MICROGRAVITY-INDUCED VASCULAR HYPORESPONSIVENESS: NITRIC OXIDE-DEPENDENT AND -INDEPENDENT MECHANISMS

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INTRODUCTION
Space-flight-adapted astronauts exhibit orthostatic intolerance. Recent evidence points to a decreased capacity to elevate peripheral resistance on standing as a major underlying mechanism. We have used the hindlimb unweighted (HU) rat to simulate microgravity, and to explore mechanisms by which vascular function could be impaired. 20 days of HU results in a marked reduction of contraction of abdominal aorta, carotid and femoral artery to norepinephrine. Our recent results have identified an up-regulation of the expression of nitric oxide synthase isoforms in vascular and nonvascular tissues, representing a chronic increase in nitric oxide-dependent vasodilator mechanisms. In addition, we have found the HU reduces or eliminates selected second messenger signaling steps associated with vascular contraction to norepinephrine.

CURRENT STATUS OF RESEARCH

Methods
Wistar rats were prepared with tail harnesses that were tethered to the top of the cage. The tether was shortened to elevate the hindlimbs 0.5cm from the cage floor when fully extended, tilting the body approximately 35°. After twenty days, tissues from these rats and their time-paired caged controls were isolated for in vitro analysis. Blood vessels were cut into 3mm rings and mounted in organ baths for isometric contraction/relaxation measurements. Blood vessels, heart, kidney and brain were subjected to Western blot analysis to determine protein mass of the endothelial constitutive, inducible and neuronal isoforms of nitric oxide synthase (ecNOS, iNOS and nNOS, respectively). Tissue content of nitrates and nitrites, the stable metabolites of nitric oxide, were isolated, chemically converted to nitric oxide and measured by chemiluminescence.

Results
Endothelium removal in the HU carotid artery ring restored the norepinephrine-induced contraction of this vessel to control levels. The sensitivity of the HU carotid artery to acetylcholine-induced relaxation was 10-fold greater than control. Protein mass of ecNOS in the HU carotid artery was greater than control. None of these HU-induced effects was seen in either abdominal aorta or femoral artery. Thus, up-regulation of endothelium-dependent nitric oxide function occurs in the carotid artery, but not abdominal aorta or femoral artery. Blockade of either cyclooxygenase or thromboxane A₂ (TP) receptors markedly depressed the already weak contraction of HU carotid artery to norepinephrine, but had no effect in control artery rings. Such blockade had no effect in endothelium-denuded HU arteries. Thus, HU treatment up-regulates endothelial nitric oxide vasodilator activity and prostaglandin-dependent vasoconstrictor activity. The overall effect of HU is a reduction in the contractile response of carotid artery to norepinephrine.

In femoral artery, HU treatment reduced the maximal contraction to norepinephrine. In order to test for a contribution of iNOS to the HU effect, vessels were exposed to 0.3 µM L-arginine and norepinephrine concentration-response curves were obtained in the presence and absence of the iNOS selective inhibitor, aminoguanidine. Aminoguanidine had no effect in control vessels, but increased the contraction to norepinephrine in the HU vessels. In phenylephrine-precontracted vessels, L-arginine induced an aminoguanidine-sensitive relaxation that was greater in HU than control vessels. These experiments strongly suggest that HU treatment up-regulates the expression of iNOS in the femoral artery, leading to an increased tissue concentration of nitric oxide. In turn, the vasodilator action of nitric oxide decreases the ability of the femoral artery to contract to norepinephrine. Western blot analysis of the femoral artery demonstrated that iNOS protein mass is increased by HU treatment.

Studies of the isolated middle cerebral artery of control and HU rats were undertaken. Vessel segments were cannulated and pressurized, and vessel diameter was measured by videomicroscopy. Myogenic tone was assessed and found to be markedly increased in the HU vessels. In part, this was due to a decrease in the contribution of nitric oxide in the HU vessels, compared to control. This finding is consistent with the possibility that the increase in pressure at the level of the brain vasculature stimulated an adaptation of the cerebrovascular autoregulation to protect the brain from overperfusion. The increased myogenic tone would contribute to that protection by decreasing vessel diameter and reducing brain blood flow.
Experiments were carried out to determine whether HU is associated with a generalized increase in the role of nitric oxide. Protein masses of NOS isoforms were determined by Western blot analysis in both vascular and nonvascular tissues. In thoracic aorta, heart and kidney, iNOS was increased. The level of nNOS was also increased in both kidney and brain. There was no change in ecNOS in any of the tissues studied. In the kidney, nitric oxide is associated with regulation of natriuresis and diuresis. The observation that iNOS and nNOS are elevated in HU kidneys is consistent with the suggestion that nitric oxide levels are also elevated, leading to natriuresis, diuresis and hypovolemia, a known characteristic of both simulated and real microgravity. In the brain, nitric oxide is one factor that regulates sympathetic outflow. That nNOS is increased in the brain of HU rats, suggests that the resultant elevated nitric oxide could lower sympathetic outflow. In order to determine if the HU-induced changes in iNOS had in vivo hemodynamic effects, the blood pressure responses to I.V. injections of norepinephrine and the iNOS inhibitor, aminoguanidine were measured. Blood pressure increased more in control, than in HU rats in response to norepinephrine. In contrast, HU rats exhibited a greater aminoguanidine-induced blood pressure elevation than controls. The reduced pressor response of HU rats to norepinephrine is consistent with the known vascular hyporesponsiveness associated with simulated microgravity. The greater pressor response to aminoguanidine in HU suggests that nitric oxide production in the vasculature, via the activity of iNOS, was greater in HU compared to control. Thus, nitric oxide-dependent vasodilation would make a greater contribution to the ambient blood pressure. The results with aminoguanidine suggest that iNOS levels are generally elevated in the vasculature of HU compared to control rats.

Studies in the abdominal aorta address nitric oxide-independent mechanisms by which HU depresses the maximal contractile response of blood vessels to vasoconstrictor agents. It was found that HU treatment depresses the contraction to norepinephrine, but not serotonin. This suggested that HU may affect either receptor or second messenger events associated with vascular stimulation by norepinephrine, but not serotonin. Use of receptor antagonists did not reveal any HU-mediated changes at the receptor level. However, use of signal transduction inhibitors revealed certain second messenger pathways that are altered by HU when norepinephrine, but not serotonin, is the agonist. Both indomethacin and genistein markedly inhibited the norepinephrine-induced contraction in control, but not in HU, aorta. Both inhibitors had equal blocking effects in control and HU aortas when serotonin was used. This suggests that both a vasoconstrictor prostaglandin and a tryosine kinase second messenger pathway contribute to norepinephrine-induced contraction in control, but not HU, aorta. Because HU treatment reduced or eliminated these vasoconstrictor mechanisms when norepinephrine was used, but not when serotonin was used, HU may have uncoupled these pathways from the alpha-adrenergic receptor. Using aorta rings stimulated with norepinephrine and shock frozen, Western blot analysis was used to assess HU effects on phosphorylated MAP kinase (pMAPK) levels. Tryosine kinases are known to be involved in the phosphorylation and, therefore, activation of MAPK. The protein mass of pMAPK in control tissues was nearly double that in HU tissues. Genistein reduced the pMAPK level in control tissues to that of the HU tissues, but had no effect on pMAPK in HU tissues themselves. These differences in pMAPK levels, and the effects of genistein, mirror the results of the contractile studies described above. They support the view that HU eliminates the tyrosine kinase-associated component of the norepinephrine-induced contraction by uncoupling this pathway from the alpha adrenoceptor.

**FUTURE PLANS**

In vivo experiments will be carried out to assess orthostatic hypotension in control and HU rats. Animals will be instrumented with radiotelemetric blood pressure probes and intravenous cannulas for drug administration. At the completion of 20-day HU treatment, both control and HU rats will be placed in a tilt restrainer and blood pressure will be monitored in the horizontal and head-up vertical positions. Pressor responses to norepinephrine and aminoguanidine will also be tested in these two positions. Novel compounds, peripheral benzodiazepine receptor ligands, been shown to inhibit iNOS expression. Control and HU rats will be injected with these compounds throughout the 20-day HU treatment and the blood pressure experiments described above will be repeated. Vascular and nonvascular tissues from control and HU animals, treated with these compounds, will be subjected to Western blot analysis to determine iNOS protein levels.

**INDEX TERMS:** abdominal aorta, aminoguanidine, blood pressure, cardiovascular, carotid artery, cerebrovascular autoregulation, femoral artery, hindlimb unweighted rat, middle cerebral artery, nitric oxide, nitric oxide synthase, microgravity, orthostatic intolerance, simulated microgravity, tyrosine kinase