivefold (480.0±8.3%) compared to the control state. The effect of the alkaloid songorine on the V_3 state of respiration was further investigated. At concentrations of the alkaloid songorine at 50, 75, and 100 μM, the respiration rate of mitochondria in the V_3 state was observed to be activated by 23%, 22.3%, and 22%, respectively, compared to the control. However, increasing the concentration of this alkaloid in the solution did not alter the rate of respiration.

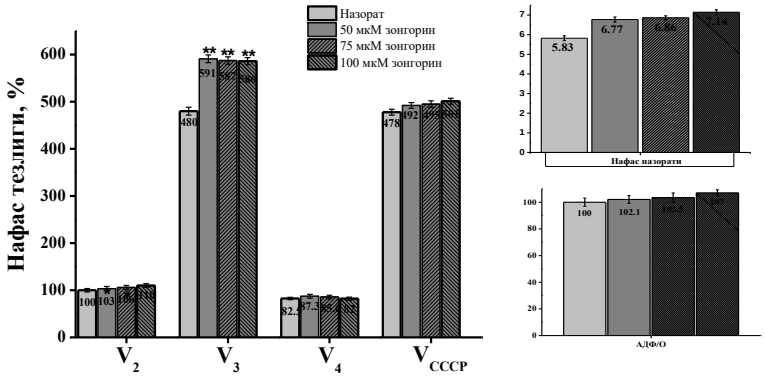

Figure 2: Effects of songorine on rat liver mitochondrial respiration and oxidative phosphorylation.

Medium containing (in mM): KCl - 120, Tris-HCl - 10, KH_2PO_4 - 1, EGTA - 1, glutamate - 5, malate - 5, pH 7.1; mitochondrial protein 1 mg/ml. n=10 for control, n=6 for all concentrations of songorine.** - R<0.01.

It is known that various factors affecting the normal activity of mitochondria, such as temperature and environmental pH, can lead to an increase in the lipid peroxidation process and disturbance in mitochondrial respiration. Subsequent in vitro studies identified one such factor: the effect of diterpene alkaloids on temperature-induced mitochondrial respiration. To investigate this, the suspension of liver mitochondria was incubated in a water bath at a temperature of 30°C for 10 minutes. It was then kept on ice and used throughout the experiments. Initially, mitochondria maintained at this temperature of 30°C were compared to controls for all states of respiration in mitochondria maintained under normal conditions. In short-term stressed mitochondria, the respiration rate decreased by 32% in the V_2 state compared to normal mitochondria in the V_2 state control. The mitochondrial respiration rate was also reduced by 29% in the V_3 state compared to the normal mitochondrial V_3 state. Temperature-induced mitochondrial respiration decreased by 14% compared to the control in the V_4 condition and by 37% in V_{CCCP} compared to its control (Figure 3). Short-term stress resulted in a decrease in respiratory rate and oxidative phosphorylation (ADP/O) ratio. In this scenario, a concentration of 50 μM of the alkaloid songorine led to an unreliable activation of the V_2 state by 9% compared to the control. At concentrations of 50 and 100 μM of this alkaloid, the V_3 state was accelerated by 23% and 16.3%, respectively, compared to the control. The above concentrations increased V_4 by 10% and 6% compared to the control. V_{CCCP} was accelerated by 50% and 53% compared to the control (Figure 3). The alkaloid songorine reliably accelerated the V_2 and V_3 metabolic states of mitochondrial respiration, the RC (respiratory control) and the ADP/O ratio compared to the control. It was observed that songorine increases the RC and the ADP/O ratio under short-term stress conditions.

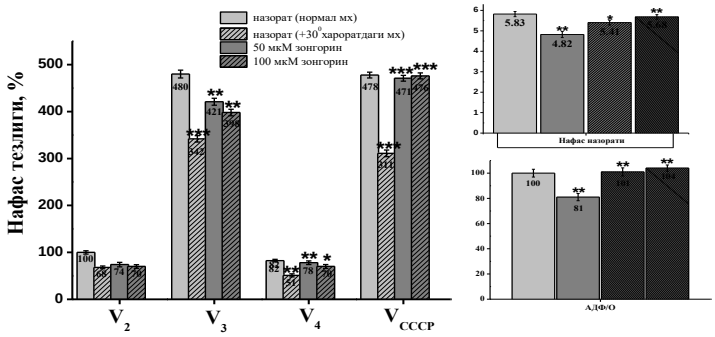

Figure 3: Effects of songorine on short-term stress-induced mitochondrial respiration and oxidative phosphorylation.

(The solution is presented in Fig. 2. Mitochondria were isolated from rat liver. n=8 for control, n=5 for all concentrations of songorine. * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$)

Thus, it was found that the alkaloid songorine has a certain activating effect on the respiration rate and oxidative phosphorylation processes of normal and stressed mitochondria. "In vivo" studies are required to determine the occurrence of changes or adaptation mechanisms in the body under stress.

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