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Hypergravity Increases the Number of Gene Transcripts of Mechanically Gated and Mechanosensitive Ion Channels in Rat Ventricular Cardiomyocytes

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Since hypergravity changes the morphological and physiological properties of the heart, it was assumed that the expression of ion channels that respond to cell stretching or compressing, mechanically gated channels (MGC) and mechanosensitive channels (MSC), would be affected. Using RNA transcriptome sequencing, the change in the number of transcripts for MGC and MSC genes was studied in isolated rat ventricular cardiomyocytes under 4*g* hypergravity for 5 days. It was shown for the first time that hypergravity induces changes in the number of transcripts of MGC genes: an increase for TRPC1, TRPC3, TRPM7, TRPP1 (PKD1), TRPP2 (PKD2), TMEM63A, TMEM63B, but a decrease for TRPV2, Piezo1, Piezo2. The number of MSC gene transcripts increases: TREK-1, Kir6.2, Nav1.5, Cav1.2, Cav1.3, Kv7.1, and Kv1.2. This potentially leads to an increase in the expression of MGC and MSC proteins leading to an increase in the net current and, as a result, pathological changes in the heart function.

Key Words: *cardiomyocytes; mechanically gated and mechanosensitive channels; hypergravity; transcripts of channel genes*

Changed gravity conditions, such as micro- and hypergravity, have a significant impact on the human and animal body, including the cardiovascular system [1]. Obviously, the work of the heart is adapted to the earth's gravity of 1*g*, and changes in gravity affect the morphology and physiological characteristics of the heart. However, the effect of hypergravity on the heart at the cellular level was addressed in a limited number of studies. In a rather old study, hypergravity was shown to cause an increase in heart weight [2]. There is also a recent work showing that calcium oscillations and calcium concentration in the cytosol of HL-1 cells increased after exposure to hypergravity [1]. At the same time, hypergravity should affect the heart at the cellular and molecular levels, including the expression of genes of ion channel proteins. In addition, cardiomyocytes are sensitive to mechanical stress of any nature, and this manifests itself in the form of pronounced changes in their electrophysiological properties (mechanoelectric feedback). Its mechanisms are based on the work of mechanically gated channels (MGC or Stretch Activated Channels: SACs) in the cardiomyocyte membranes [3], which affects the resting membrane potential and the action potentials of cardiomyocytes. Stretching of isolated ventricular cardiomyocytes triggers a current of cations through MGC that modulates the resting potential and the action potentials of these cardiomyocytes [4]. In addition to MGC, for which mechanical stress serves as a physiological stimulus, mechanosensitive ion channels (MSC) are recognized; MSC can be any

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channels (*e.g.* voltage-gated) effectively responding to mechanical stress by activation. In this regard, first of all, hypergravity should affect the expression level of MGC and MSC.

In this work, we studied changes in the number of transcripts for MGC and MSC genes in isolated rat ventricular cardiomyocytes during hypergravity.

MATERIALS AND METHODS

The effect of hypergravity was studied using a centrifuge with two 60-cm arms developed by us as a simulator of overloads (for rodents [5]). At the ends of each arm, cages with rats hang freely and were suspended during rotation of the centrifuge shaft. A constant counterclockwise rotation speed created hypergravity (*g*-forces) in animals of 4*g*. Male outbred rats weighing 200 g were exposed to hypergravity for 8 h a day (from 09.00 to 17.00) over 5 days. The control group of animals was housed in the same room. All animals were kept at 24°C with a 12/12-h light/dark cycle and had constant access to water and food.

Isolated ventricular cardiomyocytes were extracted by enzymatic dissociation of the heart tissue [6]. The RNA-seq technique was employed. RNA was extracted from freshly isolated cardiomyocytes using TRIzol, chloroform, and the RNeasy mini kit according to the manufacturers protocols (Invitrogen). The concentration, purity, quantity, and quality of RNA were determined using Nanodrop, Qi RNA kit, Qubit 4 fluorimeter (Thermo Fisher Scientific, Inc.), dsDNA high sensitivity kit (Invitrogen), and high sensitivity D5000 kit (Agilent). The samples were prepared with NEB Ultra II RNA kit and NEBNext Poly(A) mRNA magnetic isolation (New England Biolabs, Inc.). The resulting libraries were normalized, quantitatively evaluated, and applied to S2 FlowCell (Illumina) followed by loading into NovaSeq 6000 (Illumina). 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 read counting.

Differential expression was analyzed using the DESeq2 method and Galaxy software (Galaxy Project Team, Penn State University, State College, Pennsylvania), where genes with $p<0.05$ were considered differentially expressed. The experiments were carried out in triplicates (as is customary), and the data are presented as *M*±*SEM*.

RESULTS

Of the total number of transcripts of nonselective cation MGC genes in rat ventricular cardiomyocytes, the following transcripts were present in quantities exceeding 10: TRPC1, TRPC3, TRPV1, TRPV2, TRPV4, TRPM4, TRPM7, TRPP1 (PKD1), TRPP2 (PKD2) [7], Piezo1, Piezo2 [8], TMEM63A [9], and TMEM63B [10]. After 5 days of hypergravity, the number of transcripts for most of the MGC genes of the TRP channels changed (Fig. 1, *a*). We observed an increase in gene transcripts of the TRPC1 channels (from 146.66±6.69 to 518.33±65.28, $p<0.05$) that function as non-selective cation channels, primarily determining Ca^{2+} conductivity. The number of transcripts for TRPM7, cation channels permeable to Ca^{2+} at hyperpolarised potentials, increased significantly (from 333.5±31.5 to 1186.67±132.33, $p<0.005$). A pronounced increase in the number of transcripts for TRPP1/PPD1 (Polycystin-1) (from 267.4±17.9 to 1099.33±191.00, $p<0.0005$) and TRPP2/PPD2 (Polycystin-2) (from 191.6±35.0 to 445.33±44.37, $p<0.05$) was found. According to previously reported data [11] that were later confirmed [12], TRPP2 did not form functional channels by themselves, but co-expression of Polycystin-1 and TRPP2 promoted the translocation of TRPP2 to the plasma membrane and the TRPP1/TRPP2 complex produced nonselective cationic currents, with a priority for Ca^{2+} ions. The number of transcripts also increased for TRPC3 (from 3±1 to 14.67±1.45, $p<0.005$), although it is low in the control (not shown in the figure). TRPC3 is a non-selective cation channel that exhibits moderate selectivity for Ca^{2+} compared to monovalent cations with a permeability coefficient of $P_{Ca}/P_{Na}=1.6$ [7].

Hypergravity also increased the number of transcripts for the TMEM63A channels (from 63.6±17.2 to 122.67±16.50, $p<0.05$) and TMEM63B (from 546.6±48.8 to 1670±254, $p<0.05$) (Fig. 1, *a*). Among other MGC, TMEM63A [9] and TMEM63B [10] are Ca^{2+} permeable cation channels [13] activated by mechanical stimulation [10].

At the same time, the number of transcripts for TRPV1 (from 2.00±0.58 to 5.33±1.33, $p>0.05$), TRPV4 (from 5.5±0.5 to 4±2, $p>0.05$) and TRPM4 (from 179.66±5.93 to 173.00±18.73, $p>0.05$) did not change. On the contrary, the number of transcripts for TRPV2 acting as Ca^{2+} and Na^{+} permeable cation channels with a relative permeability ratio of P_{Ca}/P_{Na} equal to 2.94 [7], decreased (from 21.66±2.03 to 5.67±1.45, $p<0.05$), so did Piezo1 (from 145.3±8.0 to 72.00±24.27, $p<0.0001$) (Fig. 1, *a*). Piezo1 is opened in response to various mechanical stimuli and mediates K^{+}, Na^{+}, Ca^{2+}, and Mg^{2+} currents with a slight preference for Ca^{2+}. A decrease in the expression of the Piezo1 channel gene reduces the currents of these ions and, above all, Ca^{2+} [8]. The number of transcripts also decreased for Piezo2 present in small amounts (from 6±2 to 2.33±2.33, $p<0.05$; data not shown).

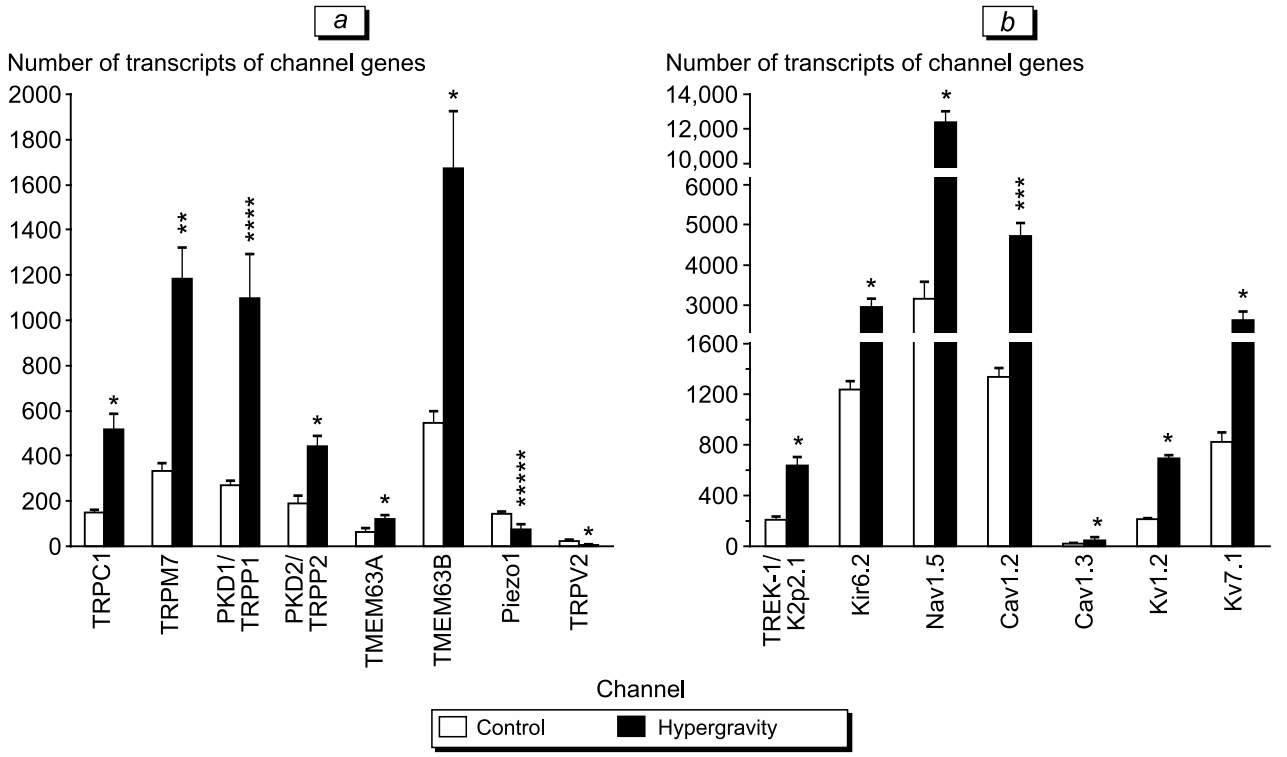

Fig. 1. Changes in the number of transcripts of MGC (*a*) and MSC (*b*) genes in rat ventricular cardiomyocytes in control and under the action of hypergravity for 5 days. *p<0.05, **p<0.005, ***p<0.001, ****p<0.0005, *****p<0.0001 in comparison with the control.

Among MSC, channels with K^+ conduction are distinguished, including TREK-1 (K_{2p}2.1). Hypergravity leads to an increase in the number of its gene transcripts (from 153.00±11.93 to 495.33±22.92, p<0.05) (Fig. 1, *b*). In addition, Kir6.2 belongs to MSC with K^+ conduction. Hypergravity led to a significant increase in the number of Kir6.2 transcripts for Kir6.2 (from 1240.66±57.17 to 2940.33±201.79, p<0.05) (Fig. 1, *b*).

MSC are also present among voltage-gated channels, and the number of their gene transcripts changes (Fig. 1, *b*). Therefore, the number of transcripts for Nav1.5 voltage gated MSC increased significantly as a result of hypergravity (from 3158.00±405.75 to 12,712.67±402.84, p<0.001). The L-type of the Ca^{2+} channel, Cav1.2, demonstrated pronounced mechanosensitivity and the number of its gene transcripts increased significantly (from 1336.0±71.80 to 4725.00±300.02, p<0.01). For Cav1.3, the number of transcripts also increased (from 17.33±2.03 to 44.00±25.06, p<0.05).

The MSC include Kv7.1 and Kv1.2, the number of their gene transcripts also increased (from 821.0±77.2 to 2607.00±220.01, p<0.05 and from 212.00±10.02 to 2607.00±220.01, respectively).

Thus, it was shown for the first time that hypergravity causes an increase in the number of transcripts for most MGC and all known MSC. This potentially leads to an increase in the expression of MGC and MSC proteins, which causes an increase in the total current through these channels even with minimal, for example, cell stretching. The expression of the channels leads to pathological changes in heart work, because Ca^{2+} and Na^+ entry through MGC into the cells increases, and MSC mediate an increased outflow of K^+ ions from cells and the influx of Na^+ and Ca^{2+}.

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