Full Paper

Pharmacological Stimulation of Type 5 Adenylyl Cyclase Stabilizes Heart Rate Under Both Microgravity and Hypergravity Induced by Parabolic Flight

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Abstract. We previously demonstrated that type 5 adenylyl cyclase (AC5) functions in autonomic regulation in the heart. Based on that work, we hypothesized that pharmacological modulation of AC5 activity could regulate the autonomic control of the heart rate under micro- and hypergravity. To test this hypothesis, we selected the approach of activating AC5 activity in mice with a selective AC5 activator (NKH477) or inhibitor (vidarabine) and examining heart rate variability during parabolic flight. The standard deviation of normal R-R intervals, a marker of total autonomic variability, was significantly greater under micro- and hypergravity in the vidarabine group, while there were no significant changes in the NKH477 group, suggesting that autonomic regulation was unstable in the vidarabine group. The ratio of low frequency and high frequency (HF) in heart rate variability analysis, a marker of sympathetic activity, became significantly decreased under micro- and hypergravity in the NKH477 group, while there was no such decrease in the vidarabine group. Normalized HF, a marker of parasympathetic activity, became significantly greater under micro- and hypergravity in the NKH477 group. In contrast, there was no such increase in the vidarabine group. This study is the first to indicate that pharmacological modulation of AC5 activity under micro- and hypergravity could be useful to regulate the autonomic control of the heart rate.

Keywords: adenylyl cyclase isoform, autonomic nerve activity, parabolic flight, heart rate variability, microgravity

Introduction

Cardiac function is regulated by the autonomic nervous system. Sympathetic regulation leads to coupling of the β -adrenergic receptor (β -AR) and Gs, the G-protein responsible for stimulating activity of adenylyl cyclase (AC), a membrane-bound enzyme that catalyzes the

conversion of ATP to cyclic AMP (cAMP), thereby stimulating protein kinase A (PKA) and ultimately increasing cardiac contractility and heart rate (HR) (1 – 5). Parasympathetic regulation counteracts these effects through the activation of the muscarinic receptor and Gi, the G-protein that can inhibit cardiac contractility and heart rate (6). The autonomic nervous system constitutes the two arms of regulation in the heart. We developed a mouse model with disruption of a major AC isoform (type 5) in the heart and demonstrated that type 5 AC (AC5) regulates cardiac function through the parasympa-

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thetic arm of the autonomic nervous system, as well as through the sympathetic arm (7–9).

The autonomic nervous system is known to be altered under microgravity (10) and hypergravity (11, 12). We have previously examined the role of AC5 in the regulation of the autonomic nervous system in the heart under micro- and hypergravity induced by parabolic flights using transgenic mouse models with AC5 overexpressed in the heart (AC5TG) or with disrupted AC5 (AC5KO) and analyzed heart rate variability during parabolic flight. Changes in heart rate variability (HRV) in response to micro- and hypergravity were augmented in AC5KO, but attenuated in AC5TG, suggesting that AC5 plays an important role in stabilizing heart rate control via autonomic regulation under gravitational stress (13).

We hypothesized that pharmacological regulation of AC5 activity could modulate the autonomic control of the heart rate under gravitational stress during parabolic flight. To test this hypothesis, we decided to examine the effect on HRV of activating or inhibiting AC5 activity in wild-type mice with a selective AC5 activator: 6-[3-(dimethylamino)propionyl]forskolin (NKH477) (14) or an AC5 inhibitor: vidarabine (15) during parabolic flight. We found that HR was stabilized by increasing AC5 activity with NKH477, but destabilized by decreasing AC5 activity with vidarabine under micro- and hypergravity during parabolic flight, in accordance with our previous findings using AC5TG and AC5KO.

We also examined the ratio between low frequency (LF) and high frequency (HF), a marker of sympathetic activity, and normalized HF (nHF), a marker of parasympathetic activity during the parabolic flight and found that AC5 activity plays an important role to show continuous responses in these autonomic parameters such as LF/HF or nHF, under micro- and hypergravity (16).

Many astronauts suffer to varying degrees from symptoms suggestive of autonomic dysfunction such as nausea, vomiting, and dizziness during the early days of space flight (17), as well as showing severe orthostatic intolerance for several days after spaceflight (18, 19). The underlying molecular mechanism and appropriate treatment for the autonomic dysfunction in space and on earth after space flight remain unknown. However, not only trained astronauts but also normal subjects will stay in space in the near future. Thus, it is important to clarify the mechanism and to establish suitable treatment of autonomic dysfunction during and after spaceflight. This study is the first to demonstrate that pharmacological activation/inhibition of AC5 activity can regulate the autonomic control of the heart rate under micro- and hypergravity, indicating the importance of regulating AC5 activity for the treatment of autonomic dysfunction in space and on earth after spaceflight.

Materials and Methods

Parabolic flight experiment

Parabolic flight experiments were performed in a jet airplane operated by the Diamond Air Service in Nagoya using the same protocol previously employed by us (13). Briefly, in one parabolic flight, which lasted for approximately 1 h, 8–12 parabolas were performed in total with a 4–6-min interval between consecutive parabolas. At the end of each parabolic flight, mice were euthanized by cervical dislocation and replaced with the next mice. Parabolic flight was divided into five phases (Fig. 1). The first phase (I) was in normogravity (1 G, G: gravity), the second phase (II) was in hypergravity (1.3 G), the third phase (III) was in microgravity (0.03 G), the fourth phase (IV) was in hypergravity (1.8 G), and the fifth phase (V) was in normogravity (1 G). Each parabola provided 15–20 s of microgravity (phase III) and hypergravity (phase IV) and we evaluated data from the first parabola, except in Figs. 3B, 4B, 5B, 6B, in which the G force in the hypergravity phase (phase II) before the microgravity phase was kept below 1.3 G to avoid excessive gravity stress on mice and the effect of previous parabolas. During the experiment, the temperature in the plane was kept at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and air pressure was 0.9 ± 0.1 atm.

Animals

Male C57BL/6CrSlc mice aged 4 months were used for this experiment (Japan SLC, Inc., Hamamatsu). The

Fig. 1. Schematic representation of parabolic flight. Each parabola provided approximately 20 s of 0.03 G (G; gravity). Parabolic flight was divided into five phases. The first phase (I) was normogravity (1 G), the second phase (II) was hypergravity (1.3 G), the third phase (III) was microgravity (0.03 G), the fourth phase (IV) was hypergravity (1.8 G), and the fifth phase (V) was normogravity (1 G). The Y-axis indicates changes in gravity.

care and treatment of the animals were carried out according to the Japanese Government Animal Protection and Management Law (no. 105) and the Guidelines for Animal Experiments of Yokohama City University School of Medicine.

Experimental design and protocol

ECG recordings were obtained with a telemetric unit (PhysioTel; Data Sciences International, St. Paul, MN, USA). Infusion of NKH477 ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) (14), vidarabine ($15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) (15), or phosphate-buffered saline (PBS) as a control was done via an osmotic minipump (Model 2001; ALZET, Cupertino, CA, USA), which was implanted 16 h before the parabolic flight.

Time-domain measures

Conscious mice were separately placed in double-walled plastic cages, which were placed on a receiver in a special rack within the aircraft, and data from the freely moving mice were recorded by the data acquisition system during flight, as described previously by us (13). HRV measurements included mean HR, R-R interval (mean, max, minimum) and the standard deviation of normal R-R intervals (SDNN). The different G phases causes important changes in the R-R interval. Therefore, we also used the coefficient of variation (CV, %) as a normalized index of SDNN (CV-SDNN). $\text{SDNN (ms)} = \text{SD of all normal R-R intervals}$; $\text{CV-SDNN (\%)} = \text{SDNN} / \text{mean R-R} \times 100$.

Frequency-domain measures

In the frequency domain, the power spectral density

was calculated by applying the fast Fourier transformation (FFT) to overlapping segments of the resampled data and by averaging the spectral results (16, 20). The FFT was calculated by using 512 points and half overlap with a Hann window. Cutoff frequencies divided the power spectrum into two main parts, LF ($0.4 - 1.5 \text{ Hz}$) and HF ($1.5 - 4.0 \text{ Hz}$) powers and were determined by multiplying the standard frequencies used in human studies by 10 to account for HR differences between mice and humans, as recommended by Gehrmann et al. (21). HF powers were normalized to account for differences in total power (TP) between animals by multiplying the power region of interest by 100 and dividing by the difference between TP and very LF power ($0.0 - 0.4 \text{ Hz}$) (16).

Statistical analyses

All data are reported as the mean $\pm$ S.E.M. Statistical comparisons were performed with the Kruskal-Wallis nonparametric test followed by the Dunn test (Figs. 2, 3, 7, 8). Differences were considered significant at $P < 0.05$.

Results

Effect of parabolic flight on HR

Mean HR in the NKH477, vidarabine, and control groups were evaluated in each phase of parabolic flight during the first parabola. The HR in phase I in the NKH477 group was significantly higher than that in the control group [control group ($n = 12$): 558 ± 81 vs. NKH477 group ($n = 8$): $681 \pm 38 \text{ bpm}$; $P < 0.01$] (Fig.

Fig. 2. Comparison of HR during parabolic flights. Comparison of HR among phases I, II, III, IV, and V in the control ($n = 12$) (A), NKH477 ($n = 8$) (B), vidarabine ($n = 8$) (C) groups during the first parabola. HR was compared between phase I and phase II, III, IV, or V in each group (** $P < 0.01$) and also between the control and NKH477 groups (+ $P < 0.05$).

2B), as previously found in AC5TG (13, 22). HR showed a significant decrease in later phases (IV – V) in the control and vidarabine groups, but there was no significant change in the NKH477 group, suggesting that HR was stabilized in the NKH477 group, compared with the other groups during parabolic flight.

Effect of parabolic flight on R-R interval of HRV

Maximum, mean, and minimum R-R intervals in the three groups were examined during the first and eighth parabolas (Fig. 3). Maximum, mean, and minimum R-R intervals, in general, increased gradually in later phases (I through IV) during the first parabola (Fig. 3A). When maximum, mean, and minimum R-R intervals were compared in each group during the first parabola, the degree of variability of the R-R interval was smaller in the NKH477 group ($n = 8$) and greater in the vidarabine group ($n = 8$) than that in the control group ($n = 8$). In the eighth parabola (Fig. 3B), the degree of variability remained unchanged in the vidarabine group ($n = 8$), suggesting that adaptations to micro- and hypergravity were impaired in the vidarabine group (10, 23).

For further analysis, the R-R intervals under normogravity (phase I), microgravity (phase III), and hypergravity (phase IV) were plotted by time (s) versus R-R

(ms) interval in each group during the first (Figs. 4A, 5A, 6A) and eighth parabolas (Figs. 4B, 5B, 6B). The R-R intervals under normogravity (phase I) during the first parabola were stable in each group (Fig. 4A), but became more variable during the eighth parabola. However, the degree of variability was greater in the vidarabine group and smaller in the NKH477 group than that in the control group (Fig. 4B).

Under microgravity (phase III), the degree of variability was less in the NKH477 group, as compared with the other groups (Fig. 5A). However, the degree of variability was greater in the vidarabine group ($n = 8$) and smaller in the NKH477 group ($n = 9$) than that in the control group ($n = 8$) during the eighth parabola (Fig. 5B).

Under hypergravity (phase IV), the degree of variability showed a similar tendency to that under microgravity in the three groups during the first (Fig. 6A) and the eighth parabolas (Fig. 6B).

Taken together, these results may indicate that pharmacological activation of AC5 stabilizes the R-R intervals not only under microgravity but also under hypergravity during parabolic flight.

Fig. 3. Comparison of R-R interval during various phases of parabolic flights. Comparison of mean (mean), maximum (Max), and minimum (min) R-R intervals (ms) among phases I, II, III, IV, and V in the control ($n = 8$), NKH477 ($n = 8 - 9$), and vidarabine ($n = 8$) during the first parabola (A) and eighth parabola (B). Maximum R-R intervals were compared with the minimum R-R interval in each group (* $P < 0.05$).

Fig. 4. Comparison of R-R interval under normogravity. R-R interval under normogravity (phase I) is shown by the plot of time (s) versus R-R interval (ms) in the control ($n = 8$), NKH477 ($n = 8 - 9$), vidarabine ($n = 8$) groups during the first (A) and eighth (B) parabolas.

Fig. 5. Comparison of R-R interval under microgravity. Representative results under the microgravity (phase III) are shown by the plot of time (s) versus R-R interval (ms) in the control ($n = 8$), NKH477 ($n = 8$), and vidarabine ($n = 8$) groups during the first (A) and eighth (B) flights.

Effect of parabolic flight on SDNN of HRV

Because HR under gravitational stress is most likely regulated by the autonomic nervous system, we thus compared SDNN (Fig. 7A) and another parameter CV-SDNN (Fig. 7B) as a measure of total autonomic instability (24). SDNN and CV-SDNN were both significantly greater under microgravity (phase III) and hypergravity (phase II and IV) than those under normogravity (phase I)

in each group. Notably, the absolute value of SDNN under microgravity (phase III) as well as hypergravity (phase IV) was strikingly greater in the vidarabine group ($n = 8$) and much smaller in the NKH477 group ($n = 9$) than that in the control group ($n = 12$). CV-SDNN showed similar behavior, indicating that increased AC5 activity stabilizes the autonomic control of the heart rate under gravitational stress.

Fig. 6. Comparison of R-R interval under hypergravity. Representative results under the hypergravity (phase IV) are shown by the plot of time (s) versus R-R interval (ms) in the control ($n = 8$), NKH477 ($n = 9$), and vidarabine ($n = 8$) groups during the first (A) and eighth (B) flights.

Fig. 7. Comparison of SDNN and CV-SDNN during parabolic flights. The standard deviation of normal R-R intervals (SDNN) (A) and coefficient of variation (CV, %) as a normalized index of SDNN (CV-SDNN) (B) during the first parabola. These indexes were compared between phase I and other phases in the control ($n = 12$), NKH477 ($n = 9$), and vidarabine ($n = 8$) groups (*, # $P < 0.05$ vs. phase I).

Effect of parabolic flight on LF/HF of HRV

To further evaluate changes in autonomic nervous activity, we compared the ratio of LF to HF (LF/HF) in HRV analysis, an index of the sympathetic nervous tone in each phase of parabolic flight during the first parabola (16). When it was compared between normogravity (phase I) and microgravity (phase III), we found that LF/HF was significantly lower in phase I than that in phase III in the control and NKH477 groups (Fig. 8A) [control group ($n = 10$): phase I vs. phase III: 1.9 ± 0.4 vs. 0.9 ± 0.1 ms, $P < 0.05$; NKH477 group ($n = 7$): phase I vs. phase III, 2.1 ± 0.04 vs. 0.7 ± 0.05 ms, $P < 0.05$]. This was in agreement, at least in part, with previous studies in which decreased sympathetic nerve activity was demonstrated under microgravity in normal individuals (25). However, the vidarabine group did not show such a decrease [vidarabine group ($n = 8$): phase I vs. phase III: 1.9 ± 0.3 vs. 1.7 ± 0.3 ms, $P = \text{NS}$, not significant], suggesting that changes in sympathetic tone be-

came small when AC5 activity was decreased with vidarabine.

Under hypergravity (phase IV), the ratio of LF/HF showed a similar tendency to that under microgravity in the three groups.

When it was compared between phase I (pre-parabolic flight) and phase V (post-parabolic flight), there was no difference in control group (phase I vs. phase V: 1.9 ± 0.4 vs. 1.7 ± 0.4 ms, $P = \text{NS}$) while it remained decreased in the NKH477 group (phase I vs. phase V: 2.1 ± 0.04 vs. 0.7 ± 0.1 ms, $P < 0.05$) (26). However, the vidarabine group showed no significant differences (phase I vs. phase V: 1.9 ± 0.3 vs. 1.9 ± 0.6 ms, $P = \text{NS}$).

Accordingly, sympathetic tone was transiently decreased under micro- and hypergravity in the control while it decreased in micro- and hypergravity and remained decreased in the NKH477 group even when gravity was normalized in the post-microgravity level flight (phase V); in contrast, such decreases were all ab-

Fig. 8. Comparison of LF/HF and nHF during parabolic flights. A) LF/HF was compared as an index of sympathetic nerve activity in each phase during the first parabola in the control ($n = 10$), NKH477 ($n = 7$), and vidarabine ($n = 8$) groups (* $P < 0.05$ vs. phase I). B) nHF was compared as an index of parasympathetic nerve activity in each phase during the first parabola in control ($n = 10$), NKH477 ($n = 7$), and vidarabine ($n = 8$) groups (* $P < 0.05$ vs. phase I).

sent in the vidarabine group.

Effect of parabolic flight on nHF of HRV

HF power was normalized to account for differences in TP and was examined as an index of parasympathetic tone (16). When nHF was compared between normogravity (phase I) and microgravity (phase III), we found that it was significantly increased in microgravity in both the control and NKH477 groups (Fig. 8B) [control group ($n = 10$): phase I vs. phase III: 31 ± 3.6 vs. 49 ± 3.2 ms, $P < 0.05$; NKH477 group ($n = 7$): phase I vs. phase III: 31 ± 1.2 vs. 47 ± 1.2 ms, $P < 0.05$), suggesting that parasympathetic nerve tone was increased under microgravity in both the control and NKH477 groups. In contrast, there was no increase in nHF in the vidarabine group under microgravity (phase I vs. phase III: 32 ± 3.5 vs. 37 ± 3.8 ms, $P = \text{NS}$, $n = 8$), suggesting that microgravity-induced enhancement of parasympathetic nerve tone was attenuated in the vidarabine group.

Under hypergravity (phase IV), nHF showed a similar tendency to that under microgravity in the three groups.

nHF in post-microgravity (phase V) was returned to a similar level to that in pre-microgravity (phase I) in WT (37 ± 4.1 ms, $P = \text{NS}$), while it further increased in the

NKH477 group (57 ± 6.2 ms, $P < 0.05$) (Fig. 8B). However, there were no such increases or decreases in the vidarabine group (38 ± 6.6 ms, $P = \text{NS}$).

Thus, parasympathetic nervous tone was transiently elevated in the control group, remained elevated in the NKH477 group, and showed no changes in the vidarabine group.

Taking these results together, in comparison to the control group, the NKH477 group showed continuous responses in the above autonomic (sympathetic and parasympathetic) parameters while the vidarabine group showed attenuated responses under micro- and hypergravity.

Discussion

Our current study demonstrated that activation of AC5 activity with NKH477 could stabilize HRV, while inhibition of AC5 with vidarabine could destabilize HRV under micro- and hypergravity during parabolic flight. Further, the absolute value of the total autonomic variability index (SDNN) was much greater in the vidarabine group than that in the control or NKH477 groups under microgravity as well as hypergravity. A marker of the sympathetic

nervous tone (LF/HF) was decreased to a greater degree in the NKH477 group relative to the control group, and a marker of parasympathetic tone (nHF) was instead increased to a greater degree in the NKH477 group; these changes were absent in the vidarabine group, indicating that pharmacological modulation of AC5 activity might be able to regulate the autonomic control of the heart rate under gravitational stress during parabolic flight.

AC consists of 9 mammalian transmembrane isoforms that differ in tissue distribution (3, 5), with type 6 AC (AC6) being the major fetal cardiac AC isoform, and AC5, the major cardiac isoform in adults (27, 28). All isoforms are stimulated by Gs, but AC5 and AC6 are the only Gi- and Ca²⁺-inhibitable AC isoforms (3, 5). AC5 is responsible for at least one-third of the cardiac AC catalytic activity and plays an important role not only in sympathetic, but also parasympathetic cardiac function and heart rate without affecting Gi-gated K⁺-channel current, which regulates normal pacing activity, as shown recently by us and others in AC5KO (7, 29). Overexpressing AC6 results in maintained basal HR (30), while overexpressing AC5 results in increased basal HR (13, 31), suggesting that AC5 may play an important role in regulating HR through the parasympathetic arm of the autonomic nervous system, as well as the sympathetic arm.

For many years, it has been recognized that many astronauts suffer from symptoms suggestive of autonomic dysfunction such as nausea, vomiting, and dizziness during the early days of space missions and their cardiovascular system subsequently adapts to the microgravity environment of space appropriately and effectively. However, on return to earth, from one-quarter to two-thirds of them have reduced orthostatic tolerance for a few weeks. The underlying molecular mechanisms remain unknown and are probably multifactorial, although alterations in the autonomic nervous system and/or neuromuscular function after spaceflight probably contribute to this problem (18, 19).

We have previously demonstrated that AC5 plays an important role in stabilizing HRV under microgravity, as well as hypergravity, during parabolic flight using genetically engineered mouse models (13). This present study has demonstrated that pharmacological activation/inhibition of AC5 activity can stabilize/destabilize the HRV not only under microgravity but also under hypergravity during parabolic flight. Importantly, NKH477 which was used in this study as a selective AC5 activator (NKH477) has been widely used for the treatment of acute heart failure in Japan since 1999. We will not need to wait for many years to get authorization for the use of NKH477 to treat autonomic dysfunction during and after space flight, if its usefulness is sufficiently confirmed by

further studies.

There are plans for suborbital spaceflight or lunar tours for normal subjects as tourists, and also for prolonged space flight to Mars. It is also likely that not only astronauts, but also normal subjects will stay for up to several months in the international space station. Therefore, it is important to clarify the mechanism of the alterations in the autonomic nervous system and to develop suitable treatment. Our studies indicate that short-term, not long-term, pharmacological activation/inhibition of AC5 activity with NKH477 or vidarabine might rescue the autonomic dysfunction in the heart under microgravity in space and on earth after space flight, especially for untrained normal subjects.

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