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INVOLVEMENT OF K_{ATP}-CHANNELS OF PLASMA AND MITOCHONDRIAL MEMBRANES IN MAINTAINING THE CONTRACTIVE FUNCTION OF MYOMETRIUM OF NON-PREGNANT RAT UTERUS

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K^+-channels, in particular, ATP-sensitive K^+-channels of plasma and inner mitochondrial membranes, play a significant role in regulating the contraction-relaxation processes in visceral smooth muscles. The specificities of these channels and their role in maintaining excitation have been well investigated for the cardiac muscle and smooth vascular muscles. However, scarce data exist about the involvement of these channels, especially the mitochondrial ones, in regulating myometrium contractions. The tenzometric methods and mechanokinetic analysis were used to study the regularities in oxytocin-induced contractions of smooth muscles of non-pregnant rats myometrium under the conditions of modulating ATP-sensitive K^+-channels of plasma and inner mitochondrial membranes. It was determined that prior incubation of myometrium in the presence of the blocking agents of K_{ATP}-channels of plasma membrane and mitochondria (glibenclamide and 5-hydroxydecanoate, respectively) was generally associated with the increase in the phase and, in some cases, tonic components of oxytocin-induced contractions. There was an increase in the amplitude of oxytocin-induced contractions at the background of the impact of glibenclamide (the range of concentrations used was 1–10 μM) without significant changes in the area under these mechanograms; the velocity of relaxation increased considerably as well. The blocking agent of mitochondrial ATP-sensitive K^+-channels, 5-hydroxydecanoate (in the concentrations of 50 μM and 200 μM) caused considerable changes in the kinetics of the processes of intensification (the increase in velocity) and decline (more than 2-fold decrease in the velocity) in comparison with the force of oxytocin-induced contractions, but it did not impact their amplitude and the area under mechanograms. The most significant effect, demonstrated by

the activator of ATP-sensitive K^+-channels of diazoxide (the range of concentrations was 50–200 μM), was the reduction in the area under oxytocin-induced contractions by half. This compound also caused a decrease in the amplitude of contractions and a considerable increase in the maximal velocities of the intensification and decline phases for the force of oxytocin-induced contractions. The combined effect of the blocking agents of K_{ATP}-channels of plasma and inner mitochondrial membranes, glibenclamide (10 μM) and 5-hydroxydecanoate (200 μM), was accompanied by the increase in the amplitude of oxytocin-induced contractions and the changes in the kinetics of contraction and relaxation processes. Thus, the data obtained demonstrate that K_{ATP}-channels of plasma membrane and mitoK_{ATP}-channels in non-pregnant rats' myometrium play a modulating role in forming the contractive response to the uterotonic hormone oxytocin. These channels are involved in the regulation of the contractive function of myometrium, modulating the amplitude of contractions, the ability of long-term strain support and the kinetics of contraction and relaxation processes.

Keywords: smooth muscles, myometrium, ATP-sensitive K^+-channels of plasma membranes and mitochondria, mechanokinetic analysis

INTRODUCTION

Smooth muscles (SM) play a vital role in the functioning of internal organs and systems of the organism, forming the structural-functional basis for the gastrointestinal tract, the vascular bed, the genito-urinary system, and respiratory pathways. In general, the regulation of the capability to maintain basal strain and active contraction of SM is ensured by changes in the intracellular concentration of Ca^{2+} ions, which is conditioned by the combination of diverse processes: the work of energy-dependent systems of Ca^{2+}-extrusion from the cell, the penetration of these ions via calcium channels from the extracellular space and the release from the intracellular depots (sarcoplasmatic reticulum and mitochondria) [4, 5, 39, 30]. The regulation of Ca^{2+}-dependent processes of SM contraction-relaxation is considerably performed by K^+-channels, including ATP-sensitive K^+-channels (K_{ATP}-channels). It was established that the activation of these channels reduced the duration of the action potential, thus decreasing the release of Ca^{2+} into the cytosol that conditioned muscle relaxation [1, 31, 36].

K_{ATP}-channels of two types are expressed in the uterus tissues: the inwardly rectifying K_{ATP}-channel of plasma membrane and mitochondrial ATP-sensitive K^+-transporter (mitoK_{ATP}-channel) [23, 25, 27]. K_{ATP}-channels of plasma membrane play one of the key roles in regulating the membrane potential of the myocyte and the contractive activity of myometrium tissue. For instance, it was demonstrated that during pregnancy and up to delivery there were changes in the properties of these channels, including the sensitivity to pharmacological and physiological regulators (Ca^{2+} ions and membrane potential) along with the decrease in the expression of its subunits [10, 23]. According to other researchers [11, 26], the expression rate of these channels depends on the age and the functional state of myometrium: on the one hand, it decreases at late stages of pregnancy, and on the other – it increases in non-pregnant uterus of a woman with age. It indicates the relevance of ATP-sensitive K^+-channels as regulators of the functional state and excitation of the uterus.

MitoK_{ATP}-channels are capable of regulating the bioenergetic state of mitochondria, affecting such relevant indices as the intensity of breathing and membrane potential of

the inner membrane, which, in its turn, impacts the physiological state of smooth muscle cells (SMC) and SM tissues in general [32, 34]. They also regulate the velocity of forming reactive oxygen intermediates and demonstrate their cytoprotective action under pathological conditions, under the influence of pro-oxidant factors and high concentration of Ca^{2+} ions [19].

Both plasma and mitochondrial K_{ATP}-channels in muscles are essential for maintaining normal functioning of myocytes and SM in general. The activation of K_{ATP}-channels leads to the decrease in muscle excitation and preservation of the energy balance in all the types of muscles, without exceptions, under both normal and pathological conditions. It was concluded that the cells of myometrium of both non-pregnant and pregnant women and female rats contain diazoxide-sensitive K_{ATP}-channels, whose activation results in inhibiting the contractions of the uterus [15, 25, 27]. The scientific data demonstrate good prospects for applying the effectors of K^+-channels (including the activators of K_{ATP}-channels) as tocolytics during the therapy of premature delivery [2, 13, 14].

The aim of our study was to investigate the involvement of K_{ATP}-channels of plasma and mitochondrial membranes in maintaining the coordinated contractive activity and their role in modulating oxytocin-induced contractions of myometrium of intact fragment of the uterus wall with preserved mucosa.

MATERIALS AND METHODS

The experiments were conducted using Wistar line rats (the average weight of animals was 200–250 g). 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 SSC Institute of Biology and Medicine No. 3 dated May 2, 2019). The animals were killed by the introduction of a lethal dose of propofol narcosis (Sigma).

The tenzometric experiments were conducted using the preparations of longitudinal smooth muscles of uterine horns. Muscle stripes (the average size – 2×10 mm) were placed into the working chamber of 2 mL with the flowing Krebs solution (the flow rate of 5 mL/min), thermostated at 37 °C. The Krebs solution contained (in mM): 20.4 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 solution was 7.4. The preparations were provided with passive tension at the rate of 10 mN and left for 1 h (until achieving stable reproduction of contractions). The contractive activity was studied in the isometric mode using the force sensing device. The signals were registered with an analogue-to-digital transformer.

The contractions were induced by the application of uterotonic hormone oxytocin (0.1 i.u., the average concentration of protein with hormonal activity is about 0.033 μM; Gideon Richter, Hungary). The activator of ATP-sensitive K^+-channels of 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 (concentrations of 50 μM and 200 μM; Sigma, USA) were used in the experiment.

Stock solutions of diazoxide and glibenclamide were prepared by preliminary dissolution of the substances in the organic solvent dimethylsulfoxide (DMSO) and added to Krebs solution to obtain the final aliquot of DMSO 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-hydroxydecanoate was prepared by preliminary dissolution of this substance in distilled water.

Non-selective blocking of ATP-sensitive K^+-channels was done by preliminary incubation for 20 min of SM preparations with glibenclamide, and their activation – with diazoxide. The blocking of ATP-sensitive K^+-channels of mitochondrial membranes was done by preliminary incubation for 20 min of SM with 5-hydroxydecanoate (5-HD). After the preliminary incubation of preparations with the above-mentioned substances, the effect of the latter on spontaneous and acetyl choline-induced contractions of SM was tested.

The mechanokinetics of the process of contractions-relaxations of muscle preparations (the calculations of normalized maximal velocities for contraction and relaxation phases, V_{nc} and V_{nr}, respectively) was studied according to the methods described in [3]. The contraction phase was defined as a fragment of contractive response from the beginning of force variation up to its maximal value (amplitude of phase contraction). The relaxation phase started with the maximum of phase contraction and lasted till achieving the stable level of the tonic contraction. The introduction of the tonic contraction into the contractive responses of smooth muscle preparations was calculated as a percentage of stationary strain relative to the maximal force of contractive responses.

The experimental data was processed by the variation statistics methods using OriginPro 8 program. The samples were checked in terms of belonging to normally distributed general populations according to Shapiro–Wilk criterion. The paired version of Student's *t*-test was used to determine the reliable differences between the mean values of samplings. In all cases the results were considered reliable on condition of the probability value *p*, under 5 % ($p < 0.05$). The validation analysis of data approximation by the linear function was performed using Fisher's F-criterion; determination coefficients (R^2) were at least 0.9. The results were presented as the arithmetic mean ± standard error of the mean value, *n* – number of experiments.

RESULTS AND DISCUSSION

The mechanokinetic parameters of contractions of rat myometrium under activation of oxytocin receptors with uterotonic oxytocin were investigated (Fig. 1***A***). This agonist is known to activate receptors, associated with $G_{q/11}$-proteins, inducing contractions via inositol triphosphate-dependent pathway [4]. The contractions of myometrium preparations, induced by oxytocin (0.1 i.u.), can be characterized by the following average kinetic parameters: the velocity of contraction phase V_{nc} (3.31 ± 0.59) min^{-1} (n = 9); the velocity of relaxation phase V_{nr} (0.23 ± 0.05) min^{-1} (n = 9).

It was determined that the prior incubation of myometrium in the presence of the blocking agents of K_{ATP}-channels of plasma membrane and mitochondria was generally accompanied by the increase in the phase and, in some cases, tonic components of oxytocin-induced contractions. For instance, under blocking of K_{ATP}-channels of SMC with glibenclamide at the cumulative increase in its concentration in the washing solution from 1 μM to 10 μM, there was a dose-dependent increase in the amplitude of oxytocin-induced contractions (Fig. 2). In the presence of glibenclamide in the concentration of 1 μM, the amplitude of contraction was 110.7 % on average, and under the increased concentration of this blocking agent up to 10 μM, it was 131.6 % as compared to the control. It should be noted that the glibenclamide concentrations used were

capable of blocking effect on K_{ATP}-channels of the plasma membrane, but they were not sufficient to induce significant changes in the conductance of these channels in mitochondria [22].

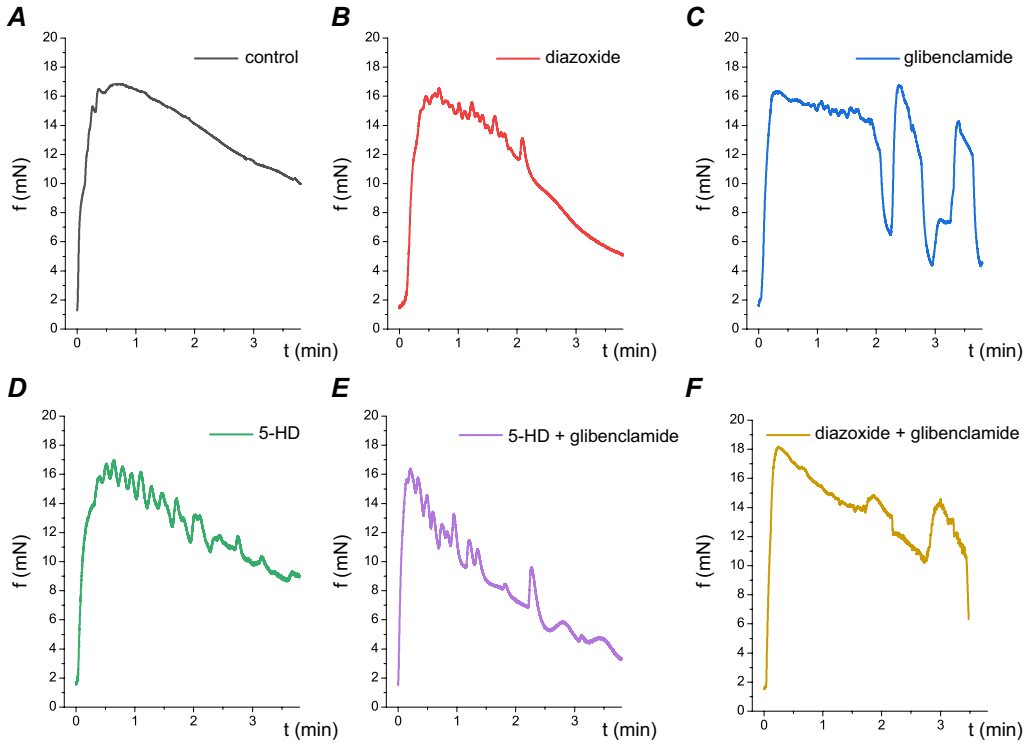

Fig. 1. Oxytocin-induced (0.1 i.u.) reduction of rat myometrium in control (***A***) and in the presence of effectors of ATP-sensitive K^+ channels: activator of ATP-sensitive K^+ channels of diazoxide (100 μM) (***B***); a blocker of ATP-sensitive K^+ channels of glibenclamide (10 μM) (***C***); blocker of ATP-sensitive K^+ channels of mitochondria of 5-hydroxydecanoate (5-HD, 200 μM) (***D***); the combined action of glibenclamide (10 μM) and 5-HD (200 μM) (***E***); the combined action of diazoxide (100 μM) and glibenclamide (10 μM) (***F***). Typical mechanograms are given

Рис. 1. Індуковані окситоцином (0,1 М.О.) скорочення міометрія щурів у контролі (***A***), а також за наявності ефекторів АТФ-чутливих K^+-каналів: активатора АТФ-чутливих K^+-каналів діазоксиду (100 мкМ) (***B***); блокатора АТФ-чутливих K^+-каналів глібенкламіду (10 мкМ) (***C***); блокатора АТФ-чутливих K^+-каналів мітохондрій 5-гідроксидеканоату (5-HD, 200 мкМ) (***D***); сукупної дії глібенкламіду (10 мкМ) і 5-HD (200 мкМ) (***E***); сукупної дії діазоксиду (100 мкМ) і глібенкламіду (10 мкМ) (***F***). Наведено типові механограми

As the efficiency of the contractive function of SM is directly related to the ability of temporal regulation of the strain, we analyzed the area under curves of oxytocin contractions (from the beginning of applying the uterotonic and during the subsequent 5 min) in the presence of glibenclamide. It was determined that the cumulative increase in this blocking agent was not accompanied by changes in the area of oxytocin-induced contractions (Fig. 2).

Under the influence of glibenclamide, there were significant changes in the normalized maximal velocities of the contraction and relaxation phases, V_{nc} and V_{nr}: the contraction process slowed down to a certain degree, whereas the relaxation of muscle

preparations considerably increased, depending on the dose (Fig. 2). For instance, at the cumulative increase in glibenclamide concentration in the washing solution up to 10 μM, the indices of V_{nc} and V_{nr} were 89.9 % on average (n = 6, p<0.05) and 185.7 % (n = 6, p<0.01) (as compared to the control values taken as 100 %).

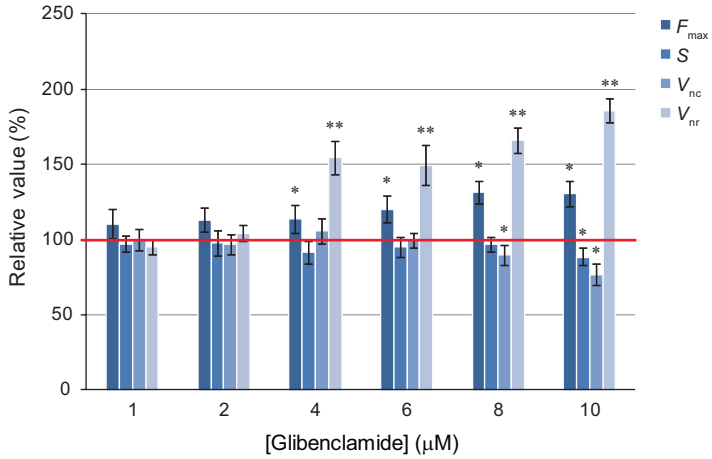

Fig. 2. Relative changes in mechanokinetic parameters (amplitude, F_{max}; area under the mechanograme within 5 min, S; normalized maximum rate of contraction phase, V_{nc}; normalized maximum rate of relaxation phase, V_{nr}) myometrial contractions of rats induced by oxytocin (0.1 i.u.) under conditions of cumulative increase in the concentration of the blocker of ATP-sensitive K^+-channels of glibenclamide (1, 2, 4, 6, 8 and 10 μM).
The corresponding control parameters are taken as 100 %; (M±m, n = 6); * – p<0.05; ** – p<0.01 – the difference is significant relative to the control

Рис. 2. Відносні зміни механокінетичних параметрів (амплітуди, F_{max}; площі під механограмою упродовж 5 хв, S; нормованої максимальної швидкості фази скорочення, V_{nc}; нормованої максимальної швидкості фази розслаблення, V_{nr}) скорочень міометрія щурів, індукованих окситоцином (0,1 М.О.) в умовах кумулятивного зростання концентрації блокатора АТФ-чутливих K^+-каналів глібенкламіду (1, 2, 4, 6, 8 і 10 мкМ).
За 100 % прийнято відповідні показники в контролі; (M±m, n = 6); * – p<0,05; ** – p<0,01 – різниця достовірна щодо контролю

At the background of the cumulative increase in the concentrations of the activator for ATP of K_{ATP}-channels of diazoxide (50, 100, 150 and 200 μM), there was a decrease in the amplitude of oxytocin-induced contractions (Fig. 3). For instance, in the presence of 200 μM of diazoxide, the average amplitude was 84.3 % as compared to the control. It should be noted that this substance caused a considerable dose-dependent decrease in the area under curves for oxytocin-induced contractions; for instance, under the influence of 200 μM of diazoxide this index decreased almost twice as compared to the control (Fig. 3).

At the background of the activation of K_{ATP}-channels with diazoxide, there were considerable changes (an increase) observed in the kinetic parameters of oxytocin-induced contractions of myometrium (Fig. 3). For instance, in the presence of 200 μM of diazoxide in the washing solution, the indices of normalized maximal velocities for the phases of contraction and relaxation, V_{nc} and V_{nr}, respectively, were as follows: 238.5 % (n = 6, p<0.01) and 183.8 % (n = 6, p<0.01) (as compared to the control values taken as 100 %).

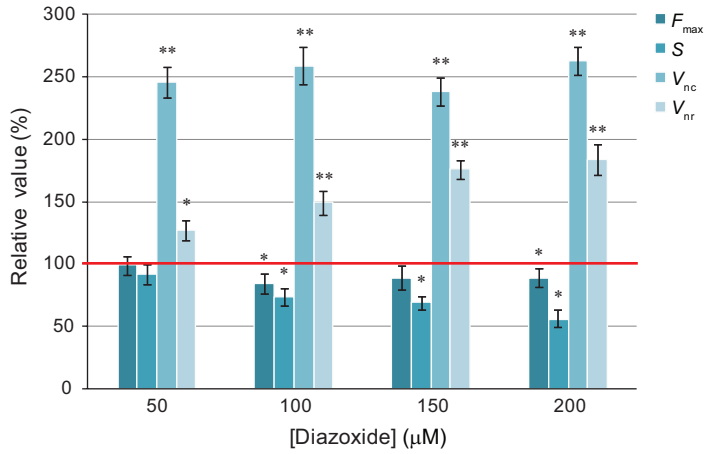

Fig. 3. Relative changes in mechanokinetic parameters (amplitude, F_{max}; area under the mechanograme within 5 min, S; normalized maximum rate of contraction phase, V_{nc}; normalized maximum rate of relaxation phase, V_{nr}) myometrial contractions of rats induced by oxytocin (0.1 i.u.) under conditions of cumulative increase in the concentration of the activator of ATP-sensitive K^+-channels of diazoxide (50, 100, 150 and 200 μM).
The corresponding control parameters are taken as 100 %; (M±m, n = 6); * – p<0.05, ** – p<0.01 – the difference is significant relative to the control

Рис. 3. Відносні зміни механокінетичних параметрів (амплітуди, F_{max}; площі під механограмою упродовж 5 хв, S; нормованої максимальної швидкості фази скорочення, V_{nc}; нормованої максимальної швидкості фази розслаблення, V_{nr}) скорочень міометрія щурів, індукованих окситоцином (0,1 М.О.) в умовах кумулятивного зростання концентрації активатора АТФ-чутливих K^+-каналів діазоксиду (50, 100, 150 і 200 мкМ).
За 100 % прийнято відповідні показники у контролі; (M±m, n = 6); * – p<0,05; ** – p<0,01 – різниця достовірна щодо контролю

In general, the blocking of K_{ATP}-channels of the inner mitochondrial membrane with 5-HD (50 μM and 200 μM) caused no changes in the amplitude or the area under the tenzometric curve for oxytocin-induced contractions (Fig. 4). However, in these conditions there were considerable transformations in the kinetics of these curves: the contraction phase accelerated greatly, whereas the relaxation phase slowed down significantly. For instance, under the influence of 50 μM and 200 μM of 5-HD the average parameter of V_{nc} was 173.2 % (n = 6, p<0.05) and 203.9 % (n = 6, p<0.05), respectively, and the normalized maximal velocities of relaxation V_{nr} were as follows: 73.8 % (n = 6, p<0.05) and 42.9 % (n = 6, p<0.05) (as compared to the control values taken as 100 %).

The combined effect of the blocking agents of K_{ATP}-channels of plasma and inner mitochondrial membranes, glibenclamide (10 μM) and 5-HD (200 μM), was accompanied with the increase in the amplitude of oxytocin-induced contractions up to 121.0 % on average (n = 6, p<0.05), but the area under the curves for contractions showed no significant changes (Fig. 5***A***). However, in these conditions, there were significant changes in the normalized maximal velocities of the contraction and relaxation phases, V_{nc} and V_{nr}: these indices were 83.6 % (n = 6, p<0.05) and 163.9 % (n = 6, p<0.05) respectively (the data presented was re-calculated in comparison to the control taken as 100 %) (Fig. 5***A***).

We also studied oxytocin-induced (0.1 i.u.) contractions of myometrium under the combined effect of the non-selective activator of K_{ATP}-channels, diazoxide (200 μM), and

the blocking agent of mitoK$_{ATP}$-channels, 5-HD (200 μM) (Fig. 1***F***). In these conditions, there were significant changes in the kinetic indices of V_{nc} and V_{nr} – up to 235.9 % (n = 6, p<0.01) and 63.7 % (n = 6, p<0.05) respectively (the data presented was re-calculated in comparison to the control taken as 100 %) (Fig. 5***B***).

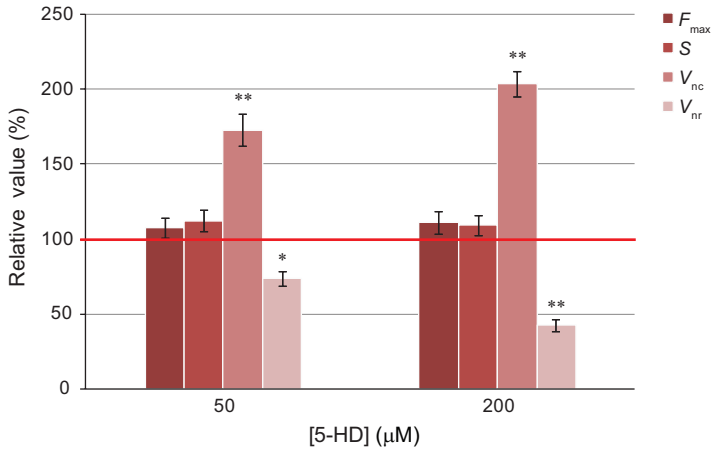

Fig. 4. Relative changes in mechanokinetic parameters (amplitude, F_{max}; area under the mechanogrаme within 5 min, S; normalized maximum rate of contraction phase, V_{nc}; normalized maximum rate of relaxation phase, V_{nr}) myometrial contractions of rats induced by oxytocin (0.1 i.u.) under conditions of cumulative increase in the concentration of the blocker of mitochondrial ATP-sensitive K$^+$-channels of 5-hydroxydecanoate (5-HD, 50 and 200 μM).
The corresponding control parameters are taken as 100 %; (M±m, n = 6); * – p<0.05; ** – p<0.01 – the difference is significant relative to the control

Рис. 4. Відносні зміни механокінетичних параметрів (амплітуди, F_{max}; площі під механограмою упродовж 5 хв, S; нормованої максимальної швидкості фази скорочення, V_{nc}; нормованої максимальної швидкості фази розслаблення, V_{nr}) скорочень міометрія щурів, індукованих окситоцином (0,1 М.О.) в умовах кумулятивного зростання концентрації блокатора мітохондріальних АТФ-чутливих K$^+$-каналів 5-гідроксидеканоату (5-HD, 50 і 200 мкМ).
За 100 % прийнято відповідні показники у контролі; (M±m, n = 6); * – p<0,05; ** – p<0,01 – різниця достовірна щодо контролю

Uterine tubes contain the areas with sources of spontaneous activity in the cervical and ovarial sections. The research group of K.V. Kazarian *et al.* [20, 21] determined that the main source of rhythm in the uterine tubes is the ovarial section. The main uterotonic hormone, oxytocin, promotes the dominance of the ovarial section and provides coordinated functioning of all the rhythmogenic parts of myometrium [20]. The control of excitation and contractive activity of SM in uterine ensures the preservation of fertility and the ability to bear the fetus and endure the delivery [35] since at least half of the cases of newborns' deaths are related to premature delivery, the cellular basis of which is the disrupted regulation of SM strain and the processes of contractions and relaxations of myometrium.

Potassium channels is an important factor of myometrium excitation regulation including K$_{ATP}$-channels of inward rectification. They have low probability of the open state, the average conductance of about 135 picosiemens, and get activated by submillimolar concentrations of ATP and ADP. Most SM (including the ones in colon, urinary bladder and vessels) are characterized by the prevalence of isoform Kir6.2/SUR2B

K_{ATP}-channel of the plasma membrane. Two isoforms of K_{ATP}-channels of the plasma membrane are expressed in the myometrium of non-pregnant rats and humans: prevailing domination is noted for isoform Kir6.1/SUR2B, whereas Kir6.2/SUR1 is detected in the insignificant amount [11, 23, 25]. During the pregnancy of women, there is a decrease in the expression of Kir6.1/SUR2B, thus, the tissues of uterus get ready to the delivery activity [11]; this regularity was not observed in the myometrium tissue of female rats.

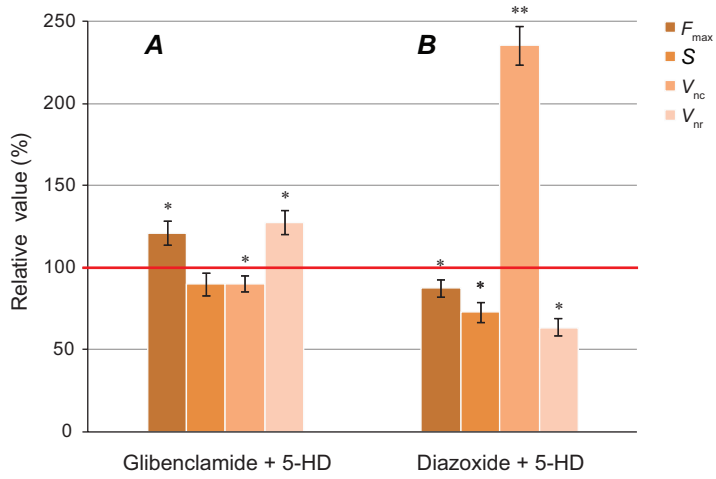

Fig. 5. Relative changes in mechanokinetic parameters (amplitude, F_{max}; area under the mechanogrammе within 5 min, S; normalized maximum rate of contraction phase, V_{nc}; normalized maximum rate of relaxation phase, V_{nr}) myometrial contractions of rats induced by oxytocin (0.1 i.u.) under the combined action of glibenclamide (10 μM) and 5-hydroxydecanoate (5-HD, 200 μM) (***A***) and the combined action of diazoxide (200 μM) and 5-HD (200 μM) (***B***).
The corresponding control parameters are taken as 100 %; (M±m, n = 6); * – p<0.05; ** – p<0.01 – the difference is significant relative to the control

Рис. 5. Відносні зміни механокінетичних параметрів (амплітуди, F_{max}; площі під механограмою впродовж 5 хв, S; нормованої максимальної швидкості фази скорочення, V_{nc}; нормованої максимальної швидкості фази розслаблення, V_{nr}) скорочень міометрія щурів, індукованих окситоцином (0,1 М.О.) в умовах сукупної дії глібенкламіду (10 мкМ) і 5-гідроксидеканоату (5-HD, 200 мкМ) (***A***) та сукупної дії діазоксиду (200 мкМ) і 5-HD (200 мкМ) (***B***).
За 100 % прийнято відповідні показники у контролі; (M±m, n = 6); * – p<0,05; ** – p<0,01 – різниця достовірна щодо контролю

ATP-sensitive K^+-channels of plasma and mitochondrial membranes of smooth muscle cells have a similar heterotetrameric structure and similar mechanisms of pharmacological and physiological regulation. The activation of both types of K_{ATP}-channels demonstrates cytoprotective effect and improves the bioenergetic state of the cell. For instance, the investigations of the processes of ischemia-reperfusion of myocardium and other tissues showed that the activation of both plasma and mitochondrial K_{ATP}-channels underlies the phenomenon of ischemic preconditioning, which resulted in the protection of cells from destruction and, in case of myocardium, the decrease in the area damaged by necrosis [5, 6, 9, 24]. Under normoxia, the activation of plasma K_{ATP}-channels in the myocardium reduces the potential of the effect and ensures relaxation of the cardiac muscle. In smooth muscles, the plasma K_{ATP}-channels also fulfill relevant

functions depending on their localization. For instance, in SMC of vascular muscles they are involved in the regulation of the vascular tone [8]. Besides vessels, K_{ATP}-channels were also identified in the plasma membrane of SMC of other muscles, including gastrointestinal and urinary tracts.

The obtained results are in agreement with the data of K. Sawada *et al*. [28] who demonstrated that the activation of K_{ATP}-channels of the plasma membrane led to inhibiting the amplitude of oxytocin-induced contractions, and the combined use of activators and blocking agents of these channels eliminated the effect of the former. Similar results were obtained using non-pregnant mice myometrium [17]. It should be noted that, in addition to impacting oxytocin-induced contractions, K_{ATP}-channels are involved in the regulation of other agonist-induced contractive responses (including the ones to the activity of acetylcholine and prostaglandin F2α) [16–18].

The scientific data related to the expression and role of mitochondrial K_{ATP}-channels in the functioning of SM, is very fragmented and mostly limited to the vascular wall [15]. MitoK_{ATP}-channels are localized in the inner mitochondrial membrane; they transport K^+ ions to the matrix, ensuring the driving force of K^+/H^+-antiporter and thus participating in the regulation of the volume and energetic homeostasis of mitochondria.

While analyzing the obtained results, one may envisage that K_{ATP}-channels of the plasma membrane and mitoK_{ATP}-channels ensure the modulation of oxytocin-induced contractions of myometrium. Their cumulative blocking leads to the increase in the amplitude of these contractions. However, this effect exert considerable impact on the total efficiency of the contractive function of myometrium, as the parameter of the area under mechanogram remains at the level of control values.

The normalized maximal velocity of relaxation, V_{nr}, is the parameter, most sensitive to the activity of the effectors of K_{ATP}-channels; in general, it increases both under non-selective blocking and activation of these channels. It is noteworthy that in case of selective blocking of mitoK_{ATP}-channels this parameter decreases considerably, and this slowing-down of the muscle relaxation process is not eliminated in the presence of the non-selective activator of K_{ATP}-channels, diazoxide. Therefore, it may be predicted that mitoK_{ATP}-channels fulfil a vital function in the myometrium tissue, defining the velocity of relaxation, thus regulating the duration of the oxytocin-induced spasm of the uterus. This process can be mediated by the regulatory effect of mitoK_{ATP}-channels on the processes of accumulation of Ca^{2+} ions by myocyte mitochondria [13].

CONCLUSIONS

Our research demonstrated that K_{ATP}-channels of plasma membrane and mitoK_{ATP}-channels in non-pregnant rats' myometrium play a modulating role in forming the contractive response to the uterotonic hormone oxytocin. These channels are involved in the regulation of the contractive function of myometrium, modulating the amplitude of contractions, the ability of long-term strain support, and the kinetics of contraction and relaxation processes.

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 institutional, national and institutional guidelines for the care and use of laboratory animals were followed.

1. *Bhatiya S., Choudhury S., Gari M., Singh P., Shukla A., Garg S.K.* Myometrial Calcium and Potassium Channels Play a Pivotal Role in Chromium-Induced Relaxation in Rat Uterus: an *In Vitro* Study. **Biol Trace Elem Res**, 2020; Feb 7.
[DOI: https://doi.org/10.1007/s12011-020-02041-x; PMID: 32034680; Google Scholar]
2. *Brainard A.M., Korovkina V.P., England S.K.* Potassium channels and uterine function. **Semin Cell Dev Biol**, 2007; 18(3): 332–339.
[DOI: https://doi.org/10.1016/j.semcdb.2007.05.008; PMID: 17596977; Google Scholar]
3. *Burdyga Th.V., Kosterin S.A.* Kinetic analysis of smooth muscle relaxation. **Gen. Physiol. Biophys**, 1991; 10: 589–598.
[PMID: 1802780; Google Scholar]
4. *Burdyga T., Paul R.J.* Chapter 86 – Calcium homeostasis and signaling in smooth Muscle / Muscle. Fundamental Biology and Mechanisms of Disease. Edited by J. A. Hill and E. N. Olson. **Elsevier Inc**, 2012; 2: 1155–1171.
5. *Chioss G., Costantine M.M., Bytautiene E., Betancourt A., Hankins G.D., Saade G.R., Longo M. In vitro* myometrial contractility profiles of different pharmacological agents used for induction of labor. **Am J Perinatol**, 2012; 29(9): 699–704.
[DOI: https://doi.org/10.1055/s-0032-1314891; PMID: 22644831; Google Scholar]
6. *Crestanello J.A., Doliba N.M., Babsky A.M., Doliba N.M., Niibori K., Osbakken M.D., Whitman G.J.* Opening of potassium channels protects mitochondrial function from calcium overload. **Journal of Surgical Research**, 2000; 94(2): 116–123.
[DOI: https://doi.org/10.1006/jsre.2000.5979; PMID: 11104651; Google Scholar]
7. *Crestanello J.A., Doliba N.M., Babsky A.M., Doliba N.M., Niibori K., Whitman G.J., Osbakken M.D.* Ischemic preconditioning improves mitochondrial tolerance to experimental calcium overload. **Journal of Surgical Research**, 2002; 103 (2): 243–251.
[DOI: https://doi.org/10.1006/jsre.2001.6361; PMID: 11922741; Google Scholar]
8. *Dogan M.F., Yildiz O., Arslan S.O., Ulusoy K.G.* Potassium channels in vascular smooth muscle: a pathophysiological and pharmacological perspective. **Fundam Clin Pharmacol**, 2019; 33 (5): 504–523.
[DOI: https://doi.org/10.1111/fcp.12461; PMID: 30851197; Google Scholar]
9. *Donato M., Goyeneche M.A., Garces M., Marchini T., Pérez V., Del Mauro J., Höcht C., Rodríguez M., Evelson P., Gelpi R.J.* Myocardial triggers involved in activation of remote ischaemic preconditioning. **Exp Physiol**, 2016; 101(6): 708–716.
[DOI: https://doi.org/10.1113/EP085535; PMID: 27028009; Google Scholar]
10. *Du Q., Jovanović S., Tulić L., Sljivančanin D., Jack D.W., Zižić V., Abdul K.S., Tulić I., Jovanović A.* K_{ATP} channels are up-regulated with increasing age in human myometrium. **Mech Ageing Dev**. 2013; 134 (3-4): 98–102.
[DOI: https://doi.org/10.1016/j.mad.2013.01.003; PMID: 23369859; Google Scholar]
11. *Du Q., Jovanović S., Tulić L., Tulić I., Jovanović A.* Pregnancy-induced hypertension is associated with down-regulation of Kir6.1 in human myometrium. **Pregnancy Hypertens**, 2019; 18: 96–98.
[DOI: https://doi.org/10.1016/j.preghy.2019.09.008; PMID: 31585348; Google Scholar]
12. *Foster M.N., Coetzee W.A.* K_{ATP} Channels in the Cardiovascular System. **Physiol Rev**, 2016; 96(1): 177–252.
[DOI: https://doi.org/10.1152/physrev.00003.2015; PMID: 26660852; Google Scholar]
13. *Greenwood I.A., Tribe R.M.* Kv7 and Kv11 channels in myometrial regulation. **Exp Physiol**, 2014; 99 (3): 503–509.
[DOI: https://doi.org/10.1113/expphysiol.2013.075754; PMID: 24121285; Google Scholar]

14. *Hamada Y., Nakaya Y., Hamada S., Kamada M., Aono T.* Activation of K^+ channels by ritodrine hydrochloride in uterine smooth muscle cells from pregnant women. **Eur J Pharmacol**, 1994; 288(1): 45–51.
[DOI: https://doi.org/10.1016/0922-4106(94)90008-6; PMID: 7705467; Google Scholar]
15. *Hantooshzadeh S., Rezaeizadeh G., Nikfar S., Ghiasi S., Abdollahi M.* A systematic review of the uterine relaxant effect of potassium channel openers. **Curr Drug Discov Technol**, 2013; 10(4): 263–269.
[DOI: https://doi.org/10.2174/15701638113109990002; PMID: 23713490; Google Scholar]
16. *Hempenstall A., Grant G.D., Anoopkumar-Dukie S., Johnson P.J.* Pyocyanin inhibits both nitric oxide-dependent and -independent relaxation in porcine coronary arteries. **Clin Exp Pharmacol Physiol**, 2015; 42(2): 186–191.
[DOI: https://doi.org/10.1111/1440-1681.12340; PMID: 25399964; Google Scholar]
17. *Hong S.H., Kyeong K.S., Kim C.H., Kim Y.C., Choi W., Yoo R.Y., Kim H.S., Park Y.J., Ji I.W., Jeong E.H., Kim H.S., Xu W.X., Lee S.J.* Regulation of myometrial contraction by ATP-sensitive potassium (K_{ATP}) channel via activation of SUR2B and Kir 6.2 in mouse. **J Vet Med Sci**, 2016; 78(7): 1153–1159.
[DOI: https://doi.org/10.1292/jvms.15-0700; PMID: 27086859; Google Scholar]
18. *Kaleli-Durman D., Alp-Yıldırım F.İ., Özdemir O., Uydeş-Doğan B.S.* Relaxant effect of atorvastatin on isolated rat gastric fundus strips: implications for Ca^{2+}-signalling mechanisms. **Can J Physiol Pharmacol**, 2019; 97 (5): 413-421.
[DOI: https://doi.org/10.1139/cjpp-2018-0723; PMID: 30730760; Google Scholar]
19. *Katakam P.V., Gordon A.O., Sure V.N., Rutkai I., Busija D.W.* Diversity of mitochondria-dependent dilator mechanisms in vascular smooth muscle of cerebral arteries from normal and insulin-resistant rats. **Am J Physiol Heart Circ Physiol**, 2014; 307(4): H493–503.
[DOI: https://doi.org/10.1152/ajpheart.00091.2014; PMID: 24929852; Google Scholar]
20. *Kazaryan K.V., Piliposyan T.A.* The ovarian region of the myometrium and its role in the regulation of spontaneous electrical activity. **Medical science of Armenia NAS RA**, 2016; LVI (2): 46–54. (In Russian)
[Google Scholar]
21. *Kazaryan K.V., Unanyan N.G., Mkrtchyan A.V.* Interaction of pacemaker activities of the extreme parts of the fallopian tubes in non-pregnant rats. **Reports of NAS of Armenia**, 2014; 114(1): 69–75. (In Russian)
[Google Scholar]
22. *Kowaltowski A.J., Seetharaman S., Paucek P., Garlid K.D.* Bioenergetic consequences of opening the ATP-sensitive K(+) channel of heart mitochondria. **Am J Physiol Heart Circ Physiol**, 2001; 280(2): H649-57.
[PMID: 11158963; Google Scholar]
23. *Kim J.Y., Wu W.H., Jun J.H., Sohn J., Seo Y.S.* Effects of corticotropin-releasing hormone on the expression of adenosine triphosphate-sensitive potassium channels (Kir6.1/SUR2B) in human term pregnant myometrium. **Obstet Gynecol Sci**, 2018; 61(1): 14–22.
[DOI: https://doi.org/10.5468/ogs.2018.61.1.14; PMID: 29372145; Google Scholar]
24. *Kraljevic J., Høydal M.A., Ljubkovic M., Moreira J.B., Jørgensen K., Ness H.O., Bækkerud F.H., Dujic Z., Wisløff U., Marinovic J.* Role of K_{ATP} Channels in Beneficial Effects of Exercise in Ischemic Heart Failure. **Med Sci Sports Exerc**, 2015; 47(12): 2504–2512.
[DOI: https://doi.org/10.1249/MSS.0000000000000714; PMID: 26057940; Google Scholar]
25. *Lovasz N., Ducza E., Gaspar R., Falkay G.* Ontogeny of sulfonylurea-binding regulatory subunits of K(ATP) channels in the pregnant rat myometrium. **Reproduction**, 2011; 142(1): 175–181.
[DOI: https://doi.org/10.1530/REP-10-0492; PMID: 21527399; Google Scholar]
26. *Lovasz N., Koncz A., Domokos D., Gaspar R., Falkay G.* ATP-sensitive potassium channels modulate *in vitro* tocolytic effects of β_2-adrenergic receptor agonists on uterine muscle rings in rats in early but not in late pregnancy. **Croat Med J**, 2015; 56(2): 114–118.
[DOI: https://doi.org/10.3325/cmj.2015.56.114; PMID: 25891870; Google Scholar]

27. *Novakovic R., Ilic B., Beleslin-Cokic B., Radunovic N., Heinle H., Scepanovic R., Gojkovic-Bukarica L.* The effect of resveratrol on contractility of non-pregnant rat uterus: the contribution of K^+ channels. **J Physiol Pharmacol**, 2013; 64: 6, 795–805.
[PMID: 24388895; Google Scholar]
28. *Sawada K., Morishige K., Hashimoto K., Tasaka K., Kurachi H., Murata Y., Kurachi Y.* Gestational change of K^+ channel opener effect is correlated with the expression of uterine K_{ATP} channel subunits. **Eur J Obstet Gynecol Reprod Biol**, 2005; 122(1): 49–56.
[DOI: https://doi.org/10.1016/j.ejogrb.2004.11.026; PMID: 16154039; Google Scholar]
29. *Tsymbalyuk O.V., Naumenko A.M., Davidovska T.L.* Influence of nano-TiO_2 on the functioning of gastric smooth muscles: an *in vitro* and *in silico* studies. **Studia Biologica**, 2019; 13(1): 3–26.
[DOI: https://doi.org/10.30970/sbi.1301.592; Google Scholar]
30. *Tsymbalyuk O.V.* Effect of calixarene C-99 on contractile activity of rat colon muscles. **Studia Biologica**, 2016; 10(3–4): 33–46. (In Ukrainian)
[DOI: https://doi.org/10.30970/sbi.1003.503; Google Scholar]
31. *Tsymbalyuk O.V.* Kinetics of relaxation of rat myometrium in conditions of inhibition of plasma membrane calcium pump and systems of active Ca^{2+} transport of intracellular Ca^{2+}-depot. **Studia Biologica**, 2018; 12(2): 3–12.
[DOI: https://doi.org/10.30970/sbi.1202.565; Google Scholar]
32. *Vadzyuk O., Kosterin S.* Activation of glybenclamide-sensitive mitochondrial swelling under induction of cyclosporin of A-sensitive mitochondrial pore. **Ukr Biochem J**, 2014; 86(4): 51–60.
[DOI: https://doi.org/10.15407/ubj86.04.051; PMID: 25509183; Google Scholar]
33. *Vadzyuk O.B., Kosterin S.O.* Mitochondria from rat uterine smooth muscle possess ATP-sensitive potassium channel. **Saudi J Biol Sci**, 2018; 25(3): 551–557.
[DOI: https://doi.org/10.1016/j.sjbs.2016.01.045; PMID: 29686518; Google Scholar]
34. *Wan X., Zhao J., Xie J.* Effects of mitochondrial ATP-sensitive K(+) channel on protein kinase C pathway and airway smooth muscle cell proliferation in asthma. **J Huazhong Univ Sci Technolog Med Sci**, 2012; 32(4): 480–484.
[DOI: https://doi.org/10.1007/s11596-012-0083-x; PMID: 22886957; Google Scholar]
35. *Wray S.* Insights from physiology into myometrial function and dysfunction. **Exp Physiol**. 2015; 100(12): 1468–1476.
[DOI: https://doi.org/10.1113/EP085131; PMID: 26289390; Google Scholar]
36. *Zhao G., Kaplan A., Greiser M., Lederer W.J.* The surprising complexity of K_{ATP} channel biology and of genetic diseases. **J Clin Invest**, 2020; 130(3): 1112–1115.
[DOI: https://doi.org/10.1172/JCI135759; PMID: 32065592; Google Scholar]

УЧАСТЬ $K_{АТФ}$-КАНАЛІВ ПЛАЗМАТИЧНОЇ І МІТОХОНДРІАЛЬНОЇ МЕМБРАН У ПІДТРИМАННІ СКОРОЧУВАЛЬНОЇ ФУНКЦІЇ МІОМЕТРІЯ НЕВАГІТНИХ ЩУРІВ

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У вісцеральних гладеньких м'язах суттєве значення в регуляції процесів скорочення-розслаблення відіграють K^+-канали, зокрема, АТФ-чутливі K^+-канали плазматичної та внутрішньої мітохондріальної мембран. Властивості цих каналів і їхня роль у підтриманні збудливості добре досліджена в серцевому м'язі та гладеньких

м'язах судин. Утім, на тепер інформації про участь цих каналів, особливо мітохондріальних, у регуляції скорочень біометрія практично немає. У роботі з використанням методів тензометрії та механокінетичного аналізу проведено дослідження закономірностей окситоцин-викликаних скорочень гладеньких м'язів невагітного міометрія щурів в умовах модуляції АТФ-чутливих K^+-каналів плазматичної та внутрішньої мітохондріальної мембран. **Встановлено**, що передінкубація міометрія у наявності блокаторів $K_{АТФ}$-каналів плазматичної мембрани і мітохондрій (глібенкламідом і 5-гідроксидеканоатом) загалом супроводжується зростанням фазного і в окремих випадках тонічного компонентів окситоцин-викликаних скорочень. На тлі дії глібенкламіду (діапазон використаних концентрацій 1–10 мкМ) спостерігали підвищення амплітуди окситоцинових скорочень без достовірних змін площі під цими механограмами; також значно зростала швидкість розслаблення. Блокатор мітохондріальних АТФ-чутливих K^+-каналів 5-гідроксидеканоат (у концентраціях 50 і 200 мкМ) спричиняв суттєві зміни кінетики процесів наростання (збільшення швидкості) і спаду (у понад два рази зменшення швидкості) сили окситоцинових скорочень, проте не впливав на їхню амплітуду і площу під механограмами. Найсуттєвішим ефектом активатора АТФ-чутливих K^+-каналів діазоксиду (діапазон використаних концентрацій 50–200 мкМ) було зменшення площі під окситоциновими скороченнями удвічі; також ця сполука зумовлювала зниження амплітуди скорочень і значне зростання максимальних швидкостей фаз наростання і спаду сили окситоцинових скорочень. Сумісна дія блокаторів $K_{АТФ}$-каналів плазматичної та внутрішньої мітохондріальної мембран глібенкламіду (10 мкМ) і 5-гідроксидеканоату (200 мкМ) супроводжувалася зростанням амплітуди окситоцинових скорочень, а також змінами кінетики процесів скорочення й розслаблення. Отже, отримані дані доводять, що у міометрії невагітних щурів $K_{АТФ}$-канали плазматичної мембрани та мітоК$_{АТФ}$-канали відіграють модуляторну роль у формуванні скорочувальної реакції на утеротонічний гормон окситоцин. Ці канали залучені до регуляції скорочувальної функції міометрія, модулюючи амплітуду скорочень, здатність до тривалого утримання напруження, а також кінетику процесів скорочення і розслаблення.

Ключові слова: гладенькі м'язи, міометрій, АТФ-чутливі K^+-канали плазматичної мембрани і мітохондрій, механокінетика

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