Progress Report
(Year 1: 1995)

"Mechanisms of Microgravity Effect on Vascular Function"

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Organization of this progress report

The present report begins with a statement of the overall goal of the project and a summary list of the specific hypotheses to be tested. These are followed by sections on results, conclusions, significance and plans for the next year.

Notice of approval of funding for this project was received in December, 1994. However, the official funding notice was received in May, 1995. Thus, the Principal Investigator requested a project start date of February 1, 1995 in order to cover the costs of fabricating harnesses and cages for the hindlimb unweighted rats. The Research Associate was hired in June, 1995, after receipt of the official funding notice, and the first experiments were performed in July, 1995. Thus, the present progress report presents results obtained over the four months of July through October, 1995.

Overall Goal

The overall goal of the project is to characterize the effects of simulated microgravity on vascular function. Microgravity is simulated using the hindlimb unweighted (HU) rat, and the following vessels are removed from HU and paired control rats for in vitro analysis: abdominal aorta, carotid and femoral arteries, jugular and femoral veins. These vessels are cut into 3 mm long rings and mounted in tissue baths for the measurement of either isometric contraction, or relaxation of pre-contracted vessels. The isolated mesenteric vascular bed is perfused for the measurement of changes in perfusion pressure as an index of arteriolar constriction or dilation.

Hypotheses

The following hypotheses were based on the observations of Delp and coworkers (1993), who found that the abdominal and thoracic aortas from HU rats exhibited decreased maximal responses to vasoconstrictor agonists and no change in vasodilator mechanisms, compared to tissues from control rats.

Hypothesis I. **HU Treatment decreases both maximal contractility and sensitivity to vasoconstrictor agonists.** Experiments to date address this hypothesis. Both norepinephrine and serotonin have been tested. The testing is complete in Sprague-Dawley and Wister rats at 20 day HU treatment. In addition, the vasoconstrictor effects of angiotensin II, endothelin and $CaCl_2$ (in K^+ depolarized vessels) will be assessed to complete the testing of hypothesis I.

The following are the remaining hypotheses to be addressed:

Hypothesis II. **Isolated blood vessels from HU rats will exhibit a generalized decrease in responsiveness to neurogenic stimulation, due to both presynaptic and postsynaptic mechanisms.** Contractile responses to nerve terminal selective, electrical field stimulation will be measured. Ring segments

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of the caudal artery will be studied for contractile responses to adrenergic nerve stimulation while the jugular vein will be studied for dilator responses to adrenergic nerve stimulation mediated by ß-adrenergic receptors. The isolated perfused mesenteric vascular bed will enable us to study responses of vasodilator, capsaicin-sensitive sensory nerves.

Hypothesis III. **Both endothelium-dependent and independent vasodilation are not altered by HU treatment.** Vasodilator responses will be characterized in perfused caudal and carotid arteries and the perfused mesenteric bed. Vasodilator agents will include acetylcholine (endothelium-dependent) and sodium nitroprusside and CGRP (endothelium-independent). The responses of isolated rings of jugular vein to isoproterenol, a ß-adrenergic agonist, will also be assessed.

Hypothesis IV. **HU-induced changes in vascular function follow the same time course for development and recovery that is found for skeletal muscle.** Once the above studies have been completed, the time course for the appearance and recovery of identified changes in vascular function will be determined. 5 and 10 day HU rats will be analyzed because these treatment times were shown to be early and intermediate with respect to hindlimb skeletal muscle alterations. 20 and 28 day HU rats will be compared to determine if the maximal effects have been achieved. Recovery of normal vascular function will be assessed in rats taken 7, 14, 21 and 28 days following termination of a 20 day HU treatment.

Modifications to the original proposal

The following parameters were not proposed in the original grant submission, but were subsequently added for the reasons given:

1)   The abdominal aorta was added to allow comparison of results with those published by Delp et al. (1993).

2)   Fourteen day HU treatment was added because this duration was used by Delp et al. (1993).

3)   The Wistar strain of rats was added for comparison of results with Sprague-Dawley rats. Initial studies with femoral artery yielded no effect of 20 day HU treatment. Thus, Wistar was added to determine if the lack of treatment effect was due to the strain of rat.

Results

Statistical analysis. Comparisons of mean contractile responses to 68 mM K^+ in vessels from control versus HU treated rats were made using one-way ANOVA. HU treatment effect on full concentration response curves to either norepinephrine or serotonin were assessed using two-way repeated measures ANOVA. Both ANOVA assessments were followed by posthoc analysis with a Scheffe's Test. $P < 0.05$ was the

criterion for significant differences and the reported N values indicate the number of experiments. Each experiment included 2-4 vascular rings per treatment.

Optimization of resting force. All vessels studied were mounted in tissue baths for the measurement of isometric contraction. In the initial experiments, optimal resting forces were determined. The optimal resting force is the magnitude of stable pre-stretch that yields the maximal development of tone in response to vasoconstrictor stimulus. Arteries and veins were initially adjusted to resting forces of 0.5 and 0.2 g, respectively. After 30 minutes, tissues were exposed to Krebs' solution containing 68 mM K^+ to depolarize tissues and elicit a contraction. After steady-state contraction was achieved, tissue baths were drained and refilled twice with normal Krebs' solution (4.9 mM K^+) and allowed to relax to resting force. The resting force was increased incrementally by 0.25-0.5 and 0.1-0.2 g in arteries and veins, respectively, and vessels were exposed to 68 mM K^+ at each increment at 30 minute intervals. The following are the optimal resting forces in the respective blood vessels:

Vessel ring	from Control Rats (g)	from HU (20-day) (g)
abdominal aorta	2	2
femoral artery	1	1
carotid artery	1.5	1
femoral vein	0.6	0.6
jugular vein	1	0.6

The isolated mesenteric vascular beds from both control and HU rats were perfused at 4 ml/minute. This yielded unstimulated perfusion pressures of 28-30 mm Hg in both control and treated vascular beds. This suggests that there was no difference between the beds in the resting level of resistance to flow.

All blood vessel rings are exposed to 68 mM K^+ twice with a 30 minute interval between. Subsequently vessels are then contracted by cumulative addition of either norepinephrine or serotonin. The second and all subsequent exposures to potassium have been found to generate uniform contractile responses. Thus, vessels from both control and HU treated rats were compared on the basis of the second potassium response, and the results are shown in figures 1-5. As shown in figure 1, the K^+-induced contraction of abdominal aorta rings from HU rats was less than that from control animals. The difference was greatest, and was statistically significant, in tissues from Wistar compared to Sprague Dawley rats.

Similar results were obtained in both carotid and femoral artery rings as shown in figures 2 and 3. Again, the K^+-induced contraction was less in artery rings from HU treated Sprague Dawley and Wistar rats. The only exception was the femoral artery from Sprague Dawley rats in which HU treatment produced no difference from control.

In contrast to artery rings, 20-day HU treatment tended to produce an increased contraction to 68 mM K$^+$ compared to control (figures 2 and 3). This trend became significant in the case of jugular veins from Wistar rats (figure 3). One exception to this trend was the femoral veins from Wistar rats in which HU treatment produced no difference from control.

The effect of 68 mM K$^+$ exposure was also analyzed in 14 day HU treated rats and their paired controls, and the results are shown in figures 4 and 5. No significant differences between HU and control vessels were observed. However, certain of the trends seen with 20 day HU treatment were apparent. Namely, HU treatment tended to reduce the magnitude of contraction in the arteries and to increase the magnitude of contraction in the veins. Insufficient numbers of experiments have been conducted to date to allow the formulation of conclusions. Time course experiments will be included in the second year of the project.

Studies of the effects of 20 day HU treatment on arterial and venous responses to norepinephrine were carried out and the results are shown in figures 6-10. As shown in figure 6, abdominal aorta rings from 20 day HU treated rats exhibited lower maximal responses to norepinephrine than control, but no difference in sensitivity was seen. The magnitude of this difference was greater in vessels from Wistar rats, consistent with the greater and significant difference observed in the contraction to 68 mM K$^+$.

Similar results were obtained in both carotid and femoral arteries. As shown in figures 7 and 8, 20 day HU treatment caused a significant reduction in the maximal response to norepinephrine compared to control. The one exception was in the case of femoral arteries from Sprague Dawley rats (figure 8). 20 day HU treatment did not produce a difference in contractile response to norepinephrine compared to control. This is consistent with the failure of 20 day HU treatment to produce a difference in contractile response to 68 mM K$^+$ compared to control. The effect of 20 day HU treatment on the response of veins to norepinephrine was studied and the results are shown in figures 9 and 10. No significant differences were found between HU treated and control in either jugular or femoral vein from either Sprague Dawley or Wistar rats. However, in contrast to arteries, there was a consistent trend toward greater maximal response in veins from HU treated rats compared to control.

A confounding factor may account for the lack of a significant effect of 20 day HU treatment in veins, at least in the case of the jugular vein. This vessel has been shown to have a predominance of beta over alpha adrenergic receptors (Cohen and Wiley, 1978). Thus, the magnitude of contraction to norepinephrine tended to be small and was followed at higher concentrations by relaxation. These experiments in both the jugular and femoral veins will be repeated in the presence of beta receptor antagonists to more fully assess alpha adrenergic receptor mediated contraction.

Contractions to norepinephrine were also assessed in vessels from 14 day hindlimb unweighted rats and their paired controls, and the results are shown in

figures 11 through 14. The number of experiments is small and more experiments will be conducted. Nevertheless, 14 day HU treatment produced a significant reduction in the maximal response to norepinephrine compared to control in vessels from Sprague Dawley rats. In contrast, no significant effects of 14 day HU treatment were seen in carotid arteries from Wistar rats or in femoral arteries from either strain. Similar studies in jugular and femoral vein are shown in figures 13 and 14. Again, 14 day HU treatment had no effect on venous contraction to norepinephrine compared to control.

The effects of perfusion of the isolated mesenteric vascular bed with 68 mM K^+ followed after washout by cumulative increase in norepinephrine in the perfusate were studied and the results are shown in figure 15. Perfusion with both 68 mM K^+ and norepinephrine increased perfusion pressure. However, HU treatment had no significant effect compared to control. This vascular preparation ruptures at perfusion pressures above 150 mm Hg. thus, maximal response cannot be determined. The present results demonstrate that HU treatment does not alter the sensitivity of the isolated mesenteric vascular bed to either 68 mM K^+ or norepinephrine.

The effect of 20 day HU treatment on arterial and venous ring response to serotonin was determined and the results are shown in figures 16 and 17. No significant differences were found between vessels from HU treated and control animals. In part, this may result from the small number of experiments conducted to date. Additional experiments are planned. However, it is of interest that in the case of the jugular vein, serotonin elicited a substantially stronger contraction in veins from 20 day HU treated animals compared to control. Additional experiments will reveal whether this trend achieves significance. This result is consistent with the effects of HU treatment on the venous contractile response to both 68 mM K^+ and norepinephrine, namely, the trend is toward a greater magnitude of contraction in veins from HU treated rats.

Conclusions

The strongest results were observed after 20 day HU treatment. 14 day HU treatment may be insufficient to yield full effect. Alternatively, insufficient numbers of experiments have been conducted to date with the 14 day HU treated animals to allow interpretation.

There was a consistent finding among the arteries studied. Namely, HU treatment depressed the magnitude of maximal contraction compared to control in the case of both 68 mM K^+ and norepinephrine. The one exception to this pattern was in the case of femoral arteries from Sprague Dawley rats. 20 day HU treatment had no effect on contraction regardless of the stimulus used. In the case of the jugular and femoral veins, 20 day HU treatment either had no effect or, in the case of the jugular vein from Wistar rats, significantly increased the maximal contractile response to potassium compared to control. In addition, while the effect of HU treatment was not significant, there was a trend toward enhancement of contraction by HU treatment.

Further experiments will be required to clarify the effect of HU treatment on contractile response of veins.

Significance

In general, HU treatment decreased the maximal contractile capacity of arterial smooth muscle, but tended to have no effect or to increase the maximal contractile capacity of venous smooth muscle. In order to understand the significance of these results, one must first address the extent to which HU treatment simulates microgravity. As summarized by Delp et al. (1993) HU treatment of rats mimics many of the changes in fluid distribution and cardiovascular function seen in humans subjected to prolonged head-down tilt or spaceflight. These include increased central venous pressure, reduced blood volume, tachycardia and decreased exercise capacity. In further support, Soyet and coworkers (1995) found that both spaceflight and HU treatment decreased the vasoconstrictor sensitivity of rat vena cava to norepinephrine by a protein kinase C-dependent mechanism. In contrast, Fagette and coworkers (1995) found no effect of 14 day HU treatment on blood pressure, heart rate or baroreceptor reflex sensitivity. These authors found no evidence for orthostatic intolerance. Taking the results of these three studies together, it can be tentatively concluded that HU treatment simulates many but not all of the effects of microgravity. Namely, HU treatment may simulate the effects of microgravity on volume distribution and vascular contractility, but not baroreceptor function. Confirmation of this proposal awaits direct testing of hemodynamic and vascular parameters in rats subjected to space flight.

If it is accepted that HU treatment mimics at least some aspects of the cardiovascular effects of spaceflight, the present results suggest a contribution of altered vascular function to microgravity-induced cardiovascular deconditioning. An impaired capacity of the arterial circuit to contract would pass the pressure-head to the venous circuit, contributing to elevated venous pressure. The enhanced capacity of veins to contract in the presence of vasoconstrictor agents might also contribute to elevated venous pressure. If these vascular changes occur in humans, they are likely to contribute to postural intolerance. Namely, exposure to gravity in the standing position in a spaceflight-adapted human may trigger sympathetic stimulation of the vasculature. However, the reduced capacity for arterial constriction would yield a reduced peripheral resistance and, therefore, a lowered blood pressure.

It is of particular significance that HU treatment had different effects on arterial and venous vasoconstrictor mechanisms. This suggests that HU treatment itself, and not some nonspecific factor such as stress, was responsible for the observed effects. Further studies to characterize the mechanisms underlying the effects of HU on arterial and venous function are clearly warranted and will be the subject of the two remaining years of this project.

Plans for the next year

In order to complete the testing of Hypothesis I, contractile responses to K^+ (10-100 mM), serotonin, angiotensin II, endothelin and Ca^{++} in K^+-depolarized vessels will be determined in arteries and veins from control and HU treated rats. Selection of strain will be based on which strain yielded the greatest HU treatment effect in each blood vessel. In addition, norepinephrine-induced contractions in jugular and femoral veins will be assessed under conditions allowing unopposed alpha adrenoceptor stimulation: in the presence of agents that block beta adrenoceptors, neuronal and extraneuronal catecholamine uptake, nitric oxide synthase and cyclooxygenase.

Experiments will also be conducted to assess vascular responsiveness to neurogenic stimulation using rings of caudal artery, jugular vein and isolated perfused mesenteric vascular bed (Hypothesis II). Vasodilator responses will be assessed in perfused caudal and carotid arteries and perfused mesenteric bed as well as jugular vein rings (Hypothesis III). Both endothelium dependent and independent vasodilation will be characterized.

The most salient effects of HU treatment identified by the studies described above will be chosen for analysis of the time course of HU effect. Five and 10 day HU treatment will be assessed. In addition, recovery of normal vascular function will be assessed in rats taken 7, 14, 21 and 28 days following termination of 20 day HU treatment.

References

Cohen, M.L. and Wiley, K.S.: Rat jugular vein relaxes to norepinephrine, phenylephrine and histamine. J. Pharmacol. Exp. Ther. 205:400-409, 1978.

Delp, M.D., Holder-Binkley, T., Laughlin, M.H. and Hasser, E.M. Vasoconstrictor properties of rat aorta are diminished by hindlimb unweighting. J. Appl. Physiol. 75(6):2620-2628, 1993.

Fagette, S., Lo, M., Gharib, C. and Gauquelin, G. Cardiovascular variability and baroreceptor reflex sensitivity over a 14-day tail suspension in rats. J. Appl. Physiol. 78(2):717-724, 1995.

Sayet, I., Neuilly, G., Mironneau, J. and Mironneau, C. Influence of spaceflight, hindlimb suspension, and venous occlusion on a_1-adrenoceptors in rat vena cava. J. Appl. Physiol. 78(5):1882-1888, 1995.

Figure 1:
Vascular contractile responses to 68mM potassium in abdominal aortas from control and twenty-day hindlimb unweighted (HU) rats: comparison of the Sprague Dawley vs. the Wistar Strains. Bars represent means +/- S.E.M. * indicates significantly different from the control. P< 0.05. N=5.
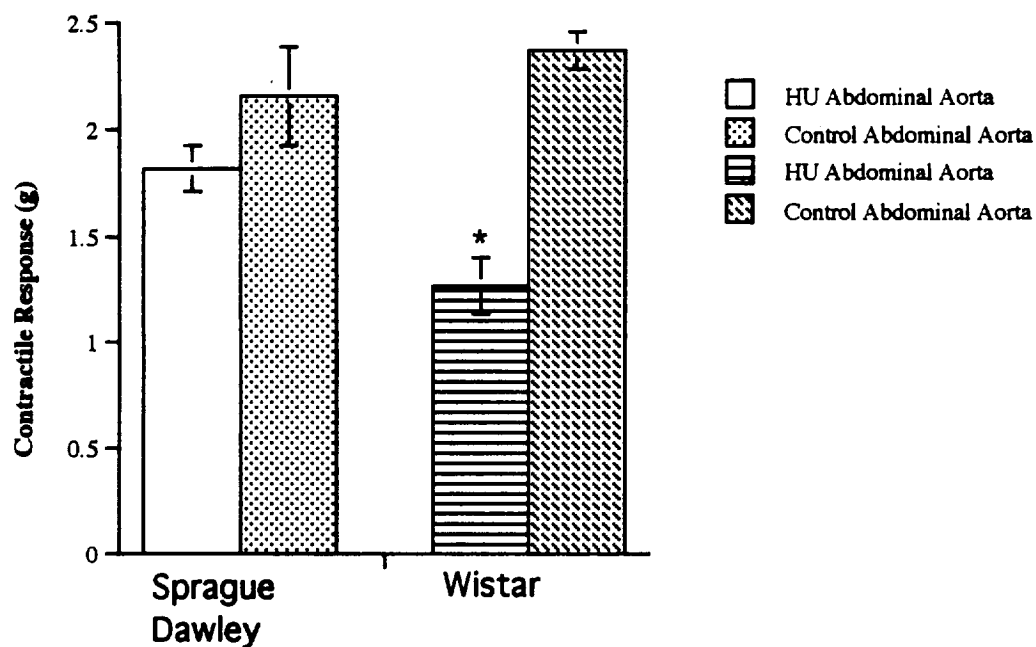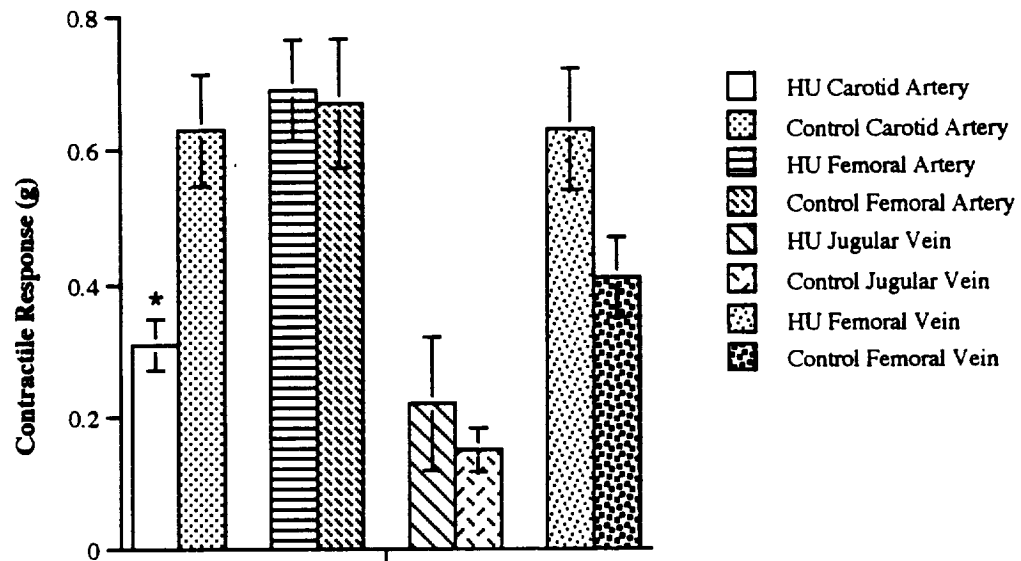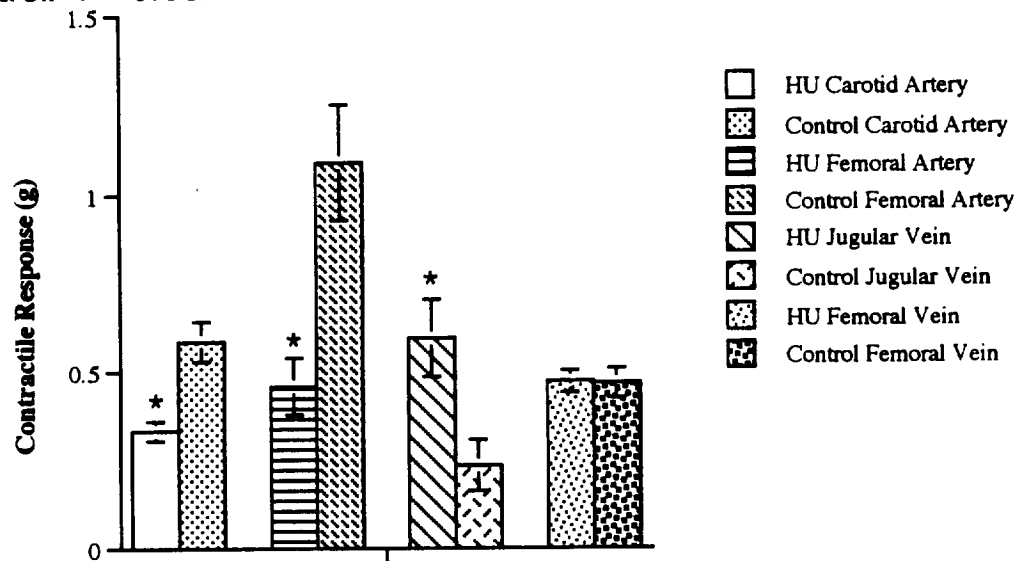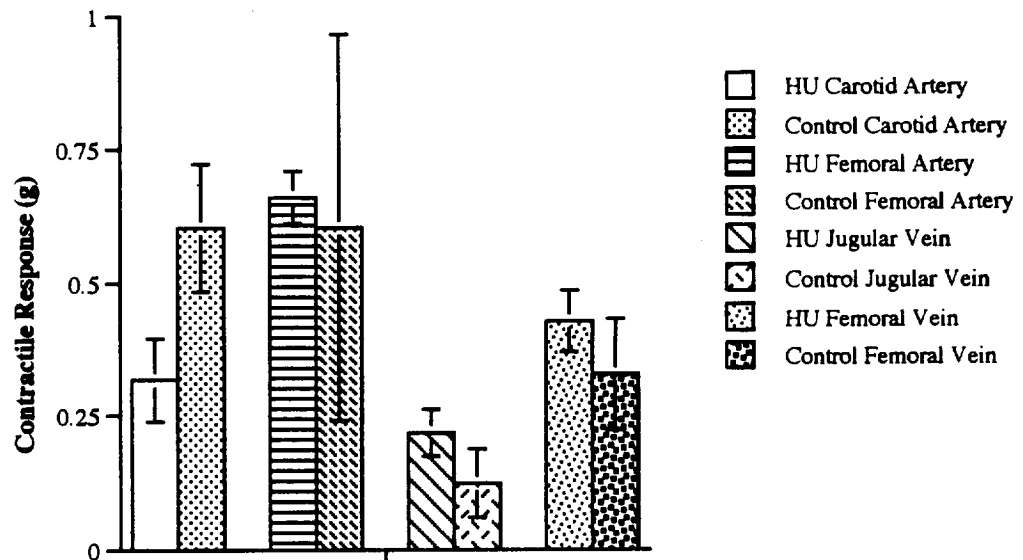
Figure 2:
Vascular contractile responses to 68mM potassium in vessels from control and twenty-day hindlimb (HU) Sprague Dawley rats. Bars represent means +/- S.E.M. * indicates significantly different from control. P< 0.05. N=7.
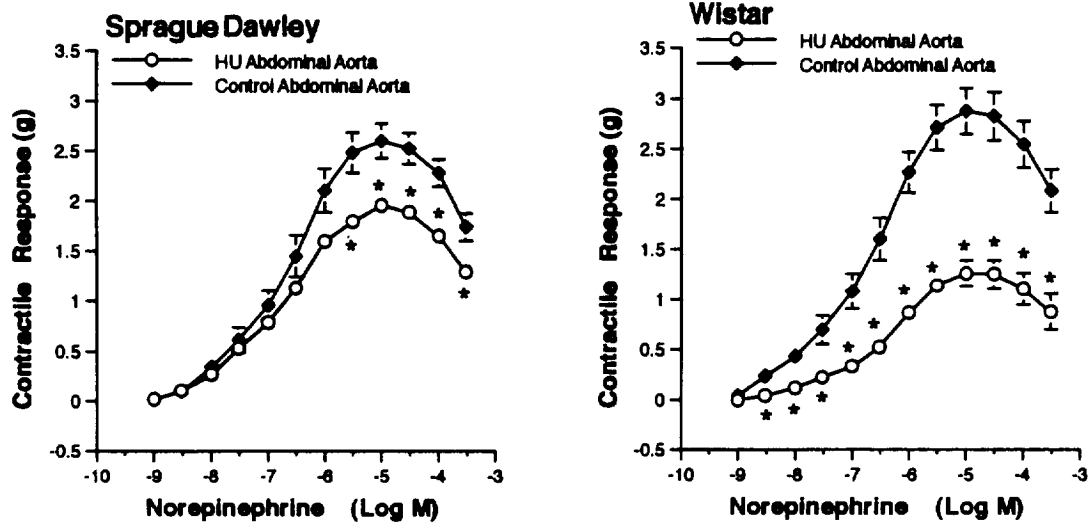
Figure 3:
Vascular contractile responses to 68mM potassium in vessels from control and twenty-day hindlimb unweighted (HU) Wistar rats. Bars represent means +/- S.E.M. * indicates significantly different from control. P< 0.05. N=6-4.

Legend:
- ☐ HU Carotid Artery
- ▨ Control Carotid Artery
- ☰ HU Femoral Artery
- ▧ Control Femoral Artery
- ◩ HU Jugular Vein
- ◪ Control Jugular Vein
- ▒ HU Femoral Vein
- ▦ Control Femoral Vein

Y-axis: Contractile Response (g)

Figure 4:
Vascular contractile responses to 68mM potassium in vessels from control and fourteen-day hindlimb unweighted (HU) Sprague Dawley rats. Bars respresents means +/- S.E.M. * indicates significantly different from control. P< 0.05. N=3.

Legend:
- HU Carotid Artery
- Control Carotid Artery
- HU Femoral Artery
- Control Femoral Artery
- HU Jugular Vein
- Control Jugular Vein
- HU Femoral Vein
- Control Femoral Vein

Figure 5:
Vascular contractile responses to 68mM potassium in vessel from control and fourteen-day hindlimb unweighted (HU) Wistar rats. Bars represent means +/- S.E.M. * indicates significantly different from control. P< 0.05. N=2-4.

Legend:
- ☐ HU Carotid Artery
- ▦ Control Carotid Artery
- ▤ HU Femoral Artery
- ▨ Control Femoral Artery
- ◩ HU Jugular Vein
- ◪ Control Jugular Vein
- ▦ HU Femoral Vein
- ▦ Control Femoral Vein

Y-axis: Contractile Response (g), 0 to 1.5
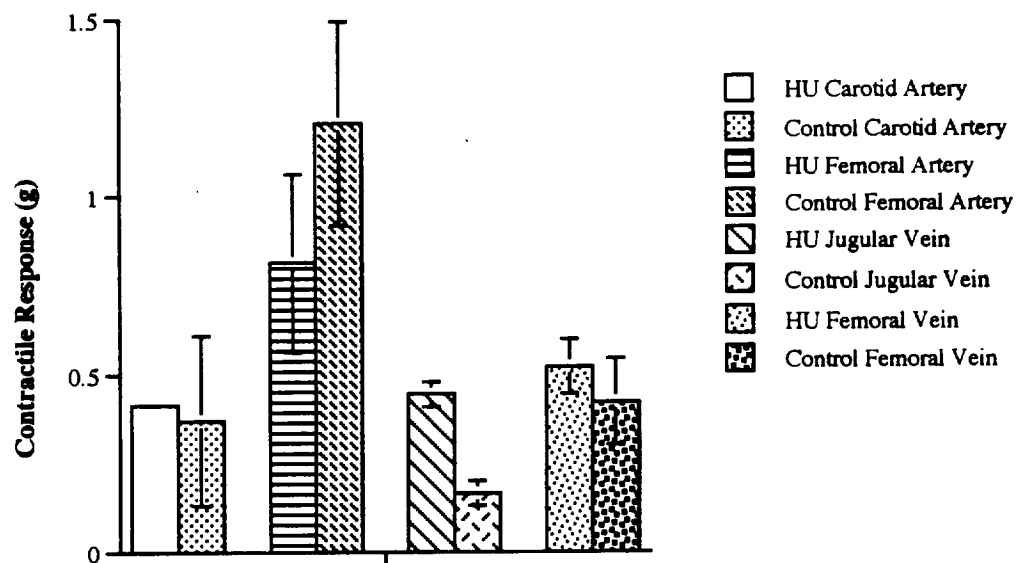
Figure 6:
Norepinephrine concentration-response curve for the contraction of the abdominal aorta from control and twenty-day hindlimb unweighted (HU) rats: comparison of the Sprague Dawley vs. the Wistar strains. Data are presented as means +/- S.E.M. * indicates significantly different from the control. P< 0.05. N=5.
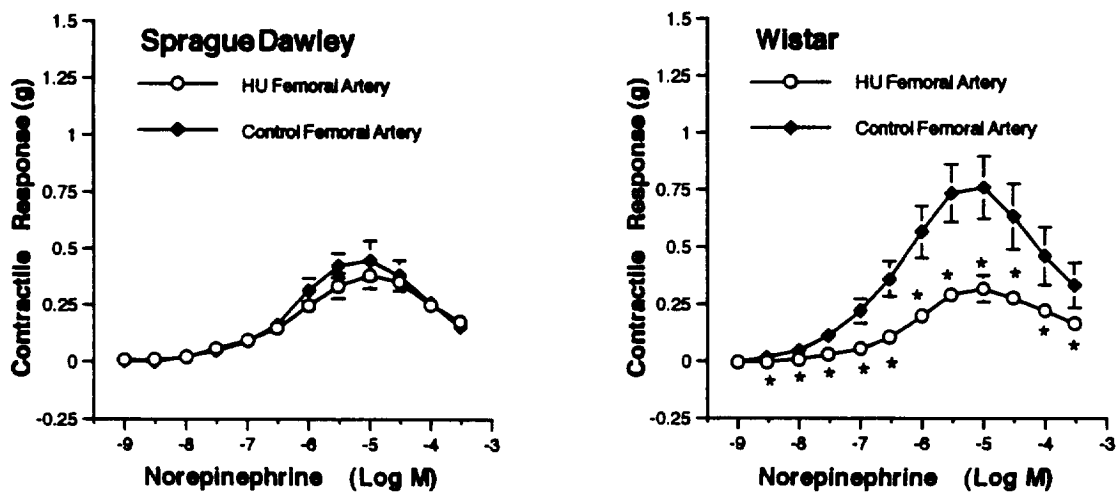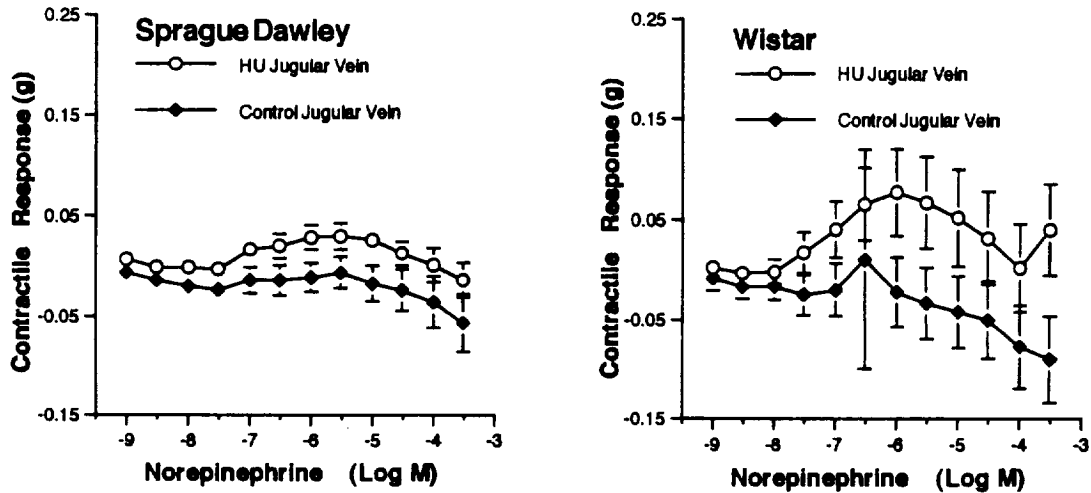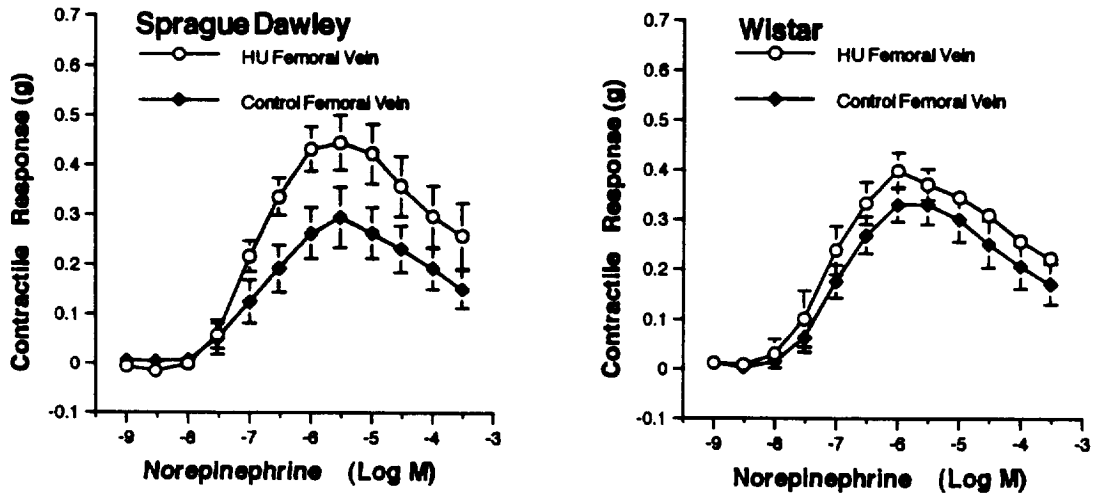
Figure 7:

Norepinephrine concentration-response curves for the contraction of the carotid artery from control and twenty-day hindlimb unweighted (HU) rats: comparison of the Sprague Dawley vs. the Wistar strains. Data are presented as means +/- S.E.M. * indicates significantly different from the control. P< 0.05. N=7-6.
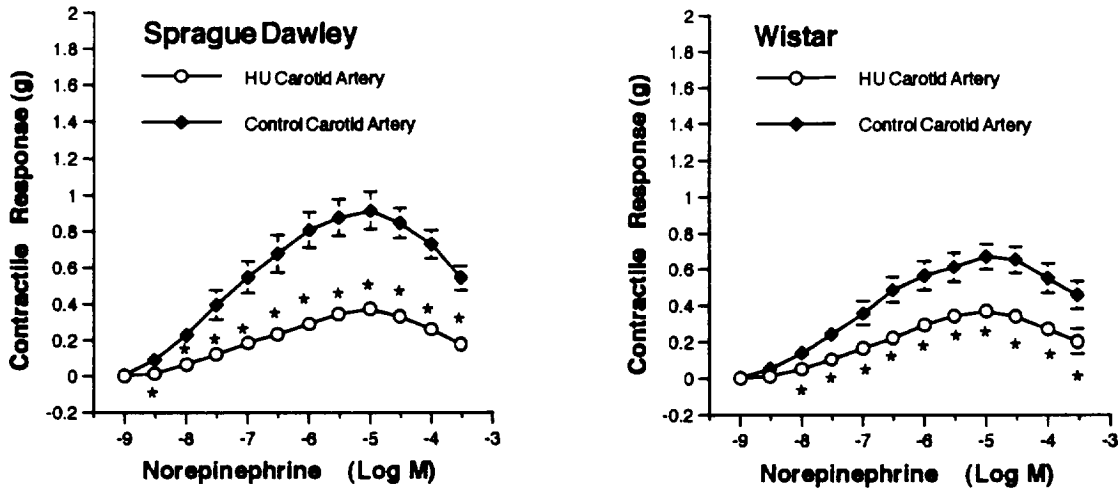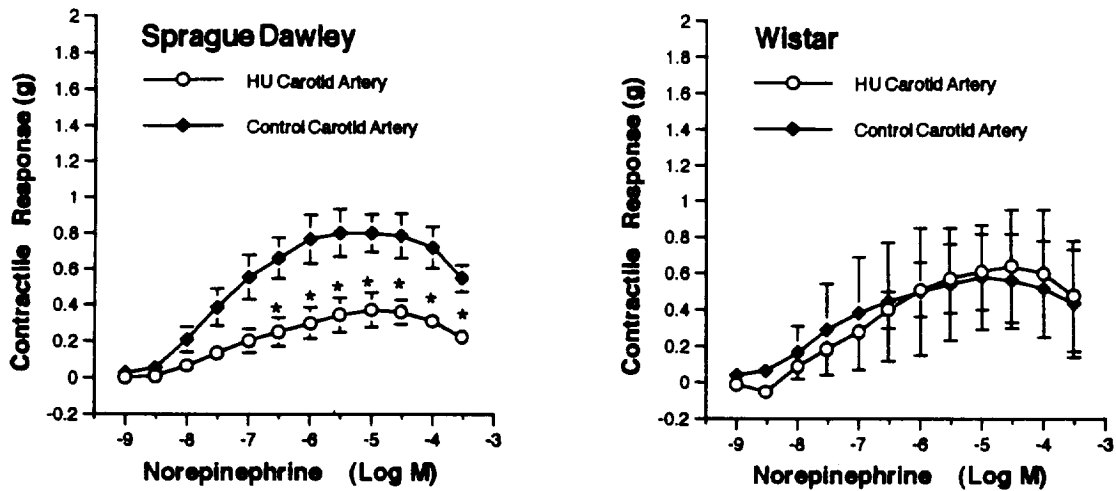

Figure 8:

Norepinephrine concentration-response curves for the contraction of the femoral artery from control and twenty-day hindlimb unweighted (HU) rats: comparison of the Sprague Dawley vs. the Wistar Strains. Data are presented as means +/- S.E.M. * indicates significantly different from control. P< 0.05. N=6-4.

Figure 9:

Norepinephrine concentration-response curves for the contraction of the jugular vein from control and twenty-day hindlimb unweighted (HU) rats: comparison of the Sprague Dawley vs. the Wistar strains. Data are presented as means +/- S.E.M. * indicates significantly different from the control. P< 0.05. N=7-6.

Figure 10:

Norepinephrine concentration-response curves for the contraction of the femoral vein from control and twenty-day hindlimb unweighted (HU) rats: comparison of the Sprague Dawley vs. the Wistar strains. Data are presented as means +/- S.E.M. P< 0.05. N=8-4.

Figure 11:

Norepinephrine concentration-response curves for the contraction of the carotid artery from control and fourteen-day hindlimb unweighted (HU) rats: comparison of the Sprague Dawley vs. the Wistar strains. Data are presented as means +/- S.E.M. * indicates significantly different from control. P< 0.05. N=2-3.
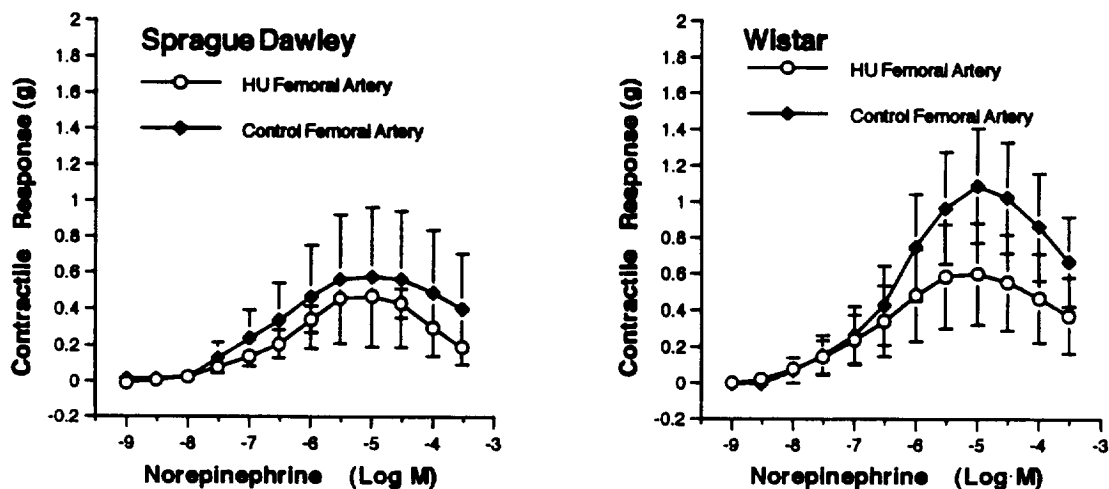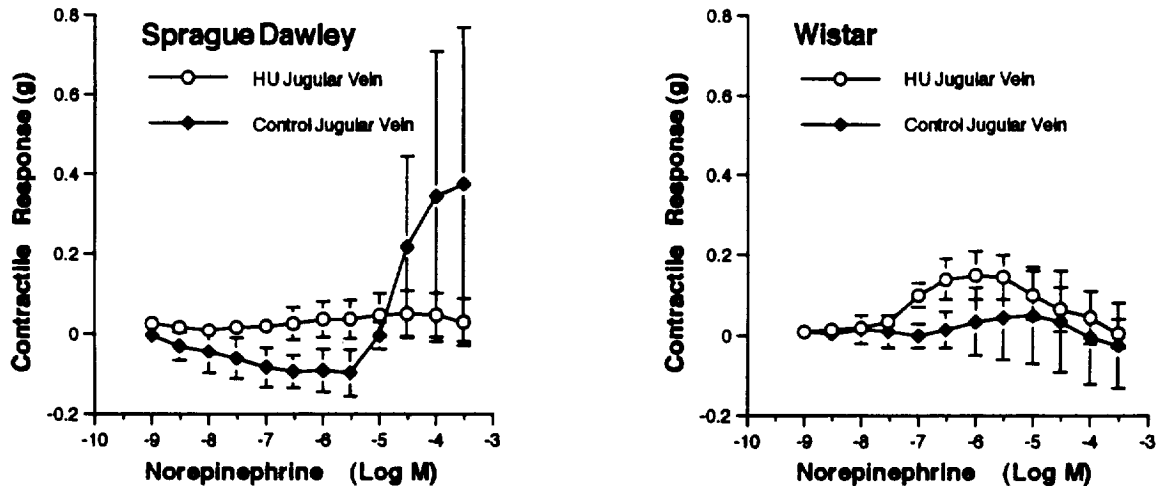

Figure 12:

Norepinephrine concentration-response curves for the contraction of the femoral artery from control and fourteen-day hindlimb unweighted (HU) rats: comparison of the Sprague Dawley vs. the Wistar strains. Data are presented as means +/- S.E.M. * indicates significantly different from control. P< 0.05. N=3-4

Figure 13:
Norepinephrine concentration-response cureves for the contraction of
the jugular vein from control and fourteen-day hindlimb unweighted (HU)
rats: comparison of the Sprague Dawley strain vs. the Wistar strain. Data
are presented as means +/- S.E.M. * indicates significantly different from
the control. P< 0.05. N=2-3.

Figure 14:
Norepinephrine concentration-response curves for the contraction of the
femoral vein from control and fourteen-day hindlimb unweighted (HU)
rats: comparison of the Sprague Dawley vs. the Wistar strains. Data are
presented as means +/- S.E.M. * indicates significantly different from the
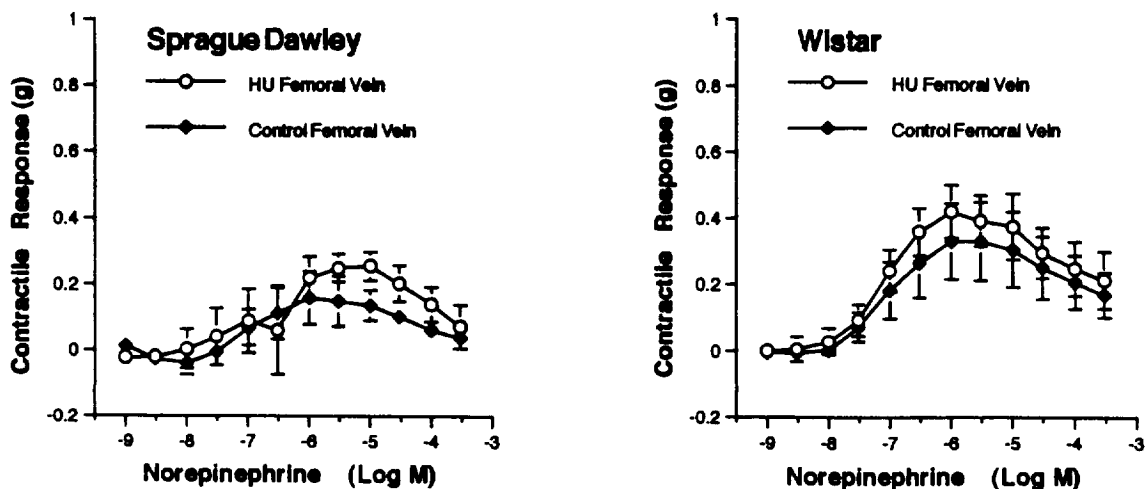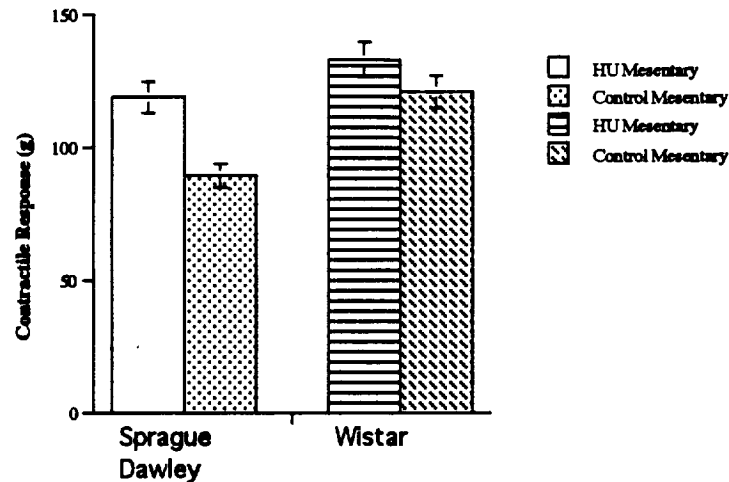control. P< 0.05. N=3-4.

Figure 1 5:

Panel A:
Vascular contractile responses to 68mM potassium during the perfusion of the mesentary arteries from control and twenty-day hindlimb unweighted (HU) rats: comparison of the Sprague Dawley vs. the Wistar Strains. Bars represent means +/- S.E.M. * indicates significantly different from the control. P< 0.05. N=9-5.

Panel B:
Norepinephrine concentration-response curves for the contraction of the mesentary artery during perfusion from control and twenty-day hindlimb unweighted (HU) rats: comparison of the Sprague Dawley vs. the Wistar strains. Data are presented as means +/- S.E.M. P< 0.05. N=9-5.
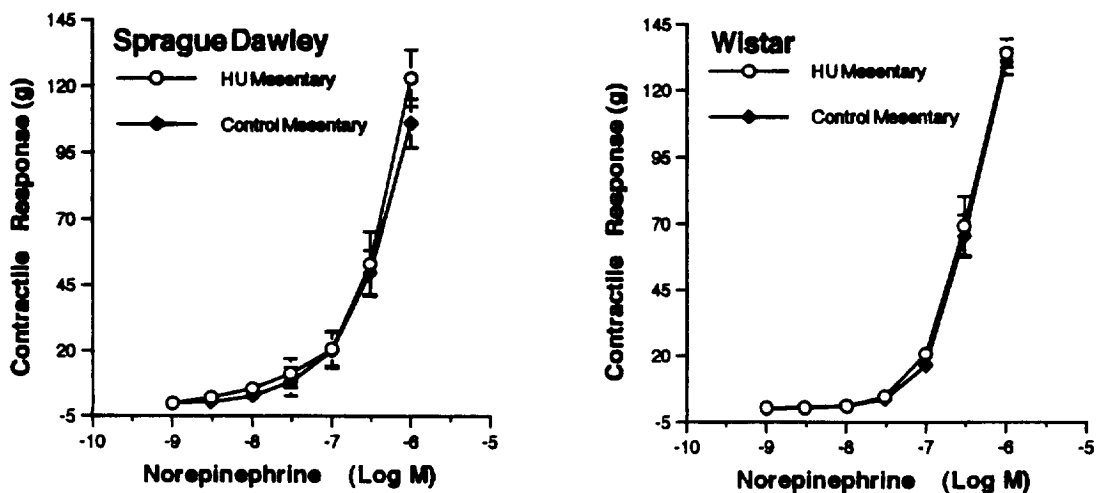
Figure 16:

Serotonin concetration-response curves for the contraction of the femoral and carotid arteries from control and twenty-day hindlimb unweighted (HU) Sprague Dawley Rats. Data are presented as means +/- S.E.M. * indicates significantly different from the control. P< 0.05. N=3.
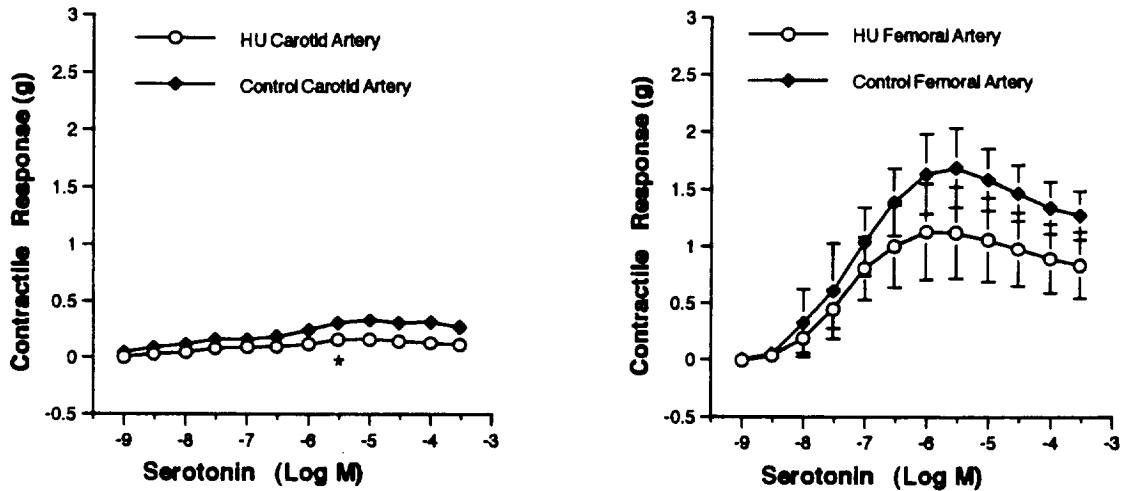

Figure 17:

Serotonin concentration-response curves for the contraction of the jugular and femoral veins from control and twenty-day hindlimb unweighted (HU) Sprague Dawley Rats. Data are presented as means +/- S.E.M. * indicates significantly different from the control. P< 0.05. N=3.