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GENETICS

Simulated Microgravity and Hypergravity Affect the Expression Level of Soluble Guanylate Cyclase, Adenylate Cyclase, and Phosphodiesterase Genes in Rat Ventricular Cardiomyocytes

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Ion channels activity is regulated through soluble guanylate cyclase (sGC) and adenylate cyclase (AC) pathways, while phosphodiesterases (PDE) control the intracellular levels of cAMP and cGMP. Here we applied RNA transcriptome sequencing to study changes in the gene expression of the sGC, AC, and PDE isoforms in isolated rat ventricular cardiomyocytes under conditions of microgravity and hypergravity. Our results demonstrate that microgravity reduces the expression of sGC isoform genes, while hypergravity increases their expression. For a subset of AC isoforms, gene expression either increased or decreased under both microgravity and hypergravity conditions. The expression of genes encoding 10 PDE isoforms decreased under microgravity, but increased under hypergravity. However, under both microgravity and hypergravity, the gene expression increased for 7 PDE isoforms and decreased for 3 PDE isoforms. Overall, our findings indicate specific gravity-dependent changes in the expression of genes of isoforms associated with the studied enzymes.

Key Words: *cardiomyocytes; soluble guanylate cyclase; adenylate cyclase; phosphodiesterase; gravity*

Microgravity and hypergravity exert significant effects on the human and animal body, and in particular on the cardiovascular system. Microgravity during space flights leads to cardiac atrophy and impairs cardiac function. In rats exposed to weightlessness for two weeks, a decrease in the mean cross-sectional area of left ventricular cardiomyocytes was revealed [1]. On the contrary, hypergravity induces an increase in heart weight [1]. Changes in gravity also affect the levels of transcripts of mechanically gated channels and mechanosensitive channels in rats [2].

Ion channels, including mechanosensitive and mechanically gated channels, are regulated through pathways involving soluble guanylate cyclase (sGC) and adenylate cyclase (AC). These pathways produce cyclic nucleotides, cGMP and cAMP acting as intricately regulated secondary messengers. Phosphodiesterase

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(PDE) isoforms control intracellular levels of cAMP and cGMP by modulating the rate at which these cyclic nucleotides are degraded, based on feedback signals from both cAMP and cGMP [3]. In cardiomyocytes, PDE1-5 are primarily responsible for the degradation of cAMP and cGMP, each of which possesses its own regulatory mechanisms. PDE1-3 can hydrolyze both cyclic nucleotides, while PDE4 is specific for cAMP, and PDE5 is specific for cGMP. Coordinated regulation of PDE1-3 activity with cAMP and cGMP functionally connects the AC/cAMP and NO/sGC/cGMP pathways [3].

The balance of cyclic nucleotide signaling is based on changes in the ratio of specific PDE isoforms. In rat ventricular cardiomyocytes, PDE3 and PDE4 are the predominant enzymes responsible for cAMP hydrolysis. However, inhibition of PDE4 shifts the balance towards PDE3. Myocardial hypertrophy, for example, is associated with decreased levels of PDE3 and PDE4, which favors PDE3 dominance [4]. Though these data were obtained through indirect methods, it is crucial to note the change in the ratio. Shifts in the balance of PDE isoform have been observed in various heart diseases.

Based on these findings, we hypothesized that microgravity and hypergravity can lead to changes in the expression of sGC, AC, and the balance between the PDE isoforms. This suggests the potential use of drugs targeting specific PDE activities to exert cardioprotective effects and mitigate the consequences of weightlessness and overload.

This study aimed to investigate changes in the gene expression of sGC, AC, and PDE isoforms in isolated rat ventricular cardiomyocytes under normal conditions and in simulated microgravity and hypergravity.

MATERIALS AND METHODS

Experimental procedures were performed in male Wistar rats (body weight 200 g, age 2 months). The animals were kept at 24°C with a 12/12-h light/dark cycle and had free access to water and food. Two control groups (6 rats in each) were housed in the same experimental facility. Microgravity was modeled by hind limb unloading, which is an adequate model to study the cardiovascular system used by many authors [5]. Six animals were subjected to simulated microgravity for 7 days. The effects of hypergravity were investigated using a custom-designed centrifuge as an overload simulator [2]. A constant counterclockwise rotation speed generated hypergravity (overloads) at a magnitude of 4*g*. Six animals were subjected to hypergravity for 7 days, 8 h per day (from 09.00 to 17.00 MSK).

Ventricular cardiomyocytes were isolated by enzymatic dissociation of cardiac cells [6]. The RNA transcriptome sequencing (RNA-seq) technique was used to determine the expression levels of enzyme genes. After performing the previously described technological procedures [2], transcript counts were further normalized and presented as Transcripts Per Kilobase Million (TPM) to account for differences in gene length and total read count across samples.

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

RESULTS

The sGC protein that triggers the regulatory pathways NO/sGC/cGMP, comprises α and β subunits, including α_1, α_2, β_1, and β_2 that can form various combinations. However, only the β subunit can be activated by the secondary messenger NO, while the role of the α subunit remains elusive. Activation of the α subunit occurs upon interaction with a synthetic substance BAY41-2272 [6]. Our experiments revealed low TPM levels for $sGC\beta_1$ (0.0224±0.0058) and lack of $sGC\beta_2$ expression (Table 1). Notably, microgravity is associated with a significant reduction in TPM for $sGC\beta_1$ (to 0.0028±0.0015; $p<0.05$ in comparison with the control), while hypergravity had minimal impact, resulting in an expression of 0.0273±0.0032. Conversely, $sGC\alpha_1$ exhibited a relatively high TPM (0.0975±0.0290), which decreased under microgravity (to 0.0575±0.0157) and increased to under hypergravity (to 0.1553±0.0231) (Table 1). The difference in TPM for $sGC\alpha_1$ under microgravity compared to the hypergravity conditions was statistically significant ($p<0.01$). The elevated TPM for $sGC\alpha_1$ is hard to explain. It is plausible that this subunit either plays an unknown physiological role or is of substantial regulatory significance. Meanwhile, $sGC\alpha_2$ displayed low TPM (0.1553±0.0231) with minimal variations due to microgravity and hypergravity ($p<0.01$). Overall, sGC isoforms demonstrated decreased gene expression levels under microgravity conditions and increased levels under hypergravity conditions.

AC is presented by 9 isoforms (AC1-9); in the myocardium, AC5 and AC6 are predominant isoforms, and AC1-3, AC9 [7], and AC8 [8] are also present. Our experimental results (Table 1) revealed gene expression for all AC isoforms except AC8 that was absent under both control and microgravity/hypergravity conditions. AC6 exhibited the highest TPM (3.7314±0.1284) and significantly increased under both microgravity (4.6424±0.1433; $p<0.005$ in comparison with the control) and hypergravity (8.9901±0.2434; $p<0.001$ in

TABLE 1. Expression Levels of Different Isoforms of sGC and AC (TPM; *M*±*SEM*)

Isoform		Control (*n*=6)	Microgravity (*n*=6)	Hypergravity (*n*=6)
sGC	$sGC\alpha_1$	0.0975±0.0290	0.0575±0.0157^{++}	0.1553±0.0231*
	$sGC\alpha_2$	0.0006±0.0004	0.0001±0.0001^{++}	0.0008±0.0001
	$sGC\beta_1$	0.0224±0.0058	0.0028±0.0015*$^{++++}$	0.0273±0.0032
	$sGC\beta_2$	0	0	0
AC	AC1	0.1901±0.0108	0.3343±0.0309**$^{++++}$	0.7360±0.0199****
	AC2	0.0937±0.0045	0.0789±0.0066	0.0804±0.0041*
	AC3	0.0024±0.0004	0.0033±0.0014^{+}	0.0053±0.0008*
	AC4	0.0963±0.0138	0.1583±0.0211*$^{++}$	0.2834±0.0106***
	AC5	0.7436±0.0397	2.2328±0.1027****	2.3875±0.1140****
	AC6	3.7314±0.1284	4.6424±0.1433***$^{++++}$	8.9901±0.2434****
	AC7	0.0297±0.0048	0.0153±0.0028*	0.0242±0.0057
	AC8	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
	AC9	0.0613±0.0092	0.1388±0.0050***$^{++}$	0.2098±0.0139****

Note. *p<0.05, **p<0.01, ***p<0.005, ****p<0.001 in comparison with the control; ^{+}p<0.05, ^{++}p<0.01, ^{+++}p<0.005, ^{++++}p<0.001 in comparison with hypergravity.

TABLE 2. Expression Levels of Different PDE Isoforms (TPM; *M*±*SEM*)

Isoform	Control (*n*=6)	Microgravity (*n*=6)	Hypergravity (*n*=6)
PDE1A	0.0002±0.0001	0.0001±0.0001^{+++}	0.0005±0.0000**
PDE1B	0.0122±0.0006	0.0084±0.0023	0.0141±0.0023
PDE1C	0.0002±0.0001	0.0001±0.0001^{++++}	0.0006±0.0001***
PDE2A	0.1232±0.0079	0.2751±0.0117****	0.2864±0.0170****
PDE3A	0.1552±0.0038	0.1186±0.0092*$^{++++}$	0.6510±0.0303****
PDE3B	0.0050±0.0001	0.0009±0.0003****$^{++++}$	0.0039±0.0004*
PDE4A	0.3181±0.0371	0.7953±0.0752***$^{+++}$	1.3347±0.0822****
PDE4B	0.0206±0.0021	0.0267±0.0014*$^{+++}$	0.0493±0.0042***
PDE4C	0.0342±0.0024	0.0398±0.0109^{+}	0.1082±0.0227*
PDE4D	0.0127±0.0016	0.0096±0.00085^{++}	0.0253±0.0028**
PDE5A	0.0040±0.0005	0.0006±0.0003***$^{+}$	0.0016±0.0003**
PDE6A	0.0002±0.0002	0.0000±0.0000	0.0005±0.0002
PDE6B	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
PDE6C	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
PDE6D	0.2815±0.0514	0.3216±0.0490^{+}	0.5104±0.0360**
PDE6G	0.0070±0.0035	0.0035±0.0035	0.0104±0.0060
PDE6H	0.0012±0.0012	0.0000±0.0000	0.0012±0.0012
PDE7A	0.0195±0.0005	0.0040±0.0011****$^{++++}$	0.0193±0.0012
PDE7B	0.0178±0.0014	0.0126±0.0008**$^{++++}$	0.0330±0.0020***
PDE8A	0.0217±0.0016	0.0296±0.0021**	0.0442±0.0079
PDE8B	0.0009±0.0003	0.0004±0.0003	0.0012±0.0003
PDE9A	0.0143±0.0026	0.0051±0.0007*$^{++++}$	0.0162±0.0013
PDE10A	0.0001±0.0000	0.0005±0.0000****$^{++}$	0.0021±0.0002***

Note. *p<0.05, **p<0.01, ***p<0.005, ****p<0.001 in comparison with the control; ^{+}p<0.05, ^{++}p<0.01, ^{+++}p<0.005, ^{++++}p<0.001 in comparison with hypergravity.

comparison with the control). AC5 demonstrated a slightly lower TPM (0.7436±0.0397), increasing under both microgravity (2.2328±0.1027) and hypergravity (2.3875±0.1140) (p<0.001 in comparison with the control). Similarly, AC1 (0.1901±0.0108), AC4 (0.0963±0.0138), AC9 (0.0613±0.0092), and AC3 (0.0024±0.0004) showed elevated TPM, which increased under both microgravity and hypergravity conditions (Table 1). However, AC2 (0.0937±0.0045) and AC7 (0.0297±0.0048) exhibited a decrease in TPM for both factors (Table 1). Thus, the gene expression levels of different AC isoforms either increased or decreased under both microgravity and hypergravity conditions, indicating nonspecific influence of gravitational changes on AC.

In relation to PDE, gene expression for PDE6B and PDE6C was not detected. TPM levels for PDE1A, PDE1C, PDE6A, and PDE10A were notably low (0.0002±0.0001 and lower; Table 2). The remaining PDE isoforms showed varying levels of expression. Consequently, in rat cardiomyocytes, degradation of cAMP and cGMP can be facilitated by PDE1B (0.0122±0.0006), PDE2A (0.1232±0.0079), PDE3A (0.1552±0.0038), PDE4A-D, and PDE5A (0.0040±0.0005), although PDE5A exhibited a low TPM (Table 2). Notably, TPM levels for 10 PDE isoforms decreased under microgravity and increased under hypergravity, indicating a specific gene expression response to gravity. However, the gene expression of 7 PDE isoforms was not gravity-specific (microgravity and hypergravity), increasing in both cases, while for 3 PDE isoforms, it decreased in both cases. It should be noted that the ratio of PDE isoforms changes under microgravity and hypergravity, as observed in some heart diseases. TPM for some isoforms increased, while for others decreased.

Overall, our findings indicate that changes in the gene expression of sGC, AC, and PDE isoforms in isolated cardiomyocytes under simulated microgravity and hypergravity conditions is generally specific and depends on gravity. However, for certain isoforms, the changes can solely depend on the presence of gravity.

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

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