Author

Alia Shbeeb says that research has dramatically influenced her career aspirations and perspectives of science, while allowing her to appreciate education and the acquisition of knowledge. One of the primary motivations behind her research project is the possibility that her results will contribute to improving the ability of astronauts to explore space and reduce orthostatic intolerance. Her experience with undergraduate research has helped pave the way toward her goal of pursuing a career in medicine while participating in clinical research. In addition to her academic pursuits, she enjoys playing tennis, swimming, and reading law and medical thriller novels.

Key Terms

- Calphostin C
- Hindlimb-Unweighting (HU)
- Nifedipine
- Protein Kinase C (PKC)
- Vascular Hyporesponsiveness
- Voltage-Operated Calcium Channels (VOCC)

Effects of Simulated Microgravity on Vascular Contractility: Role of Voltage-Operated Calcium Channels

Alia Shbeeb
Biological Sciences

Abstract

The objective of this study was to determine the effects of zero gravity on the fundamental calcium handling in vascular smooth muscle. Male Wistar rats were hindlimb unweighted (HU) for 20 days in order to simulate microgravity and its alteration of the cardiovascular system. Next, collection and analysis were performed on the contractile responses of abdominal aortas from both HU and control rats in the presence of various antagonists. HU-treated tissues displayed a reduced contractile response to norepinephrine, which indicates alteration of an a-receptor-mediated second messenger pathway. In addition, voltage-operated calcium channel function and protein mass were substantially reduced in HU-treated tissues, whereas HU-treatment seemed to have no effect on protein kinase C. This HU-induced alteration of voltage-operated calcium channels strongly suggests that reduced cytosolic calcium in HU-treated tissues may be an integral factor in the markedly reduced contractile response of the abdominal aorta to norepinephrine. Further studies are needed to determine cytosolic calcium levels in HU and control tissues and the mechanisms involved in the possible intracellular calcium level reduction.

Faculty Mentor

Alia Shbeeb has made an important contribution to our understanding of the effects of microgravity on vascular function. Using a rodent model, she showed that microgravity decreases arterial constriction, in part, by impairing the function of voltage-operated calcium channels. Vascular impairment underlies orthostatic intolerance (syncope on standing) experienced by space-adapted astronauts and 500,000 Americans who suffer from this syndrome chronically. Alia's work points to calcium channels as a potential therapeutic target for the treatment of orthostatic intolerance. Both Alia and I have found it rewarding to work together on this project, and I encourage all students to become involved in faculty-mentored undergraduate research.

Ralph Purdy
College of Medicine

Introduction

The desire of humans to explore space is severely limited by the physiological restraints induced by the adverse effects of zero gravity. Microgravity and changes in body posture (e.g., prolonged bed rest) alter hydrostatic pressure gradients, resulting in the redistribution of body fluids from the lower extremities to the upper half of the body (Hargens et al., 1984). Under the influence of gravity, the blood pressure is 70 mmHg gradient at the head and 200 mmHg at the feet, which microgravity changes to a uniform blood pressure of 100 mmHg throughout the entire body. This hemodynamic shift may trigger subsequent adaptations of the cardiovascular system. These changes include an acute increase, but chronic decrease, in central venous pressure, a decrease in blood volume, and a decrease in normal cardiovascular function characterized by orthostatic intolerance and a decrease in exercise capacity (Watenpaugh and Hargens, 1996). These adaptations experienced by astronauts can be mimicked using ground-based rodent models, namely the HU model.

The central hypothesis of this study was based on the observation that simulated microgravity as a result of HU treatment induces a generalized reduction of arterial smooth muscle contractility in response to norepinephrine (NE). Contraction of vascular smooth muscle (VSM) is dependent on numerous factors, including the sensitivity of the contractile proteins actin and myosin (Horowitz et al., 1996), vasodilator and vasoconstrictor secretion levels (Jiang and Morgan, 1989), and intracellular calcium levels (Jiang and Stephens, 1994). In smooth muscle cells, the presence of high cytosolic calcium levels allows for the formation of the Ca^{++}/calmodulin complex, which, in turn, phosphorylates myosin light chain kinase (MLCK). MLCK phosphorylates, and thereby activates, the myosin light chain allowing the myosin head to interact with actin, thus initiating the cross-bridge cycle (Kamm and Stull, 1985). The intent of this study was to investigate whether simulated microgravity adversely affects the central mechanism of contraction, thus contributing to the simulated microgravity-induced vascular hyporesponsiveness in the rat abdominal aorta. This generalized reduction in contraction may result from decreased levels of intracellular calcium or an alteration of the sensitivity of the contractile apparatus upon exposure to microgravity. The present study focused on mechanisms regulating intracellular calcium.

Two basic mechanisms are responsible for determining the concentration of cytosolic calcium: voltage-operated calcium channels in the plasma membrane and the release of stored calcium from the sarcoplasmic reticulum (Somlyo and Himpens, 1989). In effect, decreased calcium levels may result from alteration of voltage-operated calcium channels (VOCC) and/or the reduced ability to store and release significant levels of calcium from the sarcoplasmic reticulum. In the present study, the effect of HU treatment on protein kinase C, VOCC, and the contractile response to NE, serotonin (5-HT), and calcium were assessed in order to help decipher the mechanism that is altered by HU treatment.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, Irvine under protocol #97-1588. Male Wistar rats weighing approximately 200-250 g were randomly assigned to control and HU groups. The hindlimb-unweighted treatment involved suspending the animal by a swivel tail harness attached to the top of the cage, so that only the forelimbs were in contact with the ground and the

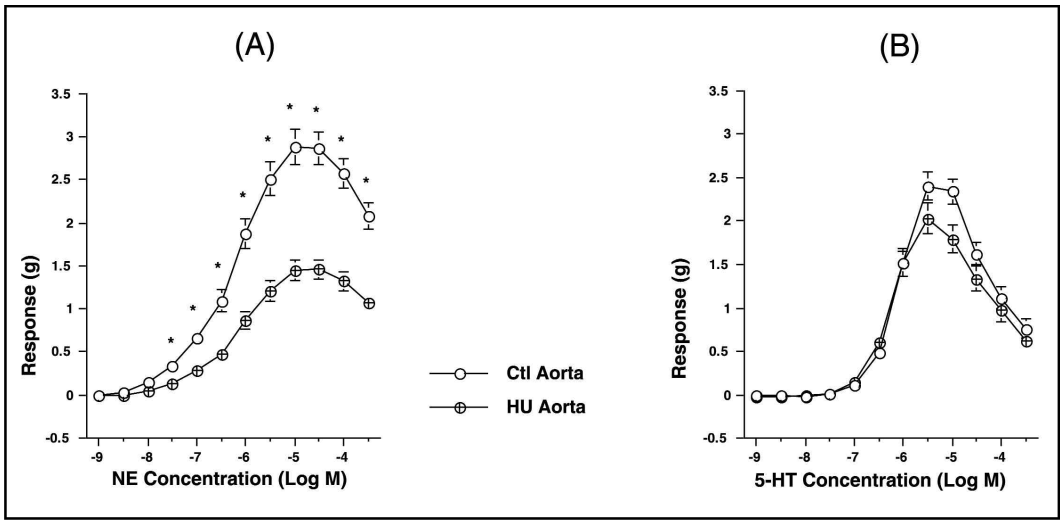

Figure 1

Concentration-Response Curves for the contractile effects of norepinephrine (A) (n=34) and seroton (B) (n=37) in control and HU abdominal aorta rings. Values are means ± SE; n=34,37. *p < 0.05.

body made a 35° angle with the floor of the cage. The preparation of the hindlimb unweighted model is as follows. The rat was placed under restraint, which allowed easy access to the tail. The tail was sprayed lightly with tincture of benzoin and allowed to air dry. One strip of Skin-Trac was applied to opposite sides of the tail. Next, three small strips of Elastoplast bandage were used to fasten the harness (Thompson et al., 1987). HU treatment was applied for 20 days, after which the animals were exposed to 100% carbon dioxide for 90 sec to produce anesthesia and then euthanised by rapidly opening the chest and removing the heart.

The abdominal aortas were isolated, removed and cleaned of extraneous tissue, cut into 3 mm rings, and mounted on luminal wires in tissue baths containing Krebs bicarbonate solution for the measurement of isometric contraction. The tissue was stretched to a resting force of 2 g and allowed to equilibrate in 37 °C, oxygenated Krebs solution. The rings were exposed twice to 100 mM K^+ solution. Tissues were allowed to equilibrate in normal Krebs solution in the presence and absence of various antagonists following recovery from their exposure to the 100 mM K^+ solution. In preparation for the calcium concentration response curves, 1 mM EGTA, a chelator, was added in each of the baths for 20 min. Next, calcium-free 100 mM K^+ Krebs solution was added in order to ensure the absence of calcium. Contractile responses to NE, serotonin, and calcium were measured in the presence and absence of the antagonists. Agonist concentration response curves (CRC) were obtained by cumulative addition of agonists in 0.5 log increments.

In preliminary experiments, some tissues were removed, isolated, cleaned, and subjected to protein analysis of VOCC using standard western blotting techniques.

Mean values were obtained from five or more independent experiments in the contractile measurements. Statistical analysis was carried out using analysis of variance, and differences between individual control versus treatment mean values were analyzed by a post hoc Scheffe's test. Differences were considered significant when $p < 0.05$.

Results

HU-induced vascular hyporesponsiveness in the rat abdominal aorta was observed in response to NE but was absent when the tissue was stimulated with serotonin (Figure 1). Factors that may be involved in this differing contractile response were assessed. Nifedipine, an L-type VOCC antagonist, was used to inhibit specific pathways that the a1 and 5-HT_{2A} receptors share in mediating smooth muscle contraction. Nifedipine produced a significant decrease of the control, but not HU, aorta response to NE. In contrast,

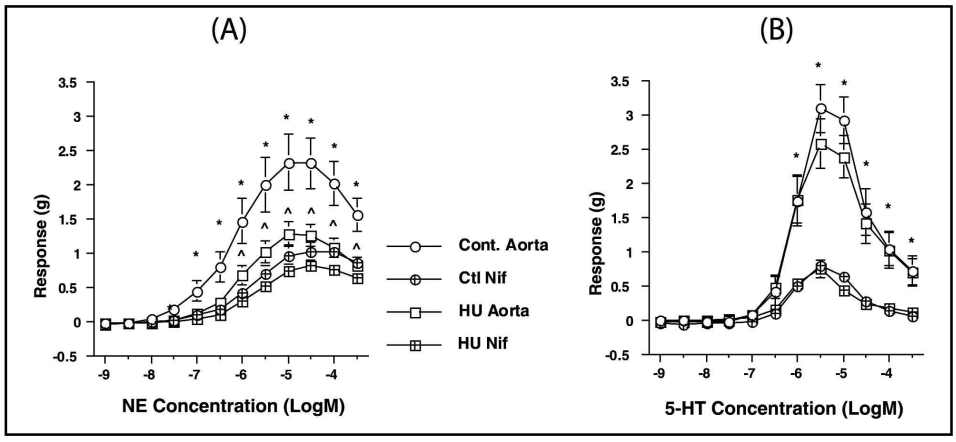

Figure 2

Concentration-response curves for the contractile effects of norepinephrine (A) and serotonin (B) in control and HU abdominal aorta rings in the presence and absence of 10-6 nifedipine. Values are means ± SE; n=8. *$p < 0.05$.

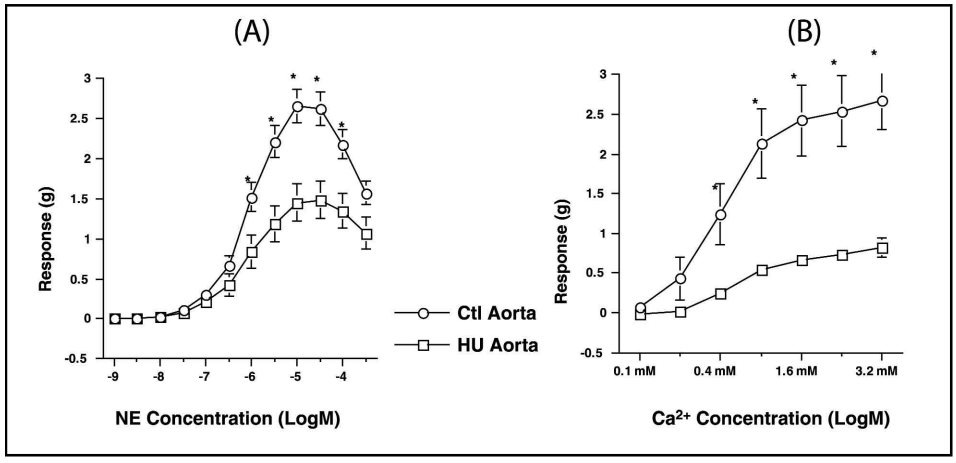

Figure 3

Concentration-response curves for the contractile effects of norepinephrine (A) and calcium (B) in control and HU abdominal aorta. Values are means ± SE; n=7,5. *$p < 0.05$.

nifedipine strongly inhibited the contractile responses of both control and HU tissues to serotonin to an equal extent (Figure 2). NE and calcium-induced contractions in both control and HU tissues were also compared. Decreased levels of HU abdominal aorta contraction in comparison to control were significantly greater in calcium-induced contraction than in the NE-induced contractile response (Figure 3). Calphostin C, a protein kinase C (PKC) inhibitor, was used in NE, 5-HT, and calcium-induced contractions. Calphostin C (0.3 μM) completely abolished contraction in both the control and HU arteries in response to NE. In addition, calphostin C (0.3 μM) significantly inhibited contraction equally in both control and HU animals in the 5-HT induced contractile response (Figure 4). However, calphostin C at 0.1 μM did not significantly inhibit the calcium concentration response curves (CRC), but did affect the NE CRC. Furthermore, preliminary data indicates that HU treatment markedly reduces total protein mass levels of VOCC (Figure 5).

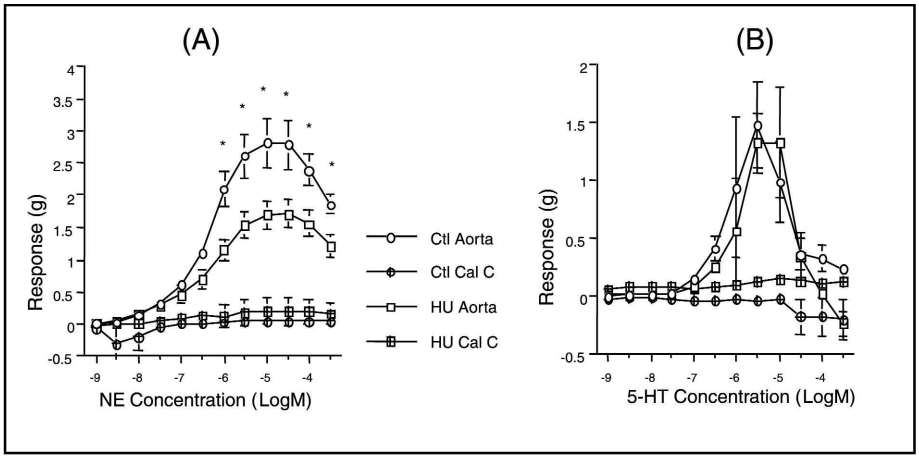

Figure 4

Concentration-response curves for the contractile effects of norepinephrine (A) and serotonin (B) in control and HU abdominal aorta rings in the presence and absence of $3x10^{-7}$ M calphostin C. Values are means ± SE. n=5. *$p < 0.05$.

Discussion

A generalized decrease in vascular contractility in response to a variety of vasoconstrictive agents including norepinephrine has been demonstrated in the HU group. It was hypothesized that the different contractile responses to serotonin and NE induced by HU may be due to an HU-induced alteration of a1-adrenoreceptor function possibly leading to either uncoupling or decreased function of second messengers associated with the a-receptor. Nifedipine, a VOCC blocker, had little effect on the NE-induced contraction in HU aorta. This implies that VOCC contributed little to the contraction (i.e., VOCC exhibited reduced function). This observation is supported by the significantly larger decrease in HU tissue contraction in response to calcium, when compared to NE. The contractile response to NE is mediated by multiple second messengers (i.e., VOCC, receptor-operated calcium channels, IP_3, intracellular Ca^{++} release, etc.). However, the contractile response induced by calcium is solely mediated by VOCC. Therefore, the substantial loss of VOCC function induced by HU treatment can account for the difference between NE and calcium-mediated contractile responses in control and HU-treated tissues.

In addition to VOCC, protein kinase C is also a key component of the signal transduction pathway in vascular smooth muscle contraction. The abolished contraction in control and HU tissues in response to both NE and 5-HT in the presence of 0.3 mM calphostin C, a PKC inhibitor, demonstrates that HU treatment does not affect PKC activity. In addition, this finding signifies that PKC is an early and integral step in the second messenger pathway. The significant reduction of response in tissues treated with 0.1 mM calphostin C to NE in control and HU, and the absence of effect of 0.1 mM calphostin C on calcium demonstrates that calphostin C selectively inhibits PKC.

The marked reduction of total VOCC protein mass in HU-treated tissues indicates a decrease in calcium channels. This finding further suggests that HU treatment affects the fundamental handling of calcium, which is a critical factor in the basic mechanism of contraction. Further studies are required to elucidate whether the ability to elevate intracellular calcium levels during agonist stimulation has been changed in HU-treated arteries, and to determine the precise mechanism for this alteration.

Conclusion

It was observed that simulated microgravity induces vascular hyporesponsiveness to NE. This decreased contractile response is one of the factors responsible for the severe adverse effects experienced by astronauts in space and upon return to normal gravitational forces. The results indicate that HU treatment alters voltage-operated calcium channel function and protein mass levels. In addition, the contractile response to calcium in HU-treated tissues was signifi-

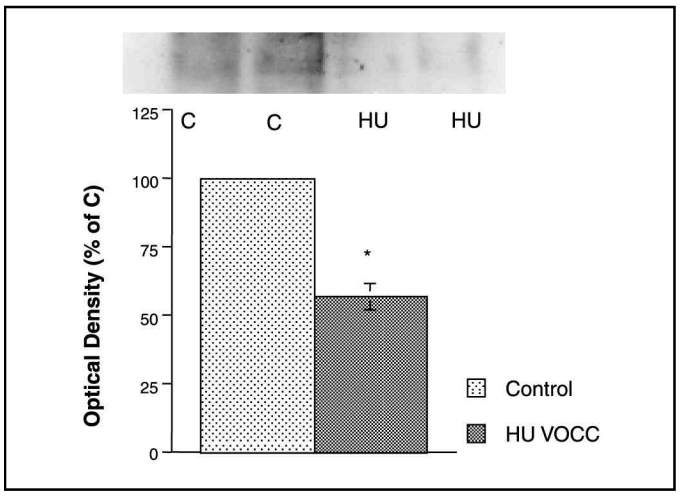

Figure 5

Representative results of western blot analysis of total VOCC protein levels in rat abdominal aorta from control and HU animals. n=3 blots. * Control different from HU at $p < 0.05$.

cantly depressed in comparison to the contractile response to norepinephrine. These results indicate that the fundamental handling of calcium in these cells may be altered. This alteration may thus contribute to reduced intracellular calcium levels, the ultimate factor leading to vascular hyporesponsiveness.

Acknowledgements

I would like to thank Dr. Purdy for his kind support, guidance and editorial advice throughout the entire project. I would especially like to thank Chadi Kahwaji, the graduate student with whom I worked, for his contribution and constant encouragement. In addition, I would like to acknowledge my fellow undergraduates Aisha Kaka, Khiem Luu and Naglaa El-Abbadi for helping me with the experiments. Moreover, I am truly thankful for the research team of the Purdy lab for supporting my project.

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