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PARTICIPATION OF K_{ATP}-CHANNELS OF PLASMA MITOCHONDRIAL MEMBRANES IN THE REGULATION OF MECHANOKINETICS OF RAT MYOMETRIUM SPONTANEOUS CONTRACTION

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Background. ATP-sensitive K^+ channels of the plasma membrane in the smooth muscles of the uterus are one of the most significant ion channels that regulate the excitability of this tissue both in the non-pregnant state and during pregnancy. MitoK_{ATP}-channels ensure regulation of the bioenergetic state of mitochondria (the intensity of mitochondrial respiration and the potential of the inner mitochondrial membrane). Thus far, there is no information on the participation of both types of these channels in the regulation of the mechanokinetics of spontaneous contractions, therefore the aim of this work was to perform a complete mechanokinetic analysis of spontaneous contractions of rat myometrium under conditions of blocking and activation of ATP-sensitive K^+-channels of the plasma membrane and blocking of mitoK_{ATP}-channels.

Materials and Methods. Experiments were performed on female Wistar rats. The spontaneous activity of smooth muscle stripes of longitudinal smooth muscles of uterine horns was registered by the tenzometric method in the isometric mode. The experiments used the activator of ATP-sensitive K^+-channels of the plasma membrane diazoxide (50, 100, 150 and 200 μM) and the blocker of these channels glibenclamide (1, 2, 4, 6, 8 and 10 μM), as well as the blocker of ATP-sensitive mitochondrial K^+-channels 5-hydroxydecanoate (5-HD, 50 μM). The study of mechanokinetics of the contraction-relaxation process of muscle preparations was carried out according to the complete mechanokinetic analysis method with the calculation of the mechanokinetic parameters of the contraction-relaxation cycle: force (F_{max}, $F_{C,}$ and F_R), time (τ_0, $\tau_{C,}$ and τ_R), impulse (I_{max}, $I_{C,}$ and I_R) and velocity parameters (V_C and V_R).

Results. It was found that both blocking and activation of plasma membrane K_{ATP}-channels cause suppression of the amplitude, probably according to different cellular mechanisms of regulation of ion conductivity. Under the influence of glibenclamide, a significant decrease in the frequency and mechanokinetic parameters of spontaneous contractions of the myometrium was observed, which confirms the contribution of plasma membrane K_{ATP}-channels to maintaining the excitability of the myometrium of non-pregnant rats. Activation of plasma membrane K_{ATP}-channels by diazoxide caused a change in individual mechanokinetic parameters of spontaneous contractions of the myometrium. Under the action of the mitoK_{ATP}-channel blocker 5-HD, suppression of the amplitude and modulation of the mechanokinetic parameters of the contraction phase was observed without changes in the kinetics of the relaxation phase of spontaneous contractions.

Conclusions. Thus, modulation of the K_{ATP}-channels of the plasma membrane and mitochondria is accompanied by the suppression of spontaneous contractions of the myometrium. Both types of K_{ATP}-channels are important regulators of myometrial excitability, however, unlike plasma membrane K_{ATP}-channels, mitoK_{ATP}-channels probably do not modulate the processes of extrusion of Ca^{2+} from the cytosol.

Keywords: myometrium, K_{ATP}-channels of the plasma membrane and mitochondria, spontaneous contractions, mechanokinetic analysis

INTRODUCTION

K^+-channels play a major role in the excitable tissues, defining the excitation level for the plasma membrane of their cells. This group of ion channels has the greatest molecular and structural diversity, a highly variable regulation and biophysical properties.

In the tissue of phasic smooth muscles, K^+-channels play a key role, regulating the membrane potential of myocytes and thus the tone and contractile activity (Aaronson *et al.*, 2006; Sikimic *et al.*, 2019; Bränström *et al.*, 2022). The main representatives of K^+-channels in uterine smooth muscles (SM) are potential-governed K^+-channels (K_V), Ca^{2+}-activated K^+-channels (BK_{Ca} and SK_{Ca}), and ATP-sensitive K^+-channels (Lorca *et al.*, 2014; Tsymbalyuk *et al.*, 2019; Na et al., 2017; Tinker *et al.*, 2018; Lee *et al.*, 2020). Up to now, the effects of K_V, $BK_{Ca,}$ and SK_{Ca} channel blockers on the spontaneous contractile activity of the myometrium of rats under different functional states (non-pregnant and different terms of pregnancy) have been studied in detail (Villar *et al.*, 1986; Zhao & MacKinnon, 2021). Also, the regulatory effects of ATP-sensitive K^+-channels on the contractile activity of rat myometrium induced by the uterotonic hormone oxytocin are known (Yoshitake *et al.*, 1991). However, there is no information about the involvement of these channels in the regulation of the mechanokinetics of spontaneous contractions of the myometrium of rats under blocking and activation of ATP-sensitive K^+-channels of the plasma membrane (using glibenclamide and diazoxide, respectively).

K_{ATP}-channels of the plasma membrane are activated by the decrease (and inhibited by the increase) in the intracellular concentration of ATP and/or the increase in ADP level (Teramoto, 2006). Both types of K_{ATP}-channels are highly relevant to the regulation of the functional activity of myometrium tissues, especially to maintain the non-excited state of myocytes in pregnancy dynamics (Khan *et al.*, 2001; Wray & Arrowsmith, 2021). K_{ATP}-channels of the plasma membrane mediate the effect of neurotransmitters: in the case of inhibition transmitters, the activation of these channels and the general tocolytic effect can be observed (Lovasz *et al.*, 2015; Tsymbalyuk, 2018); in the case of excitation

transmitters, the inhibition of the conductivity of K_{ATP}-channels and depolarization of smooth muscle cells occurs (Quayle *et al.*, 1997). MitoK_{ATP}-channels ensure the regulation of the bioenergetic state of mitochondria (in particular, the intensity of mitochondrial breathing and the potential of the inner mitochondrial membrane). They also regulate the rate of the formation of reactive oxygen intermediaries and produce a cytoprotective effect under pathological conditions (Hu *et al.*, 2017; Vadzyuk & Kosterin, 2018; Paggio *et al.*, 2019; Tatalović *et al.*, 2021).

Glibenclamide is a non-toxic compound, which is widely used for the treatment of non-insulin dependent diabetes mellitus; it is known (Löffler-Walz & Quast, 1998) that the index of half-maximal inhibition (IC_{50}) of the contractile activity detected on rat aorta smooth muscle preparations is 0.44 μM. Diazoxide is also a therapeutic agent for the management of hypoglycemia; the index of half-maximal activation of contractile activity (EC_{50}) registered on smooth muscle preparations of the rat colon is 34.2 μM (Plujà *et al.*, 1998). The IC_{50} for compound 5-HD was determined for ionic currents of isolated rat ventricular cardiomyocytes, where it is about 30 μM (Li *et al.*, 2010).

Our previous study reported the role of K_{ATP}-channels of the plasma membrane and mitoK_{ATP}-channels in the formation of the contractile reaction of the myometrium of rats on the uterotonic hormone, oxytocin (Tsymbalyuk & Vadzyuk, 2020).

As there is no information on the involvement of K_{ATP}-channels in the regulation of the mechanokinetics of spontaneous contractions of uterine SM, the aim of this work was to conduct the full mechanokinetic analysis of spontaneous contractions in the myometrium of rats under blocking of ATP-sensitive K^+-channels of plasma membranes and mitochondria (using glibenclamide and 5-hydroxydecanoate (5-HD), respectively), and under the activation of ATP-sensitive K^+-channels (using diazoxide).

MATERIALS AND METHODS

The experiments were conducted using Wistar line female rats (the average weight of animals was 200–250 g). The rats were kept in standard vivarium conditions (room temperature of 20±2 °C, relative humidity – 50–70 %, light-darkness cycle – 12:12 h). All the manipulations with animals were conducted according to the International Convention for the Protection of Animals and the Law of Ukraine “On Protection of Animals from Cruelty” (the Minutes of the meeting of bioethics commission of Education and Scientific Center Institute of Biology and Medicine No. 3 dated May 2, 2019). The animals were killed under ether anesthesia.

Functional tenzometric experiments. The spontaneous activity of smooth muscle stripes of longitudinal smooth muscles of uterine horns was registered by the tenzometric method in the isometric mode. Muscle stripes (the average size – 2×10 mm) were placed into a working chamber (the volume of 2 mL) with Krebs solution (the flow rate of 5 mL/min), and thermostated at 37 °C. The preparation was passively tensed at the rate of 10 mN and left for one hour (until achieving the stable amplitude and frequency of spontaneous contractions). The signals were registered with an analogue-to-digital transformer.

The Krebs solution was of the following composition (mM): 120.4 NaCl; 5.9 KCl; 15.5 $NaHCO_3$; 1.2 NaH_2PO_4; 1.2 $MgCl_2$; 2.5 $CaCl_2$; 11.5 glucose; pH of the solution was 7.4. The activator of ATP-sensitive K^+-channels of the plasma membrane, diazoxide (concentrations of 50, 100, 150, and 200 μM; Sigma, USA), the blocking agent for these channels, glibenclamide (concentrations of 1, 2, 4, 6, 8 and 10 μM; Sigma, USA) and

the blocking agent for ATP-sensitive K^+-channels of mitochondria, 5-hydroxydecanoate (5-HD, concentrations of 50 mM; Sigma, USA) were used in the experiment.

Stock solutions of diazoxide and glibenclamide were prepared by preliminary dissolution of substances in an organic solvent, dimethyl sulfoxide (DMSO), and added to Krebs solution to obtain the final DMSO aliquot of 0.1 % from the total volume of Krebs solution. All the other contractions, including the control ones, were studied in the solutions, containing 0.1 % DMSO. The stock solution of 5-HD was prepared by preliminary dissolution of this substance in distilled water.

The non-selective blocking of ATP-sensitive K^+-channels was done by preliminary incubation of myometrium preparations with glibenclamide and their activation – with diazoxide (in both cases, the preliminary incubation lasted for 20 min). ATP-sensitive K^+-channels of mitochondrial membranes were blocked by preliminary incubation of muscle preparations with 5-HD for 20 min. After the preliminary incubation of the preparations with the mentioned substances, the spontaneous contractions were registered with a subsequent kinetic analysis.

Comprehensive mechanokinetic analysis of spontaneous contractions. The mechanokinetics of contraction-relaxation of the muscle preparations was studied according to the method described in (Kosterin *et al.*, 2021). The contraction phase was defined as a fragment of a contractile response from the beginning of force variation up to its maximal value (F_{max}, the amplitude of the phase contraction). The relaxation phase started with the maximum phase contraction and lasted until the force decreased to its basal level.

The linearization of some contraction-relaxation cycles was done in the coordinates $\left[\ln\left(\frac{F_R}{F_C}\right); \ln\left(1+\frac{\Delta t}{t}\right)\right]$, where F and t – are instant values of force and time at the level of the contraction cycle (here C is the indication of the contraction phase; R indicates the relaxation phase); F_C – the value of the force at the inflexion point of the contraction phase (the maximal velocity V_C is observed here) and it has the corresponding time τ_C; F_R – the value of the force at the inflexion point of the relaxation phase (here the maximal velocity V_R is observed) and it has the corresponding time τ_R; Δt – constant time interval, chosen at random. The linearization charts were used to determine the characteristic constants k and n, which were further used to calculate the mechanokinetic parameters of the contraction-relaxation cycle: force (F_{max}, $F_{C,}$ and F_R), time (τ_0, $\tau_{C,}$ and τ_R), impulse (I_{max}, $I_{C,}$ and I_R) and velocity parameters (V_C and V_R) (Kosterin *et al.*, 2021).

Statistical analysis. The data were processed by variation statistics methods using Origin2018 program. The samples were checked in terms of belonging to normally distributed general populations according to the Shapiro-Wilk's test. The parametrical one-way analysis of variance (one-way ANOVA) was used to determine reliable differences between the mean values of samplings; a post-hoc analysis was made using the Tukey test.

The validation analysis of data approximation by the linear function was performed using Fisher's F-test; determination coefficients (R^2) were at least 0.98 for the linearized charts.

In all cases, the results were considered reliable on the condition of the probability value p under 5 % ($p < 0.05$). The results were presented as mean ± standard error of mean, n – number of experiments.

RESULTS AND DISCUSSION

The mechanokinetics of spontaneous contractions of the myometrium in case of blocking K_{ATP}-channels of the plasma membrane. In the first stage of the investigation, we studied the spontaneous contractile activity of the myometrium against the cumulative increase in the concentrations of glibenclamide, the blocking agent of K_{ATP}-channels (1, 2, 4, 6, 8, and 10 μM); separate spontaneous contractions were analyzed 20 min after the impact of each concentration of the substance started. It was found that in the entire range of the investigated concentrations, glibenclamide caused dose-dependent inhibition of the amplitude of spontaneous contractions (**Fig. 1**), which, against the background of the highest applied concentration, on average, amounted to 51.3 % compared to the control, accepted as 100 % (n = 6, p<0.001). The frequency of contractions decreased considerably under these conditions as well.

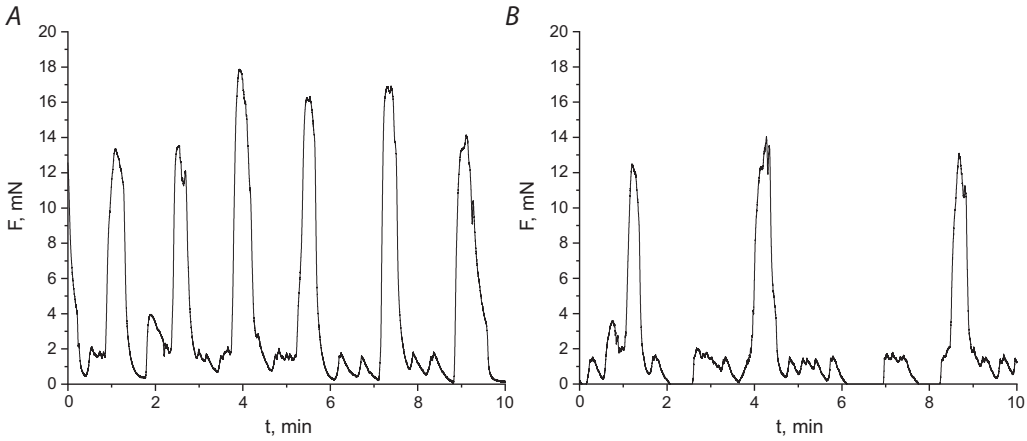

Fig. 1. The spontaneous contractive activity of the myometrium of rats in the control (***A***) and under a prior 20-minute-long incubation with the blocking agent of K_{ATP}-channels of the plasma membrane – glibenclamide (6 μM) (***B***). Typical mechanograms are presented

A complete mechanokinetic analysis of specific spontaneous contractions was further conducted. According to the method (Kosterin *et al.*, 2021), the force (F_{max}, $F_{C,}$ and F_R), time (τ_0, $\tau_{C,}$ and τ_R), impulse (I_{max}, $I_{C,}$ and I_R), and velocity parameters (V_C і V_R) were calculated.

It was determined that glibenclamide caused a decrease in some force parameters (**Fig. 2*A***). For instance, although all of the applied concentrations of this blocking agent of K_{ATP}-channels caused a reliable decrease in F_{max}, the inhibition of F_C was observed only under its impact in the concentrations of 8 and 10 μM. As for parameter F_R, its reduction was registered along the entire range of the applied concentrations of glibenclamide, but there was virtually no dependence on the dose.

In the case of time parameters of spontaneous contractions of the myometrium (τ_0, τ_C, and τ_R), there was a reliable decrease against the highest investigated concentration of glibenclamide (10 μM). The value of the characteristic time of the maximal force of spontaneous contractions τ_0 against the blocking agent (1–8 μM) was on the control level. In both cases, a significant decrease in τ_C, and τ_R parameters was observed only in the presence of high concentrations of glibenclamide (8 and 10 μM) (**Fig. 2*B***).

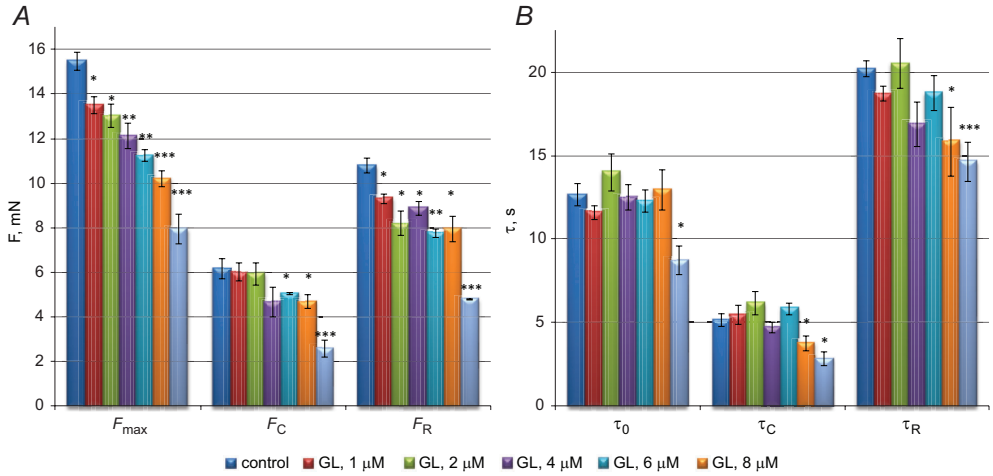

Fig. 2. The force (***A***) and force (***B***) parameters of spontaneous contractile activity of the myometrium of rats in control and under a prior incubation with glibenclamide (GL), the blocking agent of K_{ATP}-channels of the plasma membrane (1, 2, 4, 6, 8, and 10 μM). The relevant indices of spontaneous activity in control (n = 6, * – p<0.05, ** – p<0.01, and *** – p<0.001 – the difference is reliable regarding the control) were accepted as 100 %

The values of velocity and impulse of the force depend directly on the values of the force (*F*) and time (*t*), in particular, in the inflexion points of mechanograms, thus further on, we calculated the force parameters (V_C and V_R). It was determined that the maximal velocity of the contraction phase V_C considerably decreased depending on the dose in the presence of glibenclamide in the concentrations of 2–10 μM in the solution used for washing smooth muscle preparations. The maximal velocity of the relaxation phase V_R demonstrated no dependence on the dose either; it reliably decreased under the action of 8 and 10 μM of glibenclamide (**Fig. 3*A***).

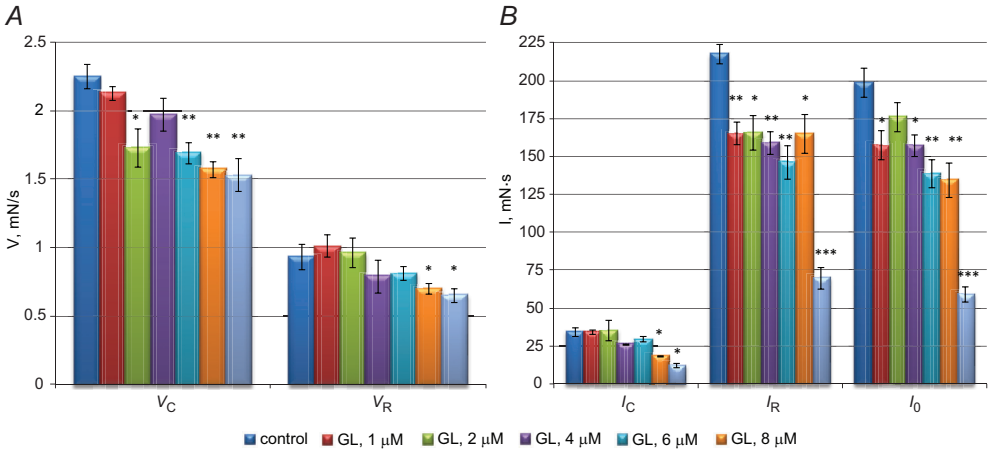

Fig. 3. The velocity (***A***) and impulse (***B***) parameters of spontaneous contractile activity of the myometrium of rats in control and under a prior incubation with glibenclamide, the blocking agent of K_{ATP}-channels of the plasma membrane (1, 2, 4, 6, 8, and 10 μM). The relevant indices of spontaneous activity in control (n = 6, * – p<0.05 and ** – p<0.001 – the difference is reliable regarding the control) were accepted as 100 %

The most significant changes among the calculated mechanokinetic parameters were observed for impulse (I_{max}, $I_{C,}$ and I_R) parameters of the contractile reactions of the myometrium (**Fig. 3*B***). For instance, in the presence of 10 μM of glibenclamide, this value for the amplitude of contractions was on average 30.3 % compared to the control ($n = 6$, $p<0.001$); in case of I_C and I_R the relative values were 23.0 % and 32.2 %, respectively ($n = 6$, $p<0.001$ in both cases).

Reliable changes of I_{max} were observed under the impact of 4–10 μM of glibenclamide, and in case of the force impulse in the inflexion points I_C and I_R, – under all the applied concentrations of this blocking agent.

Our results regarding the inhibition of the contractile activity of the myometrium of rats using glibenclamide are in agreement with the data of other investigators (Villar *et al*., 1986a, 1986b; Yoshitake *et al*., 1991; D'Ocón *et al*., 1986).

The obtained results suggest that the mechanisms for the blocking of K_{ATP}-channels of the plasma membrane by glibenclamide cause amplitude inhibition, a decrease in the frequency and modulation of mechanokinetic parameters of spontaneous contractions in the myometrium. When K^+-channels are constitutively open, their blocking should cause an increase in the membrane potential of the plasma membrane (depolarization). The depolarization of the plasma membrane can activate the potential-governed ion channels, including Ca^{2+}-channels. In this case, we could have expected to register the activation of the spontaneous activity, whereas we observed the opposite effect. Actually, as per the results of K. Yoshitake *et al*. (1991), the application of glibenclamide to the smooth muscle preparations of the aorta was accompanied by a decrease in the intracellular concentration of Ca^{2+} and inhibition of the contractions induced by the hyperkalemic solution. Also, the studies by A. Villar *et al.* (1986a, 1986b) demonstrated that sulfonylurea derivatives, tolbutamid and glibenclamide, the blocking agents of K_{ATP}-channels of the plasma membrane, induce some decrease in spontaneous contractions and considerably inhibit the coupling of myometrium contractions, induced by different means (by oxytocin, acetylcholine, hyperkalemic solution); these effects are also accompanied by a decrease in the concentration of ionized Ca in the cytoplasm of myocytes. Considering the results of additional experiments, both research groups (Villar *et al*., 1986a, 1986b; Yoshitake *et al*., 1991; D'Ocón *et al*., 1986) come to the conclusion that the abovementioned effects are caused by an increased sequestration of Ca^{2+} ions to the intracellular depots.

Among the parameters evaluated in this study, the processes of energy-dependent decrease in the cytosolic concentration of Ca^{2+} are indicated by the velocity of the relaxation phase. In this case, although the absolute values of V_R decreased in a considerable and dose-dependent manner (**Fig. 3*A***), and this decrease was more rapid as compared to the amplitude of F_{max} (**Fig. 2*A***), the normalization of V_R on F_{max} had a dose-independent reliable increase in the relative velocity of relaxation on average up to 121.2 % compared to the control. At the same time, the normalized maximal velocity of the contraction phase reliably changed only in the case of the maximal applied concentration of glibenclamide of 10 μM (so, it is possible that, in general, the energy-dependent processes of the increase in the cytosol concentration of Ca^{2+} are insensitive to the effect of this substance).

Thus, we can presume that under the blocking of K_{ATP}-channels of the plasma membrane by glibenclamide the inhibition of the spontaneous contractions in the myometrium is related to the activation of the processes of the energy-dependent decrease in the concentration of Ca^{2+} ions in the cytosol of smooth muscle cells.

The mechanokinetics of spontaneous contractions of the myometrium under the activation of K_{ATP}-channels of the plasma membrane. On the background of diazoxide, the activator of ATP of K_{ATP}-channels (the applied concentrations of 50, 100, 150, and 200 μM), there was a decrease in the amplitude of spontaneous contractions of the myometrium (**Fig. 4**), and the concentrations over 100 μM caused a complete inhibition of the spontaneous contractile activity. Thus, further analysis of the mechanokinetics of spontaneous contractions was conducted for the modulations of the myometrium using diazoxide in concentrations of 50 and 100 μM.

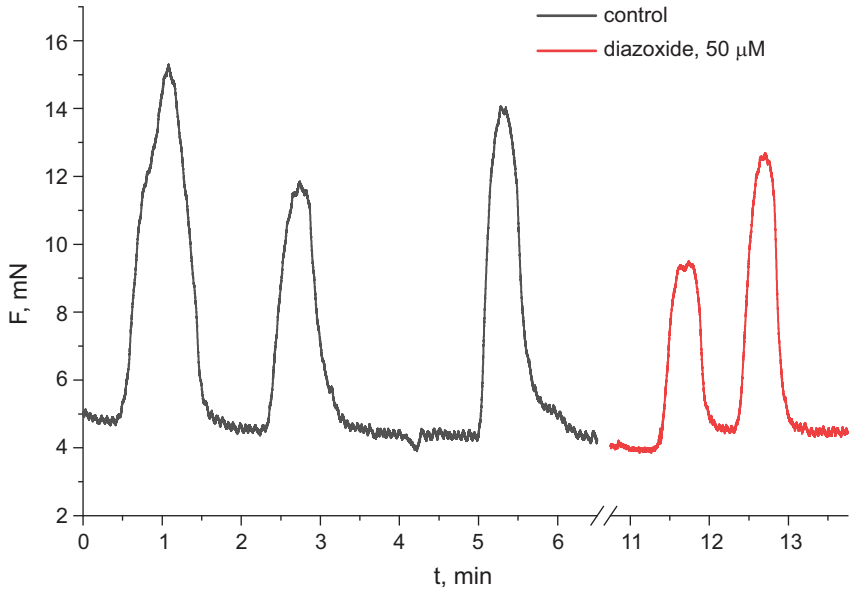

Fig. 4. The spontaneous contractile activity of the myometrium of rats in control and under a prior incubation with diazoxide, the activator of K_{ATP}-channels of the plasma membrane (50 μM). Typical mechanograms are presented

On the background of diazoxide, there was a considerable decrease in the force parameters (F_{max}, $F_{C,}$ and F_R) of spontaneous contractions (**Fig. 5*A***). Actually, similar inhibition was observed for F_{max} and F_C, while the force value at which the velocity of the relaxation phase was maximal, changed only under the impact of 100 μM.

On the background of the activation of K_{ATP}-channels using diazoxide, there were changes only in some temporal parameters which characterize the amplitude and phase of relaxation (τ_0 and τ_R), while the characteristic time for the relaxation phase τ_C remained at the control level (**Fig. 5*B***).

Besides, under the effect of diazoxide, there was a decrease in the values of impulse parameters of the amplitude (I_{max}) and the phase of relaxation (I_R), whereas the relevant impulse parameters of the contraction phase were at the control level (**Fig. 6*A***).

We did not observe reliable differences in the velocity parameters of spontaneous contractions in the myometrium under the effect of diazoxide compared with control values (**Fig. 6*B***).

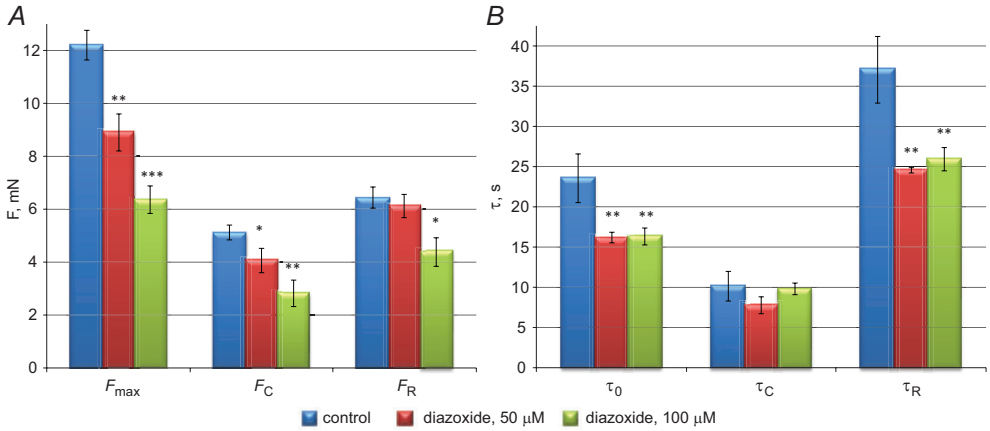

Fig. 5. The force (***A***) and time (***B***) parameters of spontaneous contractile activity of the myometrium of rats in control and under a prior incubation with diazoxide, the activator of K_{ATP}-channels of the plasma membrane (50 and 100 μM). The relevant indices of the spontaneous activity in control (n = 6, * – p<0.05, ** – p<0.01, and *** – p<0.001 – the difference is reliable regarding the control) were accepted as 100 %

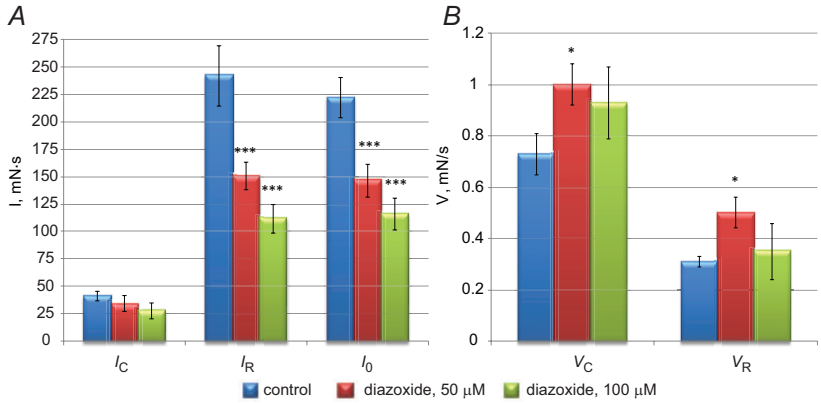

Fig. 6. The impulse (***A***) and velocity (***B***) parameters of spontaneous contractions in the myometrium of rats in control and under a prior incubation with diazoxide, the activator of K_{ATP}-channels of the plasma membrane (50 and 10 μM). The relevant indices of spontaneous activity in control (n = 6, * – p<0.05 and *** – p<0.001 – the difference is reliable regarding the control) were accepted as 100 %

Based on the above, we can suppose which molecular mechanisms lie in the foundation of the changes in spontaneous contractions of the myometrium in case of the activation of K_{ATP}-channels of the plasma membrane. In general, the activation of K^+-channels leads to the hyperpolarization of the plasma membrane (the membrane potential shifts to the value close to the equilibrium potassium potential) and the relaxation of myocytes, including the myometrium (Cheuk *et al*., 1993; Morrison *et al*., 1993; Piper *et al*., 1990; Sawada *et al*., 2005; Wray & Arrowsmith, 2021). In the case of K_{ATP}-channels, their expression increases considerably during pregnancy, and the activating preparations are used during the hypertonicity of the uterus to prevent early delivery (Cheuk *et al*., 1993; Morrison *et al*., 1993; Piper *et al*., 1990). Thus, the inhibition of the amplitude of spontaneous contractions in the myometrium of rats under

the effect of diazoxide is likely to be related to the changes in the membrane potential of myocytes. It should also be emphasized that in the background of this substance, we observe similar effects on the mechanokinetic parameters of contraction and relaxation phases which demonstrates the non-specific impact of diazoxide on Ca^{2+}-transporting systems of myocytes.

The mechanokinetics of spontaneous contractions of the myometrium in case of blocking mitoK_{ATP}-channels. The blocking of K_{ATP}-channels of the inner mitochondrial membrane by 5-HD (200 μM) was accompanied by the inhibition of spontaneous contractions in the myometrium of rats (**Fig. 7**).

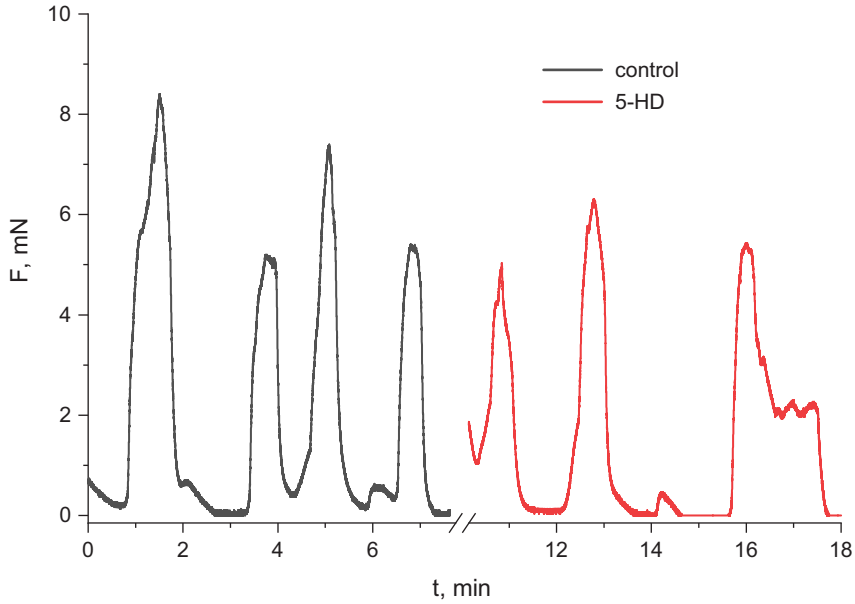

Fig. 7. The spontaneous contractile activity of the myometrium of rats in control and under a prior 20-minute-long incubation with 5-HD, the blocking agent of K_{ATP}-channels of the mitochondria (200 μM). Typical mechanograms are presented

In these conditions, there were also considerable reconstructions in the kinetics of some contraction-relaxation cycles: the contraction phase accelerated greatly, whereas the relaxation phase slowed down significantly. For instance, under the effect of 5-HD, the time parameters were: τ_C – on average 52.5 % (n = 6, p<0.01) and τ_R – on average 127.1 % as compared with the relevant control values (n = 6, p<0.05) (**Fig. 8*A***). At these points of contractile responses, the force parameters were: F_C on average 43.2 % (n = 6, p<0.01) and F_R on average 62.5 % (n = 6, p<0.001) respectively (**Fig. 8*B***).

Besides, on the background of 5-HD (200 μM), there was a reliable decrease in the value of the maximal velocity on the level of the contraction phase (up to 45.8 % as compared with the control, on average, n=6, p<0.001), whereas the maximal velocity of the relaxation phase did not have reliable differences as compared to the respective parameter in control (**Fig. 9*A***). While normalizing the velocity indices as per amplitude, the rate of contraction velocity inhibition is about 75 % as compared with the control, while the relaxation velocity remains on the control level.

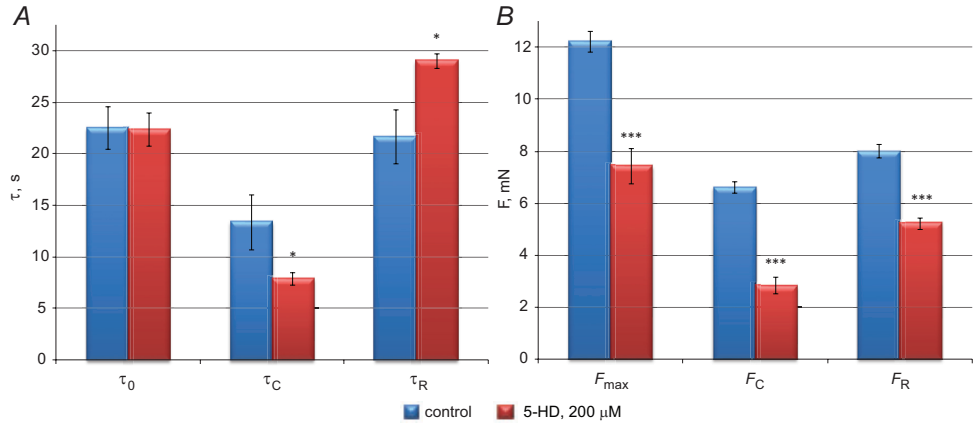

Fig. 8. The time (***A***) and force (***B***) parameters of spontaneous contractions in the myometrium of rats in control and under a prior incubation with 5-HD, the blocking agent of mitoK$_{ATP}$-channels (200 μM). The relevant indices of spontaneous activity in control (n = 6, * – p<0.05 and *** – p<0.001 – the difference is reliable regarding the control) were accepted as 100 %

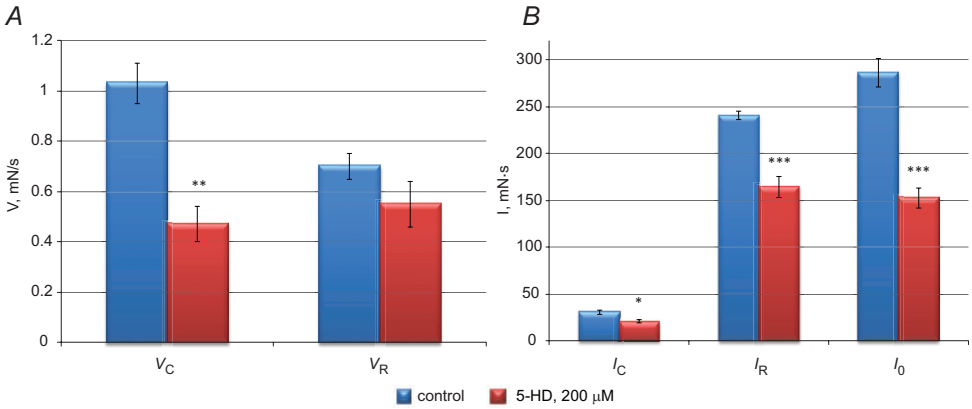

Fig. 9. The velocity (***A***) and impulse (***B***) parameters of spontaneous contractions in rat myometrium in control and under a prior incubation with 5-HD, the blocking agent of mitoK$_{ATP}$-channels (200 μM). The relevant indices of spontaneous activity in control (n=6, * – p<0.05 and *** – p<0.001 – the difference is reliable regarding the control) were accepted as 100 %

In the case of impulse parameters, considerably lower indices of the amplitude impulses (I_{max}) and maximal velocity of the relaxation phase (I_R) (47.1 % and 50.0 % as compared with the control values, n = 6, p<0.001, respectively) were observed, while the impulse of the maximal velocity of the contraction phase (I_C) was 62.8 % on average as compared with the control (n = 6, p<0.001) (**Fig. 9*B***).

Comparing the mechanokinetic effects of glibenclamide and 5-HD (blockers of K_{ATP}-channels of the plasma and inner mitochondrial membranes), we can firmly state that, in general, these two compounds cause inhibition of the contractile function of the myometrium and change the mechanokinetic parameters of individual spontaneous contractions in a rather similar way. However, a considerable difference between the effects of these compounds was observed regarding τ_R – in the case of blocking mitoK$_{ATP}$-channels this index increased markedly. It is also noteworthy that on the background of

5-HD, both V_R parameter and its normalized analog (normalized as per the amplitude of the maximal velocity of the relaxation phase) had no reliable differences regarding the control, so contrary to K_{ATP}-channels of the plasma membrane, mitoK_{ATP}-channels are not likely to modulate the processes of extruding Ca^{2+} ions from the cytosol.

CONCLUSIONS

Studies of the mechanokinetics of the spontaneous contractile activity of the longitudinal smooth muscles of the rat uterus revealed that the K_{ATP} channels of the plasma membrane and mitochondria are involved in maintaining the excitability of the myometrium of non-pregnant rats. Conceivably, the blocking of K_{ATP} channels activates the processes of an energy-dependent decrease in the concentration of Ca^{2+} ions in the cytosol of smooth muscle cells. Since the inhibition of the conductivity of mitoK_{ATP}-channels, contrary to K_{ATP}-channels of the plasma membrane, did not induce any changes in the kinetics of the relaxation phase of the spontaneous contractions, these channels are not involved in the regulation of the processes of extruding Ca^{2+} ions from the cytosol.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: This article does not contain any studies with human subjects performed by the any of the authors.

Animal studies: All international, national and institutional guidelines for the care and use of laboratory animals were followed.

AUTHOR CONTRIBUTIONS

Conceptualization, [O.V.T.; O.B.V.]; methodology, [O.V.T.; O.B.V.]; investigation, [O.V.T.; I.S.V.]; data analysis, [O.V.T.; I.S.V.; V.D.I.]; writing – original draft preparation, [O.V.T.]; writing – review and editing, [O.V.T.; V.D.I.]; visualization, [O.V.T.; I.S.V.]; supervision, [O.V.T.]; project administration, [O.V.T.; O.B.V.]; funding acquisition, [-].

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ЗАЛУЧЕННЯ $K_{АТФ}$-КАНАЛІВ ПЛАЗМАТИЧНОЇ МЕМБРАНИ І МІТОХОНДРІЙ У РЕГУЛЯЦІЮ МЕХАНОКІНЕТИКИ СПОНТАННИХ СКОРОЧЕНЬ МІОМЕТРІЯ ЩУРІВ

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Обґрунтування. АТР-чутливі K^+-канали плазматичної мембрани у гладеньких м'язах матки є одними з найзначущіших іонних каналів, які регулюють збудливість цієї тканини у невагітному стані й упродовж вагітності. Мітo$K_{АТФ}$-канали забезпечують регуляцію біоенергетичного стану мітохондрій (інтенсивність мітохондрійного дихання і потенціал внутрішньої мітохондрійної мембрани). Натепер немає інформації щодо участі обох типів цих каналів у регуляції механокінетики спонтанних скорочень, тому метою роботи було здійснити повний механокінетичний аналіз спонтанних скорочень міометрія щурів за умов блокування й активації АТР-чутливих K^+-каналів плазматичної мембрани та блокування мітo$K_{АТР}$-каналів.

Матеріали та Методи. Експерименти проводили на самках щурів лінії Wistar. Реєстрацію спонтанної скорочувальної активності гладенькоm'язових смужок поздовжніх гладеньких м'язів рогів матки здійснювали тензометричним методом в ізометричному режимі. У експериментах використовували активатор АТР-чутливих K^+-каналів плазматичної мембрани діазоксид (50, 100, 150 і 200 мкМ) та блокатор цих каналів глібенкламід (1, 2, 4, 6, 8 та 10 мкМ), а також блокатор АТР-чутливих K^+ каналів мітохондрій 5-гідроксидеканоат (5-HD, 50 мкМ). Дослідження механокінетики процесу скорочення–розслаблення м'язових препаратів здійснювали згідно з методом повного механокінетичного аналізу з розрахунком механокінетичних параметрів циклу скорочення–розслаблення: силових (F_{max}, F_C та F_R), часових (τ_0, τ_C і τ_R), імпульсних (I_{max}, I_C та I_R) і швидкісних параметрів (V_C і V_R).

Результати. Встановлено, що блокування, як і активування $K_{АТР}$-каналів плазматичної мембрани спричиняє пригнічення амплітуди, ймовірно, за різними клітинними механізмами регулювання іонної провідності. За дії глібенкламіду відбувалося суттєве зниження частоти і механокінетичних параметрів спонтанних скорочень міометрія, що підтверджує внесок $K_{АТР}$-каналів плазматичної мембрани у підтримання збудливості міометрія невагітних щурів. Активування $K_{АТР}$-каналів плазматичної мембрани діазоксидом спричиняло зміну окремих механокінетичних параметрів спонтанних скорочень міометрія. За дії блокатора мітоК$_{АТР}$-каналів 5-HD відбувалися пригнічення амплітуди та модуляція параметрів фази скорочення без змін кінетики фази розслаблення спонтанних скорочень.

Висновки. Модуляція $K_{АТР}$-каналів плазматичної мембрани і мітохондрій супроводжується пригніченням спонтанних скорочень міометрія. Обидва типи $K_{АТР}$-каналів важливі регулятори збудливості міометрія, однак, імовірно, мітоК$_{АТР}$-канали, на відміну від $K_{АТР}$-каналів плазматичної мембрани, не модулюють процеси екструзії іонів Ca^{2+} з цитозолю.

Ключові слова: міометрій, $K_{АТР}$-канали плазматичної мембрани і мітохондрій, спонтанні скорочення, механокінетичний аналіз

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