



Potassium chloride released from contracting skeletal muscle may stimulate development of its hypertrophy



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ABSTRACT

The effects of potassium chloride on the expression of IGF-1 splice forms and myoblast proliferation were investigated. KCl at the concentrations of 7–12 mM stimulated the synthesis of IGF-1 and mechano growth factor (MGF) in murine myoblasts as well as in myotubes both at the mRNA and protein levels. Pan-calcium channel blocker CdCl₂ completely abolished stimulation of growth factor expression, whereas blocker of HCN and Na_v1.4 channels ZD7288 drastically reduced it. In addition, potassium chloride stimulated myoblast proliferation, while IGF-1 autocrine signaling inhibition partially suppressed these mitogenic effects.

1. Introduction

Mechanisms of skeletal muscle hypertrophy development as well as muscle regeneration after injury are yet incompletely understood. Substances released from damaged muscle cells play an important role in these processes via the action on normal neighbouring myoblasts and stimulation of myogenesis. For example, skeletal muscle homogenate has been shown to contain compounds activating transcription factor CREB that in turn stimulates myoblast proliferation [1]. Myofibrillar proteins titin and myomesin, as well as their individual domains, stimulate expression of insulin-like growth factor 1 splice forms including IGF-1 itself and mechano growth factor (MGF) and activate myoblast proliferation [2]. S100 protein stimulates myoblast proliferation via protein kinase ERK1/2 activation [3]. HMGB1 protein released from injured muscle stimulates myoblast differentiation via RAGE receptor [4]. Low-molecular mass compounds can stimulate myoblast proliferation as well: ATP released by muscle fibers into the extracellular environment after mechanical contraction or electrical activity shows a mitogenic effect on myoblasts via P2 receptor activation and ROS production [5].

Among the substances released from muscle cells during intensive contraction, potassium ion occupies a prominent place. Its concentration in the interstitium may reach 10–12 mM after intense exercise bouts [6]. Performing one-leg exercise test for 30 min leads to an increase in potassium concentration in the interstitium to 7–8 mM [7]. However, a possible role of K⁺ in the onset of skeletal muscle regeneration or hypertrophy development remains poorly investigated. An increase in extracellular potassium concentration leads to a decrease

in membrane potential and cell depolarization, resulting in the opening of voltage-dependent ion channels. The most important among them are calcium channels, because Ca²⁺ signaling can stimulate expression of IGF-1 splice forms [8–10] and activate proliferation of different cell types including myoblasts [2,11–13]. On the other hand, voltage-dependent Ca²⁺ channels demonstrate refractoriness after opening [14,15] that adds more complexity to the overall picture. As a result, the involvement of K⁺ released from muscle cells during high mechanical strain in the development of muscle hypertrophy has not been yet shown.

The aim of this work was to investigate the influence of short-term exposure of cells to potassium ion on the expression of IGF-1 splice forms and on myoblast proliferation.

2. Materials and methods

Cell culture. Primary murine myoblast cultures were obtained as usual [16]. Cells were cultivated in a growth medium consisting of DMEM and 10% fetal calf serum (FCS) at 37 °C with 6% CO₂. All experiments were performed using cells from 3 to 5 passages. Purity of the myoblast cultures (> 95%) was evaluated by *anti-desmin* immunostaining.

The experiments on the induction of IGF-1 splice forms in myoblasts were performed as follows. 2 × 10⁵ primary myoblasts were seeded on a 60 mm Petri dish (Corning-Costar, USA) in 5 ml of the growth medium (DMEM with 10% FCS) and left overnight. After that, the medium was replaced with a growth medium containing potassium chloride at different concentrations, wherein cells were incubated for two hours. Then

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the medium with KCl was replaced with simple growth medium for different time intervals, after which the expression of IGF-1Ea and MGF was measured.

For the experiments with differentiated myotube cultures, primary myoblasts (10^6 cells per 60 mm Petri dish) were cultured in a differentiation medium (DMEM with 2% horse serum) for 5 days. After that, the medium was replaced by the differentiation medium containing potassium chloride at different concentrations, wherein cells were incubated for two hours. Then the medium with KCl was replaced with simple differentiation medium for different time intervals, after which the expression of IGF-1 and MGF was measured.

In some experiments, the incubation of cells in medium with potassium chloride was performed in the presence of ion channel blockers (all from Sigma-Aldrich, USA). The following blockers were used: CdCl₂ (pan-calcium channels), nifedipine (L-type calcium channels), NiCl₂ (Ca_v3.2 channels), ZD7288 (HCN channels).

MTT assay. Cell proliferation was assessed by the MTT assay that was employed routinely [17]. Briefly, 2×10^3 murine myoblasts per well were plated on a 96-well culturing plate (Corning Costar, USA) for 24 h in DMEM with 10% FCS. After that, the cells were serum-starved in DMEM supplemented with 0.5% FCS for 10 h, followed by the addition of potassium chloride and incubation of the cells for two hours. Then KCl-rich medium was replaced with DMEM containing 0.5% FCS and the cells were incubated for 72 h. In some experiments, the cultivation of cells was carried out in the presence of antibodies to IGF-1 (R&D Systems, USA). Then the medium was replaced with DMEM containing 0.5% FCS and 0.5 mg/ml MTT, and the cells were incubated for 4 h. After that, the medium was replaced with 150 μ l of dimethyl sulfoxide (Sigma-Aldrich, USA), and the absorbance at 595 nm was measured using a plate reader (Thermo Labsystems, USA). Each point represents an average value of 8 experiments.

Quantitation of mRNA of IGF-1 splice forms. The aim of this work was to investigate the influence of short-term exposure of cells to potassium ion on the expression of IGF-1 splice forms and on myoblast proliferation. Total RNA was isolated with the TRIZOL reagent (Invitrogen, USA) according to the manufacturer's protocol and reverse-transcribed using an RT kit (Sileks, Russia). Quantitative real-time PCR (QRT-PCR) was performed with a CFX96 system (Bio-Rad, USA) using a reagent kit (Syntol, Russia) containing SYBR Green. GAPDH was used as a reference gene. The following PCR primers were used: IGF-1Ea, forward, 5'-TTCAGTTCGTGTGTGGACCGAG-3', reverse, 5'-TCCACAATGCCTGTCTGAGGTG-3' [18]; IGF-1Eb (also named MGF), forward, 5'-TTCAGTTCGTGTGTGGACCG, reverse, 5'-TGTTTGTGATAGGGACGG-3' [19]; GAPDH, forward, 5'-TGGCAAAGTGGAGATTGTTGCC-3', reverse, 5'-AAGATGGTGATGGGCTTCCCG-3' [20].

The relative expression of IGF-1Ea and MGF was calculated using $2^{-\Delta\Delta CT}$ method. Four independent experiments were performed for each experimental condition and the averages (\pm SEM) of the results are presented in the figures. The quantitative values were normalized to the samples of mRNA from the untreated cells taken as a control. A single melting peak and a single band on 2% agarose gel electrophoresis were detected for each product of amplification.

Quantitation of MGF and IGF-1 proteins. In order to determine the concentration of secreted IGF-1 protein, cell culture supernatants from a 60 mm Petri dish were taken and IGF-1 levels were measured using the Quantkine ELISA kit (R&D Systems, USA) according to the manufacturer's instructions.

The MGF concentration was measured in cell lysates because the sensitivity of the test system used was insufficient for determining MGF concentrations in culture supernatants. The cells on a 60 mm Petri dish were lysed by pipetting in 0.2 ml PBS containing 0.05% Tween 20 (PBS-T) and a mixture of protease inhibitors (Sigma-Aldrich, USA). The MGF concentration was measured with a sandwich ELISA assay based on monoclonal antibodies as described earlier [21] using an amplification system. Microtiter plates (Corning-Costar, USA) were coated with 8B9 monoclonal antibody solution (10 μ g/ml) overnight at 4 °C. After

washing with PBS-T, the plates were incubated with 100 μ l of cell lysates for 1 h at 37 °C. After washing the plates with PBS-T, the conjugate of alkaline phosphatase with 4F3 monoclonal antibody (1:10,000 dilution) was added for 1 h at 37 °C, and then washed away. The plates were developed using an ELISA amplification system (Invitrogen, USA) according to the manufacturer's instructions. The absorbance was measured at 492 nm using a plate reader (Thermo Labsystems, USA).

Four independent experiments were performed for each experimental condition and the averages (\pm SEM) of the results are shown in the figures.

MGF concentrations were normalized per mg of total protein. Total protein concentration was determined using the Lowry method with the Bio-Rad DC Protein Assay kit.

Western blotting. Electrophoresis and Western blotting were performed as described earlier [21]. 8B9 monoclonal antibodies were used for detection of MGF protein bands. These antibodies recognized 87–111 amino acids in the MGF structure that are present in MGF protein only and are absent in the IGF-1 protein. Cells were lysed in 62.5 mM Tris-HCl buffer, pH 6.8, with 2% SDS, 1% β -mercaptoethanol and a mixture of protease inhibitors (Sigma-Aldrich, USA). After heating at 95 °C for 5 min, 10 μ g of total protein was separated by 12% SDS PAGE and transferred to a nitrocellulose membrane. The membrane was incubated in 3% BSA solution in PBS overnight, then in the solution of 8B9 monoclonal antibody conjugated with peroxidase in 3% BSA in PBS for 1 h at 37 °C. MGF bands were visualized by exposure to X-ray film after incubation with the Amersham ECL Western Blotting Detection Kit (GE Healthcare, USA). Then membrane was twice stripped in 0.2 M glycine with 0.1% SDS and 1% Tween 20, pH 2.2, for 20 min. After washing, the membrane was blocked with 3% BSA in PBS again, incubated in a 1 μ g/ml GAPDH antibody solution (Thermo Fisher Scientific, USA) for 1 h at 37 °C. After washing the membrane was incubated in the solution of secondary anti-mouse IgG antibody-peroxidase conjugate (Imtek, Russia). GAPDH bands were visualized with diaminobenzidine. Quantification was done with GelAnalyzer 2010 software.

Statistical analysis. Data are presented as mean \pm standard error of the mean. Statistical significance of difference between each experimental group and the control was determined using the two-tailed Student's *t*-test. The difference among the means of multiple groups was analyzed by one-way analysis of variance followed by the Tukey test. A difference was defined as significant at $p < 0.05$.

3. Results and discussion

Incubation of muscle cells in the medium containing potassium chloride at the concentration of 12 mM for two hours triggers activation of expression of both IGF-1 splice forms. In the case of myoblasts, the maximal elevation of expression was observed 2 h after the incubation, accompanied by an almost 2.4-fold increase in IGF-1 splice form mRNA levels (Fig. 1A). 4 h after the incubation, the levels of these two mRNAs remained markedly increased. 12 h after the incubation, stimulation became less pronounced with MGF and IGF-1Ea mRNA levels increased 1.7–1.8-fold. No statistically significant stimulation effect was observed 24 h after the incubation.

In the case of myotubes, activation of expression took place 4 h after the incubation; MGF and IGF-1Ea mRNA levels increased 1.8–1.9-fold. 12 h after the incubation, the stimulation effect diminished with MGF and IGF-1Ea mRNA levels increased only approximately 1.7-fold.

Experiments with incubation of muscle cells for two hours in media containing different amounts of potassium chloride demonstrated that statistically significant stimulation of growth factor mRNA expression in myoblasts occurred at the KCl concentration of 8 mM with MGF and IGF-1Ea mRNA levels increased 1.7–1.9-fold (Fig. 1B). At this concentration, stimulation effects in myotubes are statistically insignificant. Incubation of cells of both types at the KCl concentration of 9 mM leads to a reliable increase in MGF and IGF-1 mRNA expression levels:

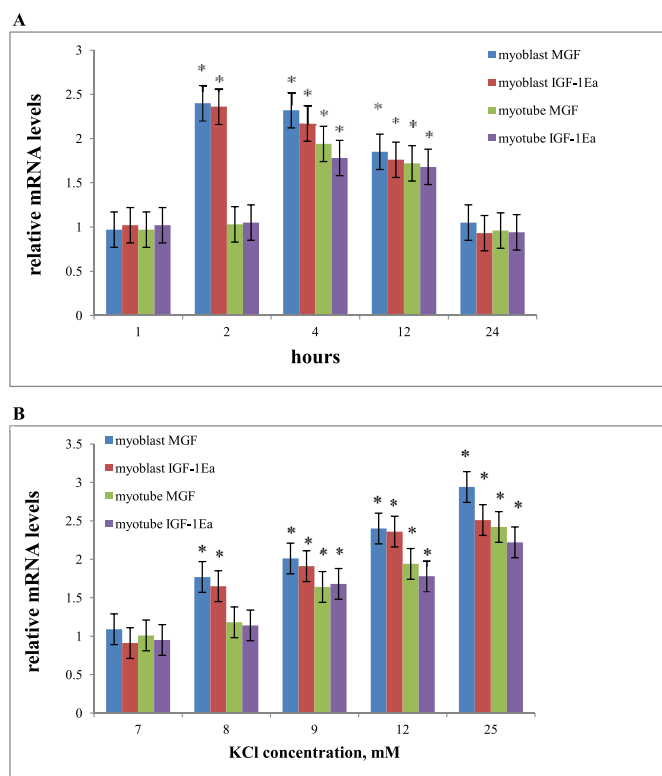


Fig. 1. Stimulation of IGF-1 splice form mRNA expression by potassium chloride. Myoblasts or myotubes were incubated for two hours with 12 mM KCl and cultured in growth (myoblasts) or differentiation (myotubes) media for different time intervals (A) or incubated with different concentrations of KCl and cultured for 2 h in the case of myoblasts and 4 h in the case of myotubes (B). Once total RNA was isolated and reverse-transcribed, the relative amount of MGF mRNA was determined by QRT-PCR (the reference gene was GAPDH). The quantitative values were normalized to the samples of mRNA from the cells incubated in media without additives taken as control. Data are presented as mean \pm S.E.M. Significantly different from control values: * $p < 0.05$.

1.9 – 2-fold in the case of myoblasts and 1.6–1.7-fold in the case of myotubes. Raising KCl concentration to 12 mM enhances the stimulation effects (values for mRNA levels are given above). The maximal induction of IGF-1 splice forms was achieved at the KCl concentration of 25 mM: MGF mRNA levels increased 2.5–2.9-fold, IGF-1Ea mRNA levels – 2.2–2.4-fold.

Incubation of myoblasts and myotubes in the medium containing sodium chloride at the same concentrations does not lead to activation of IGF-1 splice form expression (data not shown). Trypan blue staining showed the absence of cytotoxic effects of media containing KCl or NaCl at these concentrations.

Expression of IGF-1 splice forms on protein level demonstrated similar results. No measurable expression of MGF was observed in non-stimulated murine myoblasts or myotubes. Incubation of myoblasts for two hours in a medium supplemented with 12 mM KCl increased the concentration of secreted IGF-1 protein from 94 pg/ml in control cells to 181 pg/ml in treated ones 12 h later (Fig. 2A). Such incubation stimulated the expression of MGF protein to 68 pg per mg of the total protein also (Fig. 2B). In myotubes, the expression of IGF-1 and MGF proteins behaved similarly. The maximal stimulation was observed 24 h after the incubation. The MGF protein level reached 120–135 pg per mg of total protein, while the concentration of secreted IGF-1 rose to 280–300 pg/ml 36 h after the incubation, stimulation effect became less pronounced. The MGF level fell to 72–77 pg per mg, whereas the IGF-1 concentration decreased to 156–163 pg/ml. No stimulation effect was observed 48 h after the incubation.

Experiments with incubation of muscle cells for two hours in media

containing different amounts of potassium chloride demonstrated that statistically significant stimulation of IGF-1 protein expression took place in myoblasts at the KCl concentration of 7 mM, when its level reached 136 pg/ml (Fig. 2C). At these conditions, there was no statistically significant activation of IGF-1 expression in myotubes. An increase in KCl concentration to 8 mM led to elevation of secreted IGF-1 level to 187 pg/ml in myoblast cultures and to 139 pg/ml in myotube ones. The higher KCl concentration, the higher secreted IGF-1 level. The maximal stimulation was observed at the KCl concentration of 25 mM with the concentration of secreted IGF-1 exceeding 320 pg/ml.

The MGF protein behaved in a similar manner (Fig. 2D). At the KCl concentration of 7 mM, the MGF protein level in myoblasts comprised only 34 pg per mg of total protein (Fig. 2C). At the KCl concentration of 8 mM, statistically reliable stimulation was observed both in myoblasts and myotubes. An increase in KCl concentration caused an enhancement of the activation effect. The maximal stimulation was observed at the KCl concentration of 25 mM with MGF level comprising 150–160 pg/ml.

The stimulation effect of potassium chloride on the MGF protein expression was confirmed by Western blotting (Supplementary data). Protein bands were detected by monoclonal antibodies recognizing MGF in the lysates of myoblasts treated with KCl at the concentrations of 7–12 mM (lanes 1–4). Molecular masses of detected protein bands were about 12 kDa, whereas m.m. of pro-IGF-1Eb protein is 12.5 kDa, which is in good agreement with our results. At the same time, such protein band was absent in the lysates of untreated cells (lane 5) or cells treated with NaCl (lanes 6–8).

We investigated the effects of blockers of ion channels on the observed IGF-1 splice form induction by potassium chloride. All investigated blockers were not cytotoxic at the concentration range studied. Treatment with 100 μ M cadmium chloride completely abolished the stimulation effects of KCl. In the presence of this blocker, the levels of mRNAs of IGF-1 splice forms (Fig. 3A), secreted IGF-1 protein (Fig. 3B) and MGF protein (Fig. 3C) did not differ from the control values, whereas CdCl₂ by itself had no influence on IGF-1 splice form expression. Nifedipine at 10 μ M and NiCl₂ at 10 μ M did not reduce the stimulation effect of KCl. In contrast, ZD7288 at 100 μ M caused a significant impairment of IGF-1 splice form expression. In the case of myotubes, the inhibition was complete, and both the level of secreted IGF-1 and the level of MGF did not differ from the control. In the case of myoblasts, only a partial inhibition was observed. The IGF-1Ea and MGF mRNA levels decreased to 48% and 61% of the levels observed without the blocker, respectively. In a similar manner, the MGF protein expression and secreted IGF-1 concentration decreased to 47% and 63%, respectively.

We tested the effect of incubation at hyperkalaemic conditions on myoblast proliferation rate. Incubation of cells for two hours in the medium containing potassium chloride at the concentration of 8 mM triggered statistically significant activation of cell proliferation: MTT reduction in treated cells increased by 27% relative to the control (Fig. 4A). Increasing KCl concentration enhanced the stimulation effect. The maximal stimulation of proliferation was observed at the KCl concentration of 25 mM: the MTT reduction increased by 212 \pm 3% relative to the control. The incubation of myoblasts in the medium containing sodium chloride at the same concentrations did not result in the activation of proliferation.

The HCN channel blocker ZD7288 partially reduced the mitogenic action of potassium chloride with the MTT reduction by myoblast cultures diminished to 70% of the level observed without the blocker (Fig. 4B). Inhibition of IGF-1 signaling also led to a statistically reliable (though not complete) decrease in the observed mitogenic effect. Neutralizing antibodies against this growth factor at the concentration of 10 μ g/ml lowered the MTT reduction to 63% of the level observed without the inhibitor, while the antibodies by themselves had no effect on cell proliferation.

Skeletal muscle hypertrophy develops after intense training via

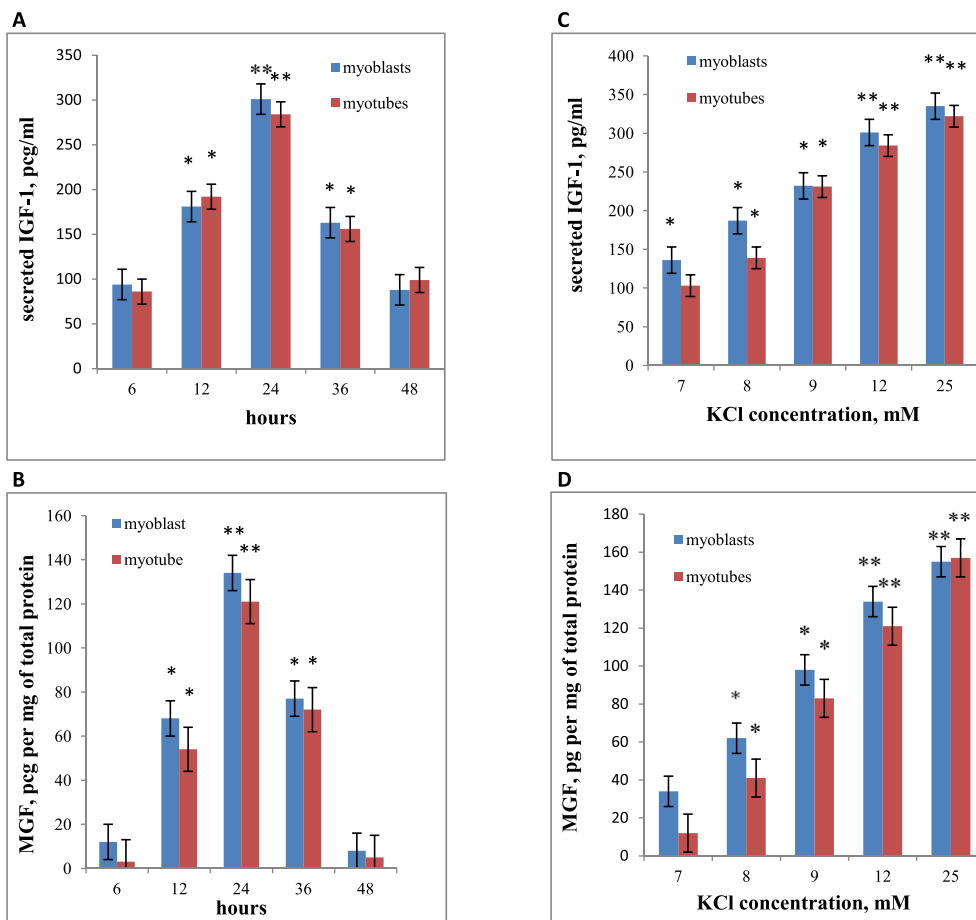


Fig. 2. Stimulation of IGF-1 (A,C) and MGF (B,D) protein expression by potassium chloride. Myoblasts or myotubes were incubated for two hours with 12 mM KCl and cultured in growth (myoblasts) or differentiation (myotubes) media for different time intervals (A,B) or incubated with different concentrations of KCl and cultured for 24 h (C,D). Secreted IGF-1 concentrations were measured in culture supernatants with the Quantikine ELISA kit (A,C). MGF protein level was measured in cell lysates by sandwich ELISA with signal amplification (B,D). Data are presented as mean \pm S.E.M. Significantly different from control values: * $p < 0.05$, ** $p < 0.01$.

many different mechanisms. Despite active investigations, they remain not fully understood. It stands to reason that compounds released from muscle cells during vigorous contraction may be used in the process of skeletal muscle growth. We demonstrated that potassium chloride activates the expression of two splice forms of IGF-1 in muscle cells. Stimulation took place both at the mRNA and at the protein levels; it was observed in myoblasts and myotubes. Activation of growth factor expression was seen after incubation of cells at the KCl concentration of 7–12 mM for two hours, which is consistent with conditions existing during intensive muscle work. It should be noted that muscle exercise needs to be quite vigorous to achieve sufficiently high KCl concentrations for a relatively long time interval, which helps prevent unneeded myoblast proliferation in response to accidental noise.

The observed stimulation was specific for potassium cation and not caused by an increase in osmotic pressure because sodium chloride at the same concentration did not activate growth factor expression or myoblast proliferation.

Myoblasts are not involved in muscle contraction but they play the key role in the processes of muscle hypertrophy development and regeneration after injury. Potassium chloride released from contracting myotubes may affect neighbouring myoblasts. In a similar manner, IGF-1 secreted by myotubes under the influence of KCl may activate the proliferation of myoblasts located in the proximity via a paracrine mechanism. We can not measure the secreted MGF concentration in our experiments because the sensitivity of used ELISA test does not achieve 20 pg/ml. However we suppose that this growth factor can also act via the same mechanism. Myoblasts may begin to secrete IGF-1 splice forms in response to KCl released from myotubes, and these growth factors would activate the proliferation of myoblasts themselves via an autocrine mechanism.

We observed that opening of calcium channels is absolutely

necessary for the mitogenic effect of potassium chloride. Cadmium chloride that totally blocks all Ca^{2+} channels completely eliminated the stimulation of growth factor expression caused by KCl. The mitogenic effect of the activation of calcium signaling has earlier been described on skeletal [2] and smooth muscle cells [22].

Neither L-type calcium channels nor $\text{Ca}_v3.2$ channels were involved in realization of potassium chloride effects because their specific blockers nifedipine and nickel chloride had no influence on the stimulation of IGF-1 splice form synthesis. The key role in the activation cascade can be played by HCN channels. A blocker of these channels, ZD7288, completely prevented an elevation of the IGF-1 splice form levels in myotubes and drastically reduced it in myoblasts. However, it was shown that ZD7288 can also block voltage-dependent $\text{Na}_v1.4$ channels [23]. If sodium channels play a role in the implementation of potassium chloride effects, Na^+ entrance in the cytoplasm would lead to activation of Ca^{2+} transport via $\text{Na}^+/\text{Ca}^{2+}$ exchanger. Cadmium chloride inhibits this exchanger also.

We demonstrated that potassium chloride not only activates the growth factor expression, but also stimulates myoblast proliferation. KCl appears to retain a pronounced proliferative effect under the conditions of IGF-1 signaling suppression by antibodies against this protein. At the same time, this suppression leads to a significant decrease in the mitogenic potential of this compound, so it may be hypothesized that IGF-1 signaling is partially involved in proliferative effects of potassium chloride.

It is notable that stimulation of IGF-1 and MGF protein expression was observed at the KCl concentration of 7 mM, whereas the activation of myoblast proliferation begins only at 8 mM KCl. It is possible that the growth factor concentration that is reached at 7 mM KCl is too low for stimulation of cell proliferation. On the other hand, IGF-1-independent mechanisms can be involved in the activation of myoblast divisions and

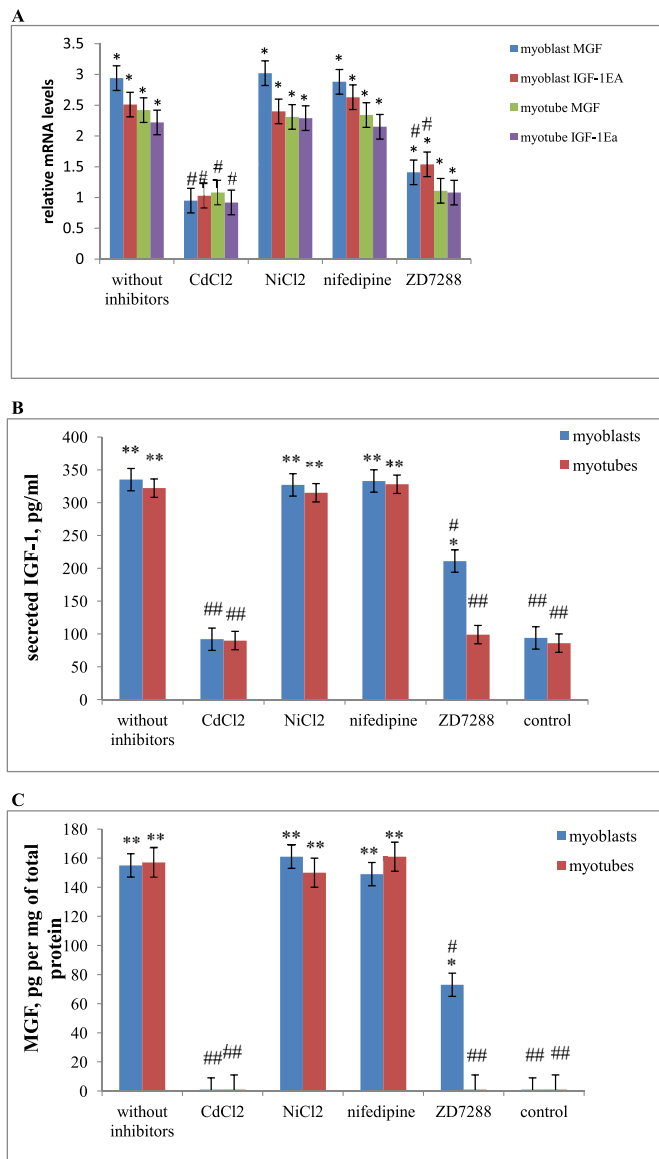


Fig. 3. Effects of several channel blockers on the IGF-1 (A,B) and MGF (A,C) induction by potassium chloride. Myoblasts or myotubes were incubated for two hours with 25 mM KCl with or without 100 μ M CdCl₂ or 10 μ M nifedipine or 10 μ M NiCl₂ or 100 μ M ZD7288. Then myoblasts were cultured for 2 h (A) or 24 h (B,C) in growth medium, and myotubes were cultured for 4 h (A) or 24 h (B,C) in differentiation medium. After that, the levels of mRNA (A) or secreted IGF-1 protein (B) or MGF protein (C) were measured. Data are presented as mean \pm S.E.M. Significantly different from control values: * p < 0.05, ** p < 0.01. Significantly different from values of samples treated with KCl without blockers: # p < 0.05, ## p < 0.01.

higher concentration of potassium chloride may be required for triggering of these mechanisms. An incomplete attenuation of the stimulation effect by antibodies to IGF-1 provides an argument in favor of the second hypothesis.

Mechanisms of development of muscle hypertrophy caused by intense exercise are still not completely understood. The observed activation of growth factor synthesis and myoblast proliferation by potassium chloride released from contracting muscle may reasonably be an important part of the physiological mechanism regulating skeletal muscle growth in response to intense muscle exercise.

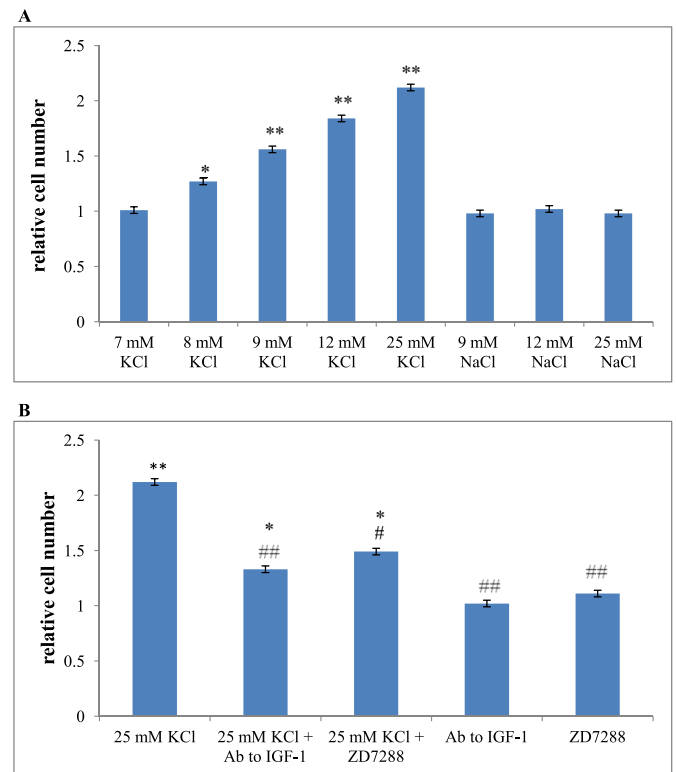


Fig. 4. Stimulation of myoblast proliferation by potassium chloride. Myoblasts were incubated for two hours with different concentrations of KCl or NaCl and cultured for 72 h without (A) or with (B) antibodies to IGF-1 at 10 μ g/ml or 100 μ M ZD7288, after which the MTT-test was performed. The relative cell number was calculated as $A_{\text{test cells}}/A_{\text{control cells}}$, where $A_{\text{test cells}}$ and $A_{\text{control cells}}$ are the absorbance of formazan for treated and untreated control cells, respectively. Data are presented as mean \pm S.E.M. Significantly different from control values: * p < 0.05, ** p < 0.01. Significantly different from values of samples treated with KCl without antibodies and blockers: # p < 0.05, ## p < 0.01.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrep.2019.100627>.

References

- [1] R. Stewart, L. Flechner, M. Montminy, R. Berdeux, CREB is activated by muscle injury and promotes muscle regeneration, *PLoS One* 6 (9) (2011) e24714.
- [2] I.V. Kravchenko, V.A. Furalyov, V.O. Popov, Specific titin and myomesin domains stimulate myoblast proliferation, *Biochem. Biophys. Rep.* 9 (2017) 226–231.
- [3] F. Riuizi, G. Sorci, R. Donato, S100B stimulates myoblast proliferation and inhibits myoblast differentiation by independently stimulating ERK1/2 and inhibiting p38 MAPK, *J. Cell. Physiol.* 207 (2006) 461–470.
- [4] F. Riuizi, G. Sorci, R. Sagheddu, R. Donato, HMGB1-RAGE regulates muscle satellite cell homeostasis through p38-MAPK- and myogenin-dependent repression of Pax7 transcription, *J. Cell Sci.* 125 (Pt 6) (2012) 1440–1454.
- [5] M. Sciancalepore, E. Luin, G. Parato, E. Ren, R. Giniatullin, E. Fabbretti, P. Lorenzon, Reactive oxygen species contribute to the promotion of the ATP-mediated proliferation of mouse skeletal myoblasts, *Free Radic. Biol. Med.* 53 (2012) 1392–1398.
- [6] M. Mohr, N. Nordsborg, J.J. Nielsen, L.D. Pedersen, C. Fischer, P. Krstrup, J. Bangsbo, Potassium kinetics in human muscle interstitium during repeated intense exercise in relation to fatigue, *Pflugers Arch.* 448 (2004) 452–456.
- [7] J.J. Nielsen, M. Mohr, C. Klarskov, M. Kristensen, P. Krstrup, C. Juel, J. Bangsbo, Effects of high-intensity intermittent training on potassium kinetics and performance in human skeletal muscle, *J. Physiol.* 554 (Pt 3) (2004) 857–870.
- [8] Y. Mikuni-Takagaki, Mechanical responses and signal transduction pathways in stretched osteocytes, *J. Bone Miner. Metab.* 17 (1999) 57–60.
- [9] I. Nagaoka, B.C. Trapnell, R.G. Crystal, Regulation of insulin-like growth factor I gene expression in the human macrophage-like cell line U937, *J. Clin. Invest.* 85 (1990) 448–455.
- [10] I.V. Kravchenko, V.A. Furalyov, S. Chatziefthimiou, M. Wilmanns, V.O. Popov,

- Induction of insulin-like growth factor 1 splice forms by subfragments of myofibrillar proteins, *Mol. Cell. Endocrinol.* 399 (2015) 69–77.
- [11] M. Illario, A.L. Cavallo, S. Monaco, E. Di Vito, F. Mueller, L.A. Marzano, G. Troncone, G. Fenzi, G. Rossi, M. Vitale, Fibronectin-induced proliferation in thyroid cells is mediated by α 5 β 1 integrin through Ras/Raf-1/MEK/ERK and calcium/CaMKII signals, *Clin. Endocrinol. Metab.* 90 (2005) 2865–2873.
- [12] S. Monaco, M. Illario, M.R. Rusciano, G. Gragnaniello, G. Di Spigna, E. Leggiero, L. Pastore, G. Fenzi, G. Rossi, M. Vitale, Insulin stimulates fibroblast proliferation through calcium-calmodulin-dependent kinase II, *Cell Cycle* 8 (2009) 2024–2030.
- [13] M. Rodríguez-Moyano, I. Díaz, N. Dionisio, X. Zhang, J. Avila-Medina, E. Calderón-Sánchez, M. Trebak, J.A. Rosado, A. Ordóñez, T. Smani, Urotensin-II promotes vascular smooth muscle cell proliferation through store-operated calcium entry and EGFR transactivation, *Cardiovasc. Res.* 100 (2013) 297–306.
- [14] E.A. Sobie, L.S. Song, W.J. Lederer, Local recovery of Ca²⁺ release in rat ventricular myocytes, *J. Physiol.* 565 (2005) 441–447.
- [15] E.D. Fowler, C.H. T Kong, J.C. Hancox, M.B. Cannell, Late Ca²⁺ sparks and ripples during the systolic Ca²⁺ transient in heart muscle cells, *Circ. Res.* 122 (2018) 473–478.
- [16] T.A. Rando, H.M. Blau, Primary mouse myoblast purification, characterization and transplantation for cell-mediated gene therapy, *J. Cell Biol.* 125 (1994) 1275–1287.
- [17] F. Denizot, R. Lang, Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability, *J. Immunol. Methods* 89 (1986) 271–277.
- [18] K. Waters, S. Safe, K.W. Gaido, Differential gene expression in response to methoxychlor and estradiol through ER α , ER β , and AR in reproductive tissues of female mice, *Toxicol. Sci.* 63 (2001) 47–56.
- [19] C. De Bari, F. Dell'Accio, F. Vandenabeele, J.R. Vermeesch, J.M. Raymakers, F.P. Luyten, Skeletal muscle repair by adult human mesenchymal stem cells from synovial membrane, *J. Cell Biol.* 160 (2003) 909–918.
- [20] J.C. Mills, A.J. Syder, C.V. Hong, J.L. Guruge, F. Raaij, J.I. Gordon, A molecular profile of the mouse gastric parietal cell with and without exposure to *Helicobacter pylori*, *Proc. Natl. Acad. Sci. U.S.A.* 98 (2001) 13687–13692.
- [21] I.V. Kravchenko, V.A. Furalyov, V.P. Khotchenkov, V.O. Popov, Monoclonal antibodies to mechano-growth factor, *Hybridoma* 25 (2006) 300–305.
- [22] M. Rodríguez-Moyano, I. Díaz, N. Dionisio, X. Zhang, J. Avila-Medina, E. Calderón-Sánchez, M. Trebak, J.A. Rosado, A. Ordóñez, T. Smani, Urotensin-II promotes vascular smooth muscle cell proliferation through store-operated calcium entry and EGFR transactivation, *Cardiovasc. Res.* 100 (2013) 297–306.
- [23] X. Wu, L. Liao, X. Liu, F. Luo, T. Yang, C. Li, Is ZD7288 a selective blocker of hyperpolarization-activated cyclic nucleotide-gated channel currents? *Channels (Austin)* 6 (2012) 438–442.