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A COMPREHENSIVE STUDY ON HYPERGRAVITY EFFECTS ON THYROID TISSUE LIPIDS AND PROTEINS

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ABSTRACT

The thyroid gland plays a crucial role in maintaining body homeostasis, and its proper function is vital for good health. Long-term space missions have revealed that mice exhibit heightened sensitivity to hypogravity, particularly in thyroid tissue. Physiological deconditioning resulting from extended spaceflight can be mitigated through exposure to hypergravity. In this study, various lipids and proteins associated with thyroid tissue function were evaluated following hypergravity treatment. Analysis included quantification of chemoglobulin (CHO), different sphingomyelin (SM) and ceramide species, cAMP, caveolin-1, thyrotropin (TSH) receptor, as well as signaling molecules STAT3 and TH, and cholesterol (CHO). Results indicated that after hypergravity exposure, caveolin-1 and TSH receptor expression increased, while STAT3 levels decreased. Interestingly, cAMP levels remained unaffected. Additionally, TSH receptor subunits were shed and dispersed throughout the cell membrane upon TSH treatment. While no significant differences were observed in SM and ceramide species, a noteworthy finding was the substantial reduction in CHO levels. This study underscores the impact of hypergravity on CHO and TSH receptor dynamics within the thyroid gland, suggesting that hypergravity-induced CHO depletion may disrupt TSH-TSH receptor interactions, leading to significant alterations in lipid rafts.

Key words: Thyroid gland, Hypergravity, Lipid rafts, TSH receptor, Spaceflight effects.

INTRODUCTION

The environment on earth has changed over time due to the variations of different factors, except for the gravitational force, which remains constant. Cells of all living organisms are shaped and function according to gravity. In vitro as well as in vivo, the differences in its composition may affect cellular behavior. Studies of microgravity on board the International Space Station and parabolic flights are the primary subjects of scientific research. Hypergravity and vibrations were nevertheless transient during parabolic flights and space travel in general [1]. The rocket acceleration resulted in hypergravity forces and launch vibrations during the launch phase. Hypergravity should not be an important factor during long missions in Space, but astronauts themselves as well as machines, for instance, cause vibration during workouts [2]. The 31 normal parabolas flown during the parabolic flight included 22 seconds of microgravity, normal gravity, and hyper gravity periods [3, 4]. Therefore, the impact of hyper gravity on biological systems needs to be considered. During hyper gravity conditions, plants were able to modify their body shape and regulate the rigidity of their cell walls through changes in the orientation of microtubules and the metabolism of anti-gravitational polysaccharides [5]. As the function of hyper gravity-exposed platelets increased, glycoprotein Ib-alpha was seen to be expressed significantly on the surface and associated with the cytoskeleton [6].

A ground-based study to investigate the effects of hypergravity on cell cycle control signaling in human T cells didn't reveal any effects. Both proliferation and differentiation were stimulated by hypergravity in myoblasts [7]. It is commonly accepted that the thyroid gland regulates the function of the musculoskeletal system, the cardiovascular system, the immune system, and the nervous system.

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The mechanical forces generated by hypergravity and vibration significantly affected the mRNA concentrations of growth factors in follicular thyroid cells. Physical force had a strong effect on activation of the IL6 gene in thyroid cells grown in vitro as monolayers. The human endocrine system, particularly the thyroid gland, changed significantly as a result of space travel. Compared with control laboratory-kept mice, thyroid glands isolated from spaceflight mice showed structural and functional modifications. Long-term exposure to real microgravity environment resulted in more homogeneous thyroid tissues with larger and thicker follicles, containing thicker and more nucleated thyrocyte cells. Caveolin-1 and the thyrotropin receptor (TSHR) were overexpressed both basally and in response to thyrotropin (TSH) [8]. The expression levels of sphingomyelinase (SMase) and sphingomyelin synthase (SM-synthase), enzymes that participate in cell signaling, were both elevated. Our previous hypogravity studies showed that some key thyroid gland results were remarkably similar despite the apparent opposite effects of hypogravity and hypergravity. There was no change in SMase expression in hypergravity as in hypogravity, but there was a similar nucleus-cytoplasm translocation between the two experimental conditions. In light of this, it is possible that changing gravity conditions might affect molecular remodellings and, thus, change the fate of cells [9].

The thyroid gland was also shown to lose parafollicular cells under both hypogravity and hypergravity, resulting in a reduction in calcitonin production, suggesting that mechanical forces may be involved in bone homeostasis regulation via the thyroid gland [10]. In terms of thyroid hormone production, hypergravity differs from hypogravity only partially. This study investigated how hypergravity impacted signal transduction proteins and lipids in the thyroid gland in vivo.

MATERIALS AND METHODS

Reagent

There were obtained secondary antibodies conjugated with fluorescein isothiocyanate (FITC) against STAT3, TSHR, Caveolin 1, and TSHR.

Animal care and experimental design

A Public Veterinary Health Department approval was obtained for all experimental procedures. According to the guidelines and regulation for the care and use of laboratory animals, the experiment also followed these guidelines. A 26g centrifuge was used in the lab to maintain seven mice for 90 days in hypergravity (2 gravity samples, 2 grams). A control sample of six mice was maintained in the Vivarium under identical environmental conditions, fed the same diet, and were fed the same diet. A similar group of mice was used in previous hypogravity studies as control mice. In accordance with various experimental protocols, thyroid tissue was immediately processed or frozen after mice were killed by inhaling carbon dioxide.

Thyroid tissue treatment

To test the effect of stimulation with TSH on cAMP and TSHR amount, right thyroid lobes of all mice under study were excised and used. In immunofluorescence analyses, left lobes of three 2 g and 3 V mice were analyzed by UFLC-MS/MS, and four 2 g mice were analyzed by UFLC-MS/MS.

TSH stimulation

A thyroid lobe fragment was removed from the right side of the body. Following the manufacturing instructions, the following steps were carried out: adding cAMP acetylcholinesterase (AChE), which is capable of binding specific antibodies in an inverse proportion to the amount of free cAMP in the sample, followed by adding Ellman's reagent containing AChE's substrate. In order to determine the intensity of color at 412 nm, spectrophotometric measurements were conducted on the product of the reaction. Protein amounts and immunoblotting analyses were performed on the pellets [11].

Pellet treatment

During all procedures, a dounce homogenizer was used to homogenize the pellets obtained after centrifugation reported in the cAMP assay. Throughout all the procedures, the temperature was maintained at 4 degrees Celsius. In addition to using the suspension for protein dosage, FRTL-5 cells were used as positive controls for immunoblotting analysis [12].

Western immunoblotting

In order to detect TSHR and STAT3, we electrophoresed 30 mg of pellet proteins on polyacrylamide slab gels 10% and 12%, respectively. Using Coomassie-blue stained gels, electrophoresis images were analyzed. In accordance with our previous description, the proteins were transferred into nitrocellulose for 90 minutes. A molecular size standard migration was used to calculate the apparent molecular weight of the proteins. Densitometry scanning and analysis were used to evaluate the area density of the bands [13].

Lipid extraction

A methanol solution of 1 mL was used to extract lipids from NFL and N pellets. MTBE and ultra-pure water were added to an ultra-pure water solution by 3 mL each. A vortexing step of one minute was followed by a centrifugation step of five minutes at 3000 g. Supernatants were collected. In addition to the first extraction, the supernatant from the pellet was added to the original extraction with MTBE. Methanol was added to 500 mL of

dried organic phase and resuspended under nitrogen flow [14].

Immunofluorescence analysis

A neutral phosphate-buffered formaldehyde solution containing 4% phosphate was used to fix tiroid tissues for 24 hours. Essentially random orientations were used when dropping lobes in paraffin. Four-mm-thick sections were cut from the paraffin blocks. At a 20x magnification, fluorescence microscopes equipped with OLYMPUS DP 50 cameras examined the samples.

RESULTS

TSHR content was increased in hypergravity experiments by immunoblotting. Using absolute ethanol for the fixation of tissue fragments, the pellet obtained with centrifugation was used for immunoblotting to assess cAMP levels. This study have thus tested the thyroid cells' sensitivity to hormones in hypergravity conditions by stimulating cAMP production with TSH. As shown by the results, basal and TSH-stimulated cAMP production were similar in V and 2 g samples, suggesting that despite increased TSHR activity in hypergravity, the loss of TSH-linked subunits prevents hormonal responses from occurring. A membrane remodelling could result in an easier extraction of the b subunit due to hypergravity rather than ethanol treatment, since hypergravity induces a membrane remodelling. It is therefore possible that the first step in the cell's response to hypergravity is perception from the cell membrane. TSHR was immunofluorescence analyzed with specific antibodies to verify this hypothesis. There were fluorescent receptors present on the surface of the thyrocytes surrounding the follicles in V, as indicated by the sharp brightness of the fluorescent signal. In 2 g samples, fluorescence levels were higher, confirming the immuneglotting results. Thyrocytes also showed a fluorescent signal throughout their surfaces. Our next question was whether the distribution of TSHRs across the whole cell surface could be explained by the alteration of the lipid component of the cell membrane, since TSHRs are G protein-coupled receptors that are localized in membrane microdomains, including caveolae and lipid rafts enriched in SM and CHO. Using UFLC-MS/MS, we focused on SM species and CHO species in thyroid tissue lipid fraction. To establish possible specific variations of SM species by using specific standards, we have assessed the amount of SM, PC and ceramide species. In addition to PC and ceramide species, SM species also did not change, as shown in our results. On the other hand, CHO levels decreased by 49%. Our observations were extended to SM species containing longer fatty acid chains and with greater unsaturation degrees based on the literature-known relationship SM-CHO. Based on the RT and the molecular weight, the molecules were identified. Based on the percentages of the areas, we compared the V and 2 g samples. Neither saturated/unsaturated ratios of total SM nor SM species showed significant changes.

A key protein for the caveolae is Caveolin-1, and STAT3, which suppresses thyroid tumor growth, was examined to explore the mechanism underlying gravity's effect on thyroid protein fraction. As compared to V mice, Caveolin-1 protein levels were higher in 2 g mice's thyroid tissues both when untreated with TSH and when treated with TSH. 2 g C had an apparent molecular weight of 22 kDa, 14% higher than V C, and the density value for Caveolin-1 remained stable after TSH treatment. Compared to V mice, 2 g mice had a lower level of immunopositive STAT3. After TSH treatment, STAT3 area density remained significantly unchanged after TSH treatment, being 53% lower in 2 g C than in V C.

DISCUSSION

Our previous research had suggested that thyrocytes may remodel their cell membrane under hypergravity conditions by increasing TSHR surface protein and, as a consequence, become more sensitive to TSH treatment [15]. Despite our best efforts, we were not able to demonstrate a straightforward relationship between the two. TSHR is a G proteincoupled receptor. Disulphide bridges hold together a wide b transmembrane and intracellular subunit (30-42 kDa), containing an extracellular subunit (53 kDa) that interacts with TSH. It is important to note that receptor signaling complexes were assembled on the surface of raft domains. Therefore, gravity forces may act specifically on lipid rafts to remodel cell membrane structure. TSHR shedding and extracellular release are facilitated by TSH treatment. TSHR a subunit was not significantly reduced in V mice by TSH treatment, but by 2g mice it was reduced 2.1 times by TSH treatment. A subunit reduction of the a subunit would be expected in C V mice after TSHR treatment [16]. Considering that our TSHR assessment was conducted after ethanol was added to the pellet, it is noteworthy that we have performed the TSHR analysis in the pellet after ethanol was added to the pellet. As a consequence, ethanol may have fixed the membranes by impeding shedding of the membrane-bound TSHR a subunit and extraction of the intramembrane b subunit after TSH treatment. In hypergravity samples, TSH subunits behaved differently. As a result of these findings, we concluded that hypergravity-induced CHO depletion strongly disrupted lipid rafts, thereby losing their specific localization, resulting in the spread of the TSHR throughout the cell membrane. In these circumstances, we demonstrated the presence of TSHR as well as a rise in CHO in the medium using our in vitro system. When compared with control samples, hypogravity inhibited the production of cAMP after TSH stimulation. In this case, however, we observed a different phenomenon [17].

CONCLUSION

The study highlights the impact of hypergravity on thyroid tissue function, shedding light on lipid and protein changes. Hypergravity led to alterations in caveolin-1 and TSH receptor levels, alongside changes in signaling molecules like STAT3. Notably, cholesterol levels decreased significantly, suggesting a role in TSH-TSHR interaction disruption. These findings deepen our understanding of thyroid physiology under altered gravitational conditions, offering insights into potential therapeutic avenues.

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