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Hypergravity Upregulates and Simulated Microgravity Downregulates TRPM7 Mechanosensitive Ion Channel Expression in Isolated Rat Cardiomyocytes

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Hypergravity (HG) and microgravity (MG) modulate Ca^{2+} entry into the cytosol of cardiomyocytes via both voltage-gated and mechanically gated Ca^{2+} channels. The last include members of the TRP channel family with TRPM7 being the predominant subtype. We investigated the relationship between changes in TRPM7 channel protein synthesis and the abundance of its corresponding mRNA in cardiomyocytes from rats exposed to HG or MG for 14 days. Using transcriptome sequencing with normalization of "raw reads" by the TPM (Transcripts Per Kilobase Million) method, we found that TRPM7 TPM values significantly increased after HG exposure and significantly decreased after MG. Western blotting of TRPM7 protein levels confirmed a pronounced upregulation under HG and a substantial downregulation under MG. These changes may lead to altered ion conductance through mechanically gated channels in cardiomyocytes.

Key Words: *cardiomyocytes; mechanically gated channels; hypergravity; simulated microgravity; gene transcripts and protein synthesis of TRPM7 channel*

Intracellular calcium concentration $[Ca^{2+}]_i$, which plays a central role in the mechanism of cardiac contraction, is primarily determined by Ca^{2+} influx through L-type Ca^{2+} channels and cation-nonselective mechanically gated ion channels (MGCs), which are sensitive to mechanical stress of any nature. It has long been established that ventricular cardiomyocytes respond to mechanical stretch with an increase in $[Ca^{2+}]_i$ [1,2]. This rise in $[Ca^{2+}]_i$ is attributed to Ca^{2+} entry via MGCs and subsequent activation of Ca^{2+} release from the sarcoplasmic reticulum [1,2].

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Numerous MGCs have been identified to date. Among them, a prominent group comprises members of the transient receptor potential (TRP) channel family that exhibit significant Ca^{2+} conductance, including TRPM7, TRPM4, TRPM3, TRPV4, TRPV2, TRPV1, TRPC5, TRPC3, TRPC1, TRPP1 (PKD1), TRPP2 (PKD2), and TRPA1; notably, TRPC6 is absent in rat cardiomyocytes [3]. Within this group, TRPM7 is particularly distinctive: it functions as a fully conducting channel for divalent cations at negative membrane potentials and permits monovalent cation flux only during depolarization [4]. In experimental settings, its ion selectivity follows the sequence Zn = Ni > Ba > Co > Mg > Mn > Sr > Cd > Ca. Under physiological conditions, TRPM7 primarily governs cellular permeability to Ca^{2+} and Mg^{2+} [4]. TRPM7 channels are directly activated by cellular stretch or other forms of mechanical stimulation [3].

Hypergravity (HG) and microgravity (MG) are well-recognized environmental factors that exert sig-

nificant effects on the cardiovascular system [5]. Cardiac function is evolutionarily adapted to the Earth's 1*g* gravitational environment, and deviations from this baseline induce structural and functional alterations in the heart. A primary response to such gravitational changes is the modulation of MGC conductance in cardiomyocytes, an effect potentially driven by upregulated channel protein synthesis under HG and downregulation under MG mediated through corresponding changes in the abundance of channel-encoding transcripts.

The aim of this study was to determine and quantitatively assess alterations in both gene expression and protein synthesis of the mechanically gated TRPM7 channel in rat cardiomyocytes followed by 14-day exposure to HG and MG.

MATERIALS AND METHODS

The study was approved by the Commission for the Control of Maintenance and Use of Laboratory Animals of Pirogov Russian National Research Medical University (Pirogov University) (Protocol No. 14/2023 of May 24, 2023) and was conducted in accordance with the Directive 2010/63/EU of the European Parliament and of the Council (September 22, 2010; On the Protection of Animals Used for Scientific Purposes).

The experiments were performed on 2-month-old male Wistar rats (n = 46; body weight 200 g). All animals received food and water *ad libitum*. Control animals were kept in the same room as experimental animals under standard laboratory conditions: room temperature 24°C and 12:12-h light/dark cycle.

To assess the effects of HG, rats were exposed to 4*g* gravitational load for 14 consecutive days 8 h per day (09:00-17:00 MSK), using a centrifuge with fixed counterclockwise rotation speed as an overload simulator [6]. Simulated microgravity (SMG) was modeled using the hindlimb unloading technique according to a previously established protocol [7] for 14 days.

Ventricular cardiomyocytes were isolated via enzymatic dissociation of cardiac tissue [3] followed by centrifugation to obtain purified isolated cardiomyocytes. To analyze gene expression of the mechanically gated TRPM7 channel, RNA sequencing (RNA-seq) was used. To enable accurate cross-sample comparison, raw sequencing reads were normalized using the TPM (Transcripts Per Kilobase Million) method, which accounts for gene length and total sequencing depth. Differential gene expression was assessed using the DESeq2 algorithm implemented in the Galaxy platform (Galaxy Project Team, Penn State University). RNA-seq analyses included cardiomyocytes from control rats (n = 7), rats exposed to HG (n = 6), and rats subjected to SMG (n = 4). The data are presented as the mean (M) ± standard error of the mean (*SEM*).

TRPM7 protein expression was evaluated using Western blotting. Cell lysates were prepared in RIPA buffer (Sigma-Aldrich) supplemented with protease and phosphatase inhibitors (Calbiochem). Samples were mixed with Laemmli buffer containing β-mercaptoethanol and denatured at 95°C for 5 min. Proteins were separated using SDS-PAGE on polyacrylamide gels at 80 V (stacking) and 120 V (resolving), then electrotransferred onto nitrocellulose membranes. Membranes were blocked and incubated with primary antibodies diluted in 5% non-fat dry milk: anti-TRPM7 (1:1000; #A10075, ABclonal) and anti-α-subunit of Na^+/K^+-ATPase (1:100,000; [EP1845Y], #ab76020, Abcam). The α-subunit of Na^+/K^+-ATPase was used as a loading control, as its expression remains stable under varying gravitational conditions. After incubation with HRP-conjugated secondary antibodies (1:10,000; #AS014, ABclonal) and thorough washing, immunoreactive bands were visualized by chemiluminescent detection using luminol-based substrate. Signal acquisition was performed using a ChemiDoc imaging system (Bio-Rad), and band intensities were quantified with ImageJ software (NIH). Western blotting included samples of the control rats (n = 11), HG rats (n = 10), and SMG rats (n = 8).

Statistical analysis was carried out using GraphPad Prism 10.0.3 (GraphPad Software). Normality of data distribution was determined using the Shapiro–Wilk test. Group comparisons were performed using ANOVA for repeated measures followed by the Holm–Šidák post-hoc test for pairwise comparisons. All data are presented as the mean (M) ± standard deviation (*SD*), n is the number of independent experimental replicates, each derived from cardiomyocytes isolated from a single animal. The differences were considered statistically significant at $p < 0.05$.

RESULTS

As a result of the experiments, a significant increase in TRPM7 channel gene transcript levels (expressed in TPM) were observed in the HG group in comparison with controls. Specifically, TRPM7 TPM values were 0.236 ± 0.021 in the control group (n = 7) and 0.333 ± 0.024 in the HG group (n = 6; $p < 0.005$), representing a 41% increase (Fig. 1). In contrast, SMG led to a significant decrease in TRPM7 TPM to 0.112 ± 0.029 (n = 4; $p < 0.05$ in comparison with the control) corresponding to a 52% reduction (Fig. 1).

Western blotting of TRPM7 protein levels under HG and SMG revealed significant intergroup differences (Fig. 2). When the TRPM7 protein level in cardiomyocytes of control rats normalized to the housekeeping protein was set to 1 (1 ± 0.25 rel. units;

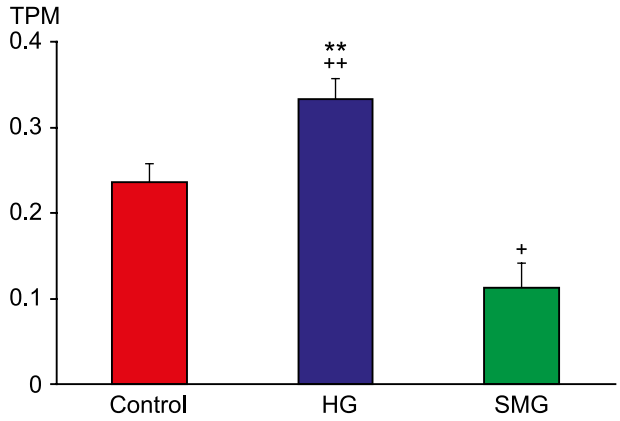

Fig. 1. TPM values of mechanically gated TRPM7 channel in cardiomyocytes of control rats (n = 7), rats subjected to HG (n = 6) and SMG (n = 4). $^{+}p < 0.05$, $^{++}p < 0.005$ in comparison with the control; $^{**}p < 0.005$ in comparison with SMG.

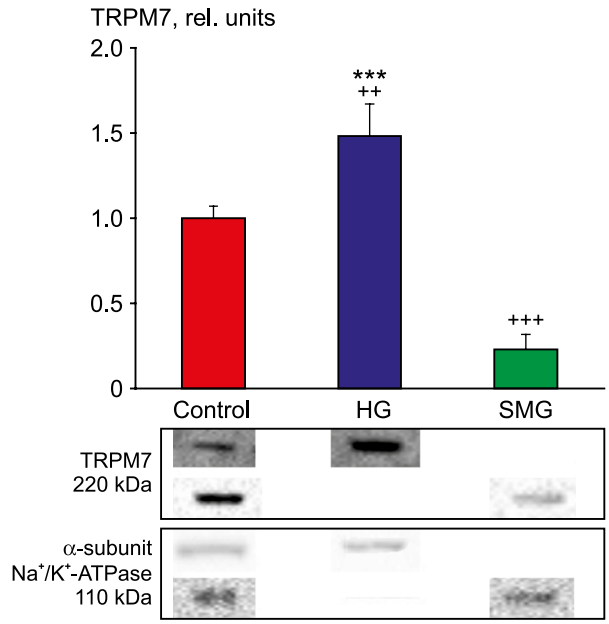

Fig. 2. TRPM7 channel protein level normalized to housekeeping protein (α-subunit of Na^+/K^+-ATPase) in cardiomyocytes of control rats (n = 11) and subjected to HG (n = 10) and SMG (n = 8). $^{++}p < 0.01$, $^{+++}p < 0.001$ in comparison with the control; $^{***}p < 0.001$ in comparison with SMG.

n = 11), it increased to 1.48 ± 0.19 rel. units in the HG group (n = 10; $p < 0.01$). Conversely, SMG markedly reduced TRPM7 protein abundance to 0.23 ± 0.09 rel. units (n = 8; $p < 0.001$).

These findings demonstrate that prolonged exposure to HG induces a coordinated upregulation of both TRPM7 mRNA and protein, whereas prolonged SMG elicits a parallel downregulation of both. The results are consistent with our earlier electrophysiological data: mechanical stretching of cardiomyocytes isolated from HG-exposed rats produced significantly enhanced ion currents through MGCs, whereas cardiomyocytes from SMG-exposed animals exhibited markedly reduced MGC currents [8]. Thus, the present study supports our hypothesis that altered transcript levels of MGC genes drive corresponding changes in channel protein synthesis, thereby directly modulating the functional ion conductance of these channels.

These data on gravity-dependent modulation of MGC protein expression, key determinants of cardiomyocytes mechanosensitivity under HG and MG, provide important insights into the cellular adaptations that occur under altered gravitational conditions.

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Conflicts of interest. The authors have no conflicts of interest to declare.

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