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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Simulated Microgravity Changes the Number of Mechanically Gated and Mechanosensitive Ion Channels Genes Transcripts in Rat Ventricular Cardiomyocytes

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Abstract—The mechanoelectrical feedback in the heart is based on the work of mechanically gated (MGCs) and mechanosensitive (MSCs) channels. Since microgravity alters the heart's morphological and physiological properties, we hypothesized that the expression of both MGCs and MSCs would be affected. We employed RNA transcriptome sequencing to investigate changes in the gene transcript levels of MGCs and MSCs in isolated rat ventricular cardiomyocytes under control conditions and in a simulated microgravity environment. For the first time, our findings demonstrated that simulated microgravity induces alterations in the gene transcript levels of specific MGCs, such as TRPM7, TRPV2, TRPP1, TRPP2, Piezo1, TMEM63A, TMEM36B, and known MSCs, including $K_{2P}2.1$, $K_{2P}3.1$, Kir6.2, Kir6.2, $Na_V1.5$, $Ca_V1.2$, $K_V7.1$. However, other voltage-gated channels and channels lacking a voltage sensor remained unaffected. These findings suggest that the altered expression of MGCs and MSCs could lead to changes in the net currents across the membrane, ultimately impacting the heart's function.

Keywords: rats, heart, ventricles, cardiomyocytes, mechanically gated and mechano-sensitive ion channels, microgravity, channel genes transcripts, genes expression

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Cardiomyocytes, as cells of many other organs, are sensitive to mechanical stress of any nature, and this manifests itself in the form of a pronounced change in their electrophysiological properties. This phenomenon, opposite to electromechanical coupling, is called mechanoelectric feedback, which plays an important role not only under normal conditions but also in heart pathologies, for example, as the basis of the mechanism of several cardiac arrhythmias [1]. Mechanoelectric feedback is realized due to the current of cations through ion channels activated by stretch (Mechanically Gated Channels: MGCs or Stretch-Activated Channels: SACs) [2], which causes a change in the resting membrane potential and the action potentials of cardiomyocytes [3, 4]. Stretching of isolated ventricular [5, 6] or atrial [7] cardiomyocytes has been shown to trigger a cation current through MGCs, which modulating the membrane potential of cardiac myocytes. Sensitivity to the stretch of heart cells is determined by its state relative to the norm. It is especially high in ventricle cardiomyocytes in rats' hearts with hypertrophy of any etiology [3–5], which the authors attribute to overexpression of MGCs. Exactly like the sensitivity of cardiomyocytes, the sensitivity of MGCs to stretch is especially high in isolated atrial cardiomyocytes from people with various cardiac pathologies [8]. In general, cardiomyocyte MGCs play an important role not only in normal heart function, but, above all, in pathological conditions.

At the same time, it is well known that alterations in gravity change the morphological and physiological properties of the heart. Therefore, microgravity in the space leads to atrophy of the heart and decreased cardiac function [9]. Studies conducted in this field during space flights revealed a redistribution of body fluid on the side of the head, as a result of which demonstrated a significant load on the cardiovascular system [10]. Prolonged exposure to microgravity leads to a decrease in the end-diastolic volume of the left ventricle and the stroke volume of the ventricle [11]. Furthermore, in rats housed in space for 14 days, a decrease in the average cross-sectional area of myocytes was shown in the left ventricular muscle, which indicated myocardial atrophy [12]. These data show that prolonged exposure to microgravity has a significant negative effect on the cardiovascular system.

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Importantly, similar changes have been found in the hearts of patients with bedridden and paraplegics [13].

We hypothesized that microgravity can affect both MGCs and MSCs of the cell plasma membrane and, above all, their expression level. There are fundamental differences between the principles of operation of these types of channels. As is known, MGCs, in essence, play the role of mechanoelectric transducer that convert mechanical energy into electrical energy using the gate mechanism of the channels. For them, mechanical stress realized through a change in the mechanical tension of the membrane (direct or through the cytoskeleton) serves as a full-fledged physiological stimulus that opens channels. The opening (activation) of MGCs lies in the microsecond range and persists throughout the duration of the mechanical factor [5, 6]. The majority of MGCs are cation nonselective, and some have potassium conductivity. Unlike MGCs, MSCs can be voltage- or ligand-gated, i.e. for them, a full-fledged physiological stimulus activating channels is a change in potential (if there is a voltage sensor in the structure of the domains of the S4 segment, for example, Na_V, Ca_V, K_V channels) or binding to a ligand (in the presence of a receptor). However, under mechanical stress, these channels change the conductivity of their ions. For example, stretching of a cardiomyocyte results in a decrease in Ca^{2+} current through the L-type Ca^{2+} channels of $Ca_V1.2$, which are MSCs.

It has been shown that stretching of ventricular cardiomyocytes of young healthy rats by 8–10 μm, which increases the sarcomere length from 1.8 to 2.2 μm, leads to the appearance of a maximum current through MGCs of -2.69 ± 0.17 nA at -80 mV [5, 6]. The increase in current can be explained by a change in the time and probability of the open state of the channel and, consequently, by the number of passing ions. However, further stretching of the cells does not lead to an increase in current. This may be due to the fact that, according to the rate theory for ion channels, no more than a certain number of ions can flow through each channel, since the channels are modeled as a sequence of energy barriers over which the ion must "jump", which determines the saturation of the transport speed phenomenon. At the same time, when heart cells are stretched in pathological conditions (for example, cells of a hypertrophied heart) by only 2–4 μm, the same current occurs as when cardiomyocytes of the hearts of healthy animals are stretched by 10 μm [5, 6], and further stretching leads to its increase. On the one hand, this could also be explained by a change in the functioning of MGCs under pathological conditions; however, on the other hand, taking into account the limitation of the ion flow through the channel in the framework of the reaction rate theory, this issue can be considered from the standpoint of an increase in channel expression [5], which increases current. In this regard, microgravity, first of all, should affect the expression level of MGCs and MSCs.

In this work, using the RNA-seq technique, we studied the change in the amount of mRNA of the MGCs and MSCs genes in isolated rat ventricular cardiomyocytes under control conditions and in a microgravity simulation. Regarding the cardiovascular system, a suitable model, used by a significant number of authors, is the unloading of the hind limbs of rodents [14]. The animal was suspended from the ceiling of the cage by its tail so that the forelimbs were supported by the floor, while the hindlimbs were positioned at an angle of 30–40 degrees from the floor without touching it. The animal could freely move within the cage [14]. Under these conditions, the level of corticosterone, an indicator of the stress level of the animal, did not exceed the levels recorded in control animals. Isolated rat ventricular cardiomyocytes were obtained according to a known method [15].

Male Wistar rats weighing 200 g, aged 2 months in the amount of 6 animals were exposed to simulated microgravity for 7 days. The control group of 6 animals was housed in the same room. All animals had constant access to food and water. The room temperature was 24°C with a 12-hour light and dark cycle.

The studies were approved by the ethics committee of the Federal State Autonomous Educational Institution of Higher Education of the Russian National Research Medical University named after N.I. Pirogov Ministry of Health of the Russian Federation. Protocol no. 14/2023, extract dated 05/24/2023.

The RNA-seq technique was employed. RNA was extracted from freshly isolated cardiomyocytes using TRIzol, chloroform, and the RNeasy mini kit according to the manufacturer's protocols. The concentration, purity, quantity and quality of RNA were determined using Nanodrop, Qi RNA kit, Qubit 4 fluorimeter, dsDNA high sensitivity kit, and high sensitivity D5000 kit. The samples were prepared with NEB Ultra II RNA kit and NEBNext Poly(A) mRNA magnetic isolation. The resulting libraries were normalized, quantitatively evaluated, and applied to S2 FlowCell followed by loading to NovaSeq 6000. RNA-seq was performed in triplicate. The raw FASTQ data were quality assessed, adapter trimmed and aligned to the reference genome mRatBN7.2.108 using FastQC, Trimmomatic, HISAT2 and SAMtools. Alignments were passed to HTSeq-count for the counting of the channels' genes transcripts.

Differential expression was analyzed using DESeq2, where genes with $p < 0.05$ were considered differentially expressed. Experiments were carried out in triplicate (as is customary), and data are presented as mean ± standard error of the mean (SE).

Of the total number of transcripts of the genes for the cation nonselective TRP channels in rat ventricular cardiomyocytes, the following MGCs are present in amounts exceeding 10 transcripts: TRPM7, PKD1

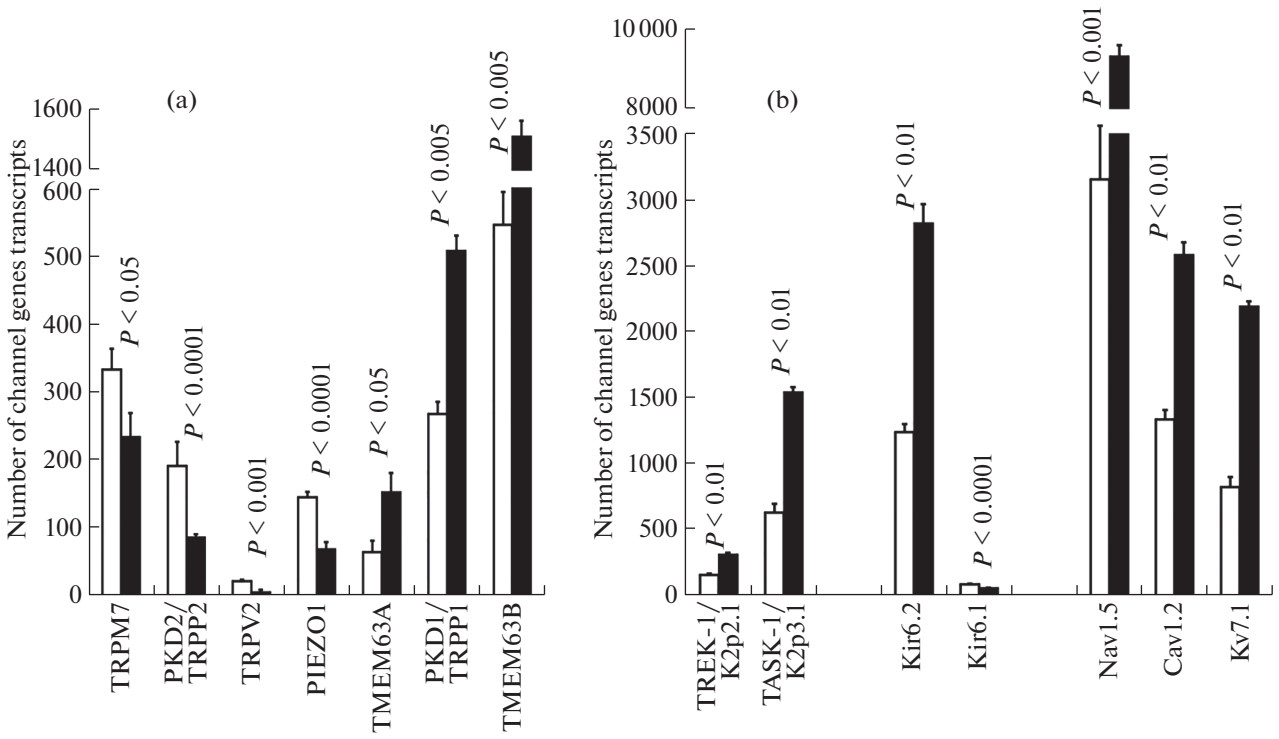

Fig. 1. Changes in the number of genes transcripts for mechanically gated (A) and mechanosensitive (B) ion channels in rat ventricular cardiomyocytes in control (white bars) and under the action of simulated microgravity for 7 days (black bars).

(TRPP1) and PKD2 (TRPP2), TRPC1, TRPM4, TRPV2. The transcripts of other MGCs, including TRPM3, TRPV4, TRPV1, TRPM3, TRPC5, TRPA1, TRPC6, are present in extremely small amounts, and their changes during microgravity simulation are also very small and are not discussed. After 7 days of simulated microgravity, the number of gene transcripts for most MGCs of TRP channels changes (Fig. 1A). There is a decrease in the transcripts of the TRPM7 channel gene (from 333 ± 31 to 234 ± 35, $P < 0.05$), which functions as a cation channel permeable to Ca^{2+} at more negative potentials. The number of TRPV2 channel (functioning as Ca^{2+} and Na^{+} permeable cation channels with a relative permeability ratio of P_{Ca}/P_{Na} equal to 2.94) gene transcripts also decreases (from 21 ± 2 to 4 ± 3, $P < 0.001$). The number of transcripts of the PKD2 channel gene (polycystin-2), named in the TRP classification as TRPP2 channel, also decreases (from 191 ± 35 to 86 ± 4, $P < 0.0001$). However, the number of transcripts of PKD1 or polycystin-1 (TRPP1) channel gene increased (from 267 ± 17 to 508 ± 22, $P < 0.005$). According to a number of authors [16], which was subsequently confirmed [17], TRPP2 did not form a functional channel by itself, but the coexpression of polycystin-1 and TRPP2 promoted TRPP2 translocation into the plasma membrane and the TRPP1/TRPP2 complex produced non-selective cationic currents, with a priority for $Ca2^{+}$ ions. In this case, it can be assumed that a 78% increase in the number of PKD1 channel gene transcripts is offset by a 55% decrease in the number of PKD2 gene transcripts, which makes it impossible to form polycystin-1/TRPP2 complexes. In general, a decrease in the expression of cation nonselective MGCs of the TRP family results in a reduced influx of Ca^{2+} and Na^{+} into cells. At the same time, the number of transcripts for the TRPC1 channel genes (from 147 ± 11 to 141 ± 11, P = NS) and TRPM4 (from 179 ± 6 to 200 ± 29, P = NS) does not change, but their expression can change at a later date.

In addition, transcripts of the cation nonselective MGCs Piezo1 gene were found (Fig. 1A). Their number, as well as TRP, decreases in simulated microgravity (from 145 ± 8 to 68 ± 10, $P < 0.0001$). Piezo1 opens to various mechanical stimuli and mediates the influx of Na^{+}, Ca^{2+}, and Mg^{2+} with a slight preference for Ca^{2+}. A decrease in the expression of Piezo1 channels reduces, respectively, the currents of these ions and, above all, Ca^{2+}. As for Piezo2 channels, the number of their gene transcripts under control conditions was insignificant, although this value, as for Piezo1, decreased (from 6 ± 1 to 2 ± 0.5, $P < 0.05$).

Among other MGCs, transcripts of the TMEM63A [18] and TMEM63B channels genes, [20], are present in significant numbers. Simulated microgravity leads to an increase in the number of TMEM63B channel gene transcripts (from 564 ± 48 to 1509 ± 51, $P < 0.005$) and TMEM63A (from 63 ±

17 to 152 ± 27, $P < 0.05$) (Fig. 1A). These are cation channels with Ca^{2+} permeability activated by mechanical stimulation [19]. Such a pronounced increase in the number of transcripts of their genes in the microgravity simulation is possibly a compensatory mechanism that is activated with a reduced expression of TRP channels.

Among MSCs, channels with K^+ conduction are distinguished, including TREK-1 ($K_{2p}2.1$). Simulated microgravity leads to an increase in the number of transcripts of their genes (from 153 ± 11 to 311 ± 13, $P < 0.01$) (Fig. 1B). Since TASK-1 channels ($K_{2P}3.1$) are predominantly expressed in the atria and are targets for the treatment of atrial fibrillation, it is possible to suggest their mechanosensitivity, which has not yet been shown. In any case, under simulated microgravity, the number of transcripts of their genes increases significantly (from 629 ± 64 to 1542 ± 34, $P < 0.01$) (Fig. 1B). An increase in the number of transcripts of the genes of these channels with K^+ conductance, which are involved in the formation of the resting membrane potential of cells, is probably aimed at maintaining and stabilizing the outflux of K^+, which underlies the resting membrane potential under conditions of an altered supply of Na^+ and Ca^{2+}.

Furthermore, Kir6.2 and Kir6.1 belong to MSCs with K^+ conduction. Simulated microgravity leads to a significant increase in the Kir6.2 channel gene transcripts (from 1240 ± 57 to 2825 ± 142, $P < 0.01$), however, the number of Kir6.1 channel gene transcripts (Fig. 1B) somewhat decreases (from 82 ± 6 to 56 ± 3, $P < 0.0001$).

MSCs are also present among voltage-gated channels and the number of transcripts of their genes varies (Fig. 1B). Therefore, the number of gene transcripts for voltage-gated $Na_V1.5$ MSCs increases significantly as a result of the simulated microgravity (from 3158 ± 405 to 9257 ± 167, $P < 0.005$). The L-type of the Ca^{2+} channel, $Ca_V1.2$, demonstrates pronounced mechanosensitivity and the number of its gene transcripts increases significantly (from 1336 ± 71 to 2582 ± 98, $P < 0.01$). $K_V7.1$ belonging to MSCs also increases its number of gene transcripts (from 821 ± 77 to 2195 ± 37, $P < 0.01$).

The overwhelming majority of voltage-gated channels that are not sensitive to the mechanical stress of cells are not significantly affected by simulated microgravity. The exception is $K_V7.2$ channels, the number of gene transcripts of which in the control is very high, but simulated microgravity causes their huge increase (from 11272 ± 671 to 27551 ± 1797, $P < 0.01$).

As for BK channels ($K_{Ca}1.1$, KCNMA1 gene), the presence of their genes transcripts in isolated cardiomyocytes was very low: 1.3 ± 0.8 in control and 1.0 ± 0.5 as a result of simulated microgravity (P = NS).

Thus, it was shown for the first time that simulated microgravity causes changes in the number of gene transcripts of some MGCs and known MSCs but, with one exception, does not affect voltage-gated channels and other channels without a voltage sensor. This potentially leads to a change in the expression of MGCs and MSCs proteins, which causes changes in the net currents through the membrane, and this leads to a change in the work of the heart.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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