REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
<small>The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.</small> PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) 08 Aug 2006		2. REPORT TYPE Final Technical Report		3. DATES COVERED (From - To) 01 Dec 2001 - 31 Dec 2005	
4. TITLE AND SUBTITLE Neural, Endocrine, and Local Mechanisms in the Effects of Environmental Stressors on the Cardiovascular Response to Blood Loss				5a. CONTRACT NUMBER N000140210162	
				5b. GRANT NUMBER N000140210162	
				5c. PROGRAM ELEMENT NUMBER	
				5d. PROJECT NUMBER	
6. AUTHOR(S) Schadt, James C.				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Curators of the University of Missouri, Office of Sponsored Programs Administration, University of Missouri-Columbia, 310 Jesse Hall, Columbia, MO 65211-1230 Phone: (573) 882-7560; Fax: (573) 884-4078; email: grantsdc@missouri.edu				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Mr. Joshua Kabisch, Contract Administrator Office of Naval Research, Chicago Region John C. Kluczynski Federal Building 230 South Dearborn Street, Suite 380 Chicago, IL 60604-1595				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Distribution Unlimited					
<div style="display: flex; justify-content: space-between; align-items: center;"> <div style="text-align: left;"> DISTRIBUTION STATEMENT A <small>Approved for Public Release</small> Distribution Unlimited </div> <div style="font-size: 2em; font-weight: bold;">20060809630</div> </div>					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Our studies have provided important, new information about the role of neurohumoral systems as well as the interaction of these systems with local mechanisms in cardiovascular control during blood loss. Although, blood loss surely alters and compromises the highly integrated cardiovascular and respiratory control of oxygen delivery and removal of carbon dioxide, there is little if any published information related to respiratory control during blood loss in conscious animals. We have done experiments to define respiratory changes during a hypotensive hemorrhage in our conscious rabbit model. Finally, despite the presence of painful sensory stimuli during most traumatic blood loss, there does not appear to be any published information related to the effects of simultaneous painful sensory stimuli on cardiorespiratory control during blood loss. Our initial experiments in this area have provided a firm starting point for further studies evaluating the mechanisms involved in the effects of visceral pain on cardiovascular control in general and during hypotensive blood loss in particular.					
15. SUBJECT TERMS hemorrhage, blood loss, conscious, rabbits, cardiovascular, respiratory, control, pain, nociception, renin-angiotensin, nitric oxide, prostaglandins, vasopressin, stress					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			McKinney, Dona R.
U	U	U	UU	8	19b. TELEPHONE NUMBER (Include area code) (573) 882-7560

FINAL TECHNICAL REPORT

GRANT #: N000140210162

PRINCIPAL INVESTIGATOR: James C. Schadt (e-mail: schadtj@missouri.edu)

INSTITUTION: Dalton Cardiovascular Research Center, University of Missouri

GRANT TITLE: Neural, Endocrine, and Local Mechanisms in the Effects of Environmental Stressors on the Cardiovascular Response to Blood Loss

AWARD PERIOD: December 1, 2001 – December 31, 2005

OBJECTIVE: To define the mechanisms responsible for the stress-improved defense of arterial blood pressure during blood loss.

APPROACH: Sensory stressors extend the blood loss necessary to produce hypotension. We used conscious, chronically prepared, male and female rabbits to examine the mechanism(s) responsible for this alteration in the response to blood loss. We surgically and/or pharmacologically eliminated neural, humoral, and local mechanisms involved in cardiovascular control. Published data as well as our own preliminary experiments indicated that blocking the effects of sympathetic nerves, vasopressin, the renin-angiotensin system, nitric oxide (NO), or prostaglandins would affect the response to stress and hemorrhage. Blockade of some of these systems should enhance the effects of stress (e.g. NO, prostaglandins) while blockade of other systems should reverse the effects of stress (e.g. blockade of angiotensin II (Ang II) AT-1 receptors). Rabbits were anesthetized and aseptically prepared with: an arterial catheter (arterial pressure); two venous catheters (central venous pressure, injection of drugs, removal of blood); and Doppler flow probes on the distal aorta (skeletal muscle blood flow) and cranial mesenteric artery (GI blood flow). In some cases electrodes were implanted in the diaphragm for measurement of diaphragm electromyographic (EMG) activity. We used a fixed pressure model of blood loss. During a hemorrhage, we removed blood from the abdominal venous catheter at a rate of 8-9 ml/min until MAP $\leq$ 40 mmHg. All experiments were done with the animals fully conscious and fully recovered from surgery.

ACCOMPLISHMENTS (throughout award period):

We examined interactions between nitric oxide (NO) and air jet stress in the cardiovascular response to blood loss (21 rabbits, 11 female). NO synthase blockade (L-NAME, 50 mg/kg) extended the blood loss necessary to produce hypotension (i.e. mean arterial pressure $\leq$ 40 mmHg) with or without simultaneous air jet stress. L-NAME also limited the vasodilation that occurs coincident with the development of hypotension. This limit on the vasodilation was enhanced by air jet stress.

We examined the interaction of vasopressin and NO. Data from these experiments (14 rabbits, 7 female) indicated that blockade of vasopressin (V1) receptors (AVPX) did not significantly affect the blood loss required to decrease mean arterial pressure to 40 mmHg (hypotensive blood loss) with or without simultaneous air jet stress. Results from

this group of experiments also indicated that there were no sex differences in the response to stress or stress + hemorrhage with or without AVPX. As was mentioned above, L-NAME and/or air jet extended the hypotensive blood loss. That is, a greater blood loss was required to produce hypotension ($\text{MAP} \leq 40 \text{ mmHg}$) in the presence of NO synthase blockade or the air jet stressor, and the effects of L-NAME were increased by simultaneous air jet. In this series of experiments, we found that blockade of vasopressin V1 receptors (AVPX) normalized this effect of L-NAME in male rabbits but not in female rabbits. Thus, there appears to be a sex difference in the interaction between vasopressin and NO during hemorrhage with and without air jet stress.

We completed experiments to evaluate the role of prostaglandins during stress and hemorrhage (20 rabbits, 7 female). Prostaglandin synthesis blockade with indomethacin (5 mg/kg) during hemorrhage increased the blood loss required to decrease mean arterial pressure to 40 mmHg (hypotensive blood loss) with or without simultaneous air jet stress. This is consistent with a role for prostaglandins in the vasodilation and transition from phase 1 to phase 2 (i.e. to hypotension) during blood loss. An interesting preliminary observation was that mesenteric denervation (i.e. removal of cranial mesenteric and celiac sympathetic ganglia) abolished the indomethacin-induced extension of the hypotensive blood loss. Since the denervation procedure sympathectomized the rostral two thirds of the gut, this finding suggests that any role of prostaglandins in the cardiovascular response to blood loss may involve an interaction (direct or indirect) with the sympathetic innervation of the gut.

Our experiments demonstrated that nitric oxide and prostaglandins were involved with the vasodilation associated the transition to hypotension (i.e. phase 2). Therefore, we examined the potential interaction between prostaglandins and NO in mediating the vasodilation associated with hypotensive hemorrhage with and without simultaneous stress (15 rabbits, 7 female). One pattern that emerged was the additive nature of blockade of these two vasodilator systems in the presence but not in the absence of air jet stress. In other words, in the absence of air jet stress, the combined blockade of NO and prostaglandins had no greater effect on the hypotensive blood loss than blockade of either system alone. In contrast, during hemorrhage in the presence of the psychological stressor, air jet, combined blockade of the synthesis of NO and prostaglandins increased the hypotensive blood loss more than blockade of either system alone.

We did a pilot study to evaluate the use of Saffan, a steroid anesthetic, in studies of hemorrhage and hemorrhagic shock. It had been suggested that the steroid anesthetic, Saffan, might have fewer effects on the hemodynamic response to hemorrhage than other anesthetic agents. To date we have only compared the response to hemorrhage in the conscious and the Saffan-anesthetized state in 3 rabbits. All of these animals were fully recovered from surgery allowing us to evaluate the effects of Saffan anesthesia in the absence of surgical trauma. The results of this pilot study indicated that if the anesthetic level is sufficient to perform surgery (i.e. a dramatically increased threshold for withdrawal reflex), the response to blood loss is altered as it is with other anesthetics. Like all other anesthetics that have been tested, Saffan reduced the rabbits' ability to compensate for blood removal as indicated by decreased skeletal muscle vasoconstriction and a decreased blood loss threshold to produce hypotension. While limited in scope, this preliminary study once again emphasizes the importance of studies in conscious animals.

We have completed experiments examining the role of the renin-angiotensin system in 10 rabbits (2 females). We found that inhibition of angiotensin converting enzyme with captopril or blockade of Ang II AT1 receptors with Losartan altered the response to blood loss with or without simultaneous air jet stress. Either drug decreased the rabbits' ability to maintain arterial pressure. That is, either drug decreased the blood loss necessary to produce hypotension with or without simultaneous air jet stress (Figure 1). The decrease (in ml/kg) in hypotensive blood loss was similar during sham stress and air jet stress. In addition to a decreased ability to defend arterial pressure, Losartan or captopril also: decreased the skeletal muscle vasoconstriction characteristic of phase 1; increased the skeletal muscle and mesenteric vasodilation during phase 2; and reduced the increase in arterial pressure caused by the air jet stressor. These effects are all likely due to loss of the actions of the renin-angiotensin system on sympathetic release of norepinephrine. Angiotensin II has been shown to augment sympathetic effects at all levels, from central sympathetic outflow to the postsynaptic effects of catecholamines on their effector tissue. Thus, it seems unlikely that the renin-angiotensin system mediates the air jet-induced increase in the hypotensive blood loss. Rather, the renin-angiotensin system, and, thus, Ang II appear to provide a background or platform for sympathetic activation during hemorrhage with or without simultaneous stress.

In order to determine if central release or actions of Ang II might be involved in the hemodynamic changes associated with stress, we have done additional experiments in 5 rabbits (2 male) prepared with lateral cerebral ventricular cannulae in addition to indwelling arterial (abdominal aorta) and venous (thoracic and abdominal vena cava) catheters. We compared the effects of peripheral (iv) and central (intraventricular, ivt) blockade of AT1 receptors with Losartan on the response to hypotensive hemorrhage. Consistent with our earlier results, iv Losartan (5 mg/kg) was equally effective during sham or air jet stress and decreased (compared to saline vehicle) the hypotensive blood loss. In contrast, ivt Losartan (80 ug in 25 ul) did not change the hypotensive blood loss either with or without simultaneous air jet stress. We can conclude from these experiments that the role of Ang II during hemorrhage with or without simultaneous air jet stress appears to involve providing a peripheral platform or starting point for the effects of other pressor systems (e.g. sympathetic nervous system).

During the last year and a half of the funding period, we examined the respiratory effects of blood loss with the aid of chronically implanted diaphragmatic EMG electrodes. While the role of arterial baroreceptors in the cardiovascular response to blood loss is axiomatic, the role of arterial chemoreceptors is less clear. It has been suggested that stimulation of arterial chemoreceptors is responsible, at least in part, for the vasoconstriction seen during phase 1. In addition, we previously observed that, at least in some rabbits, there is an increase in respiratory rate during blood loss. This would be consistent with stimulation of peripheral and/or central chemoreceptors. Therefore, we undertook a study to characterize the respiratory response to blood loss in the conscious rabbit.

We completed experiments in 14 rabbits (6 female) to evaluate the effects of hypotensive hemorrhage with or without simultaneous air jet stress on respiratory rate and arterial blood gases. Respiratory rate was calculated from diaphragmatic EMG activity (chronically implanted electrodes). Using chronically implanted catheters, we also measured arterial (abdominal aorta) and venous (thoracic vena cava) blood gases at three

time points during blood loss: 1) before hemorrhage; 2) after normotensive hemorrhage (25% increase in heart rate and no decrease in MAP; phase 1); and 3) after hypotensive hemorrhage ($BP \leq 40$ mmHg; phase 2). We completed experiments in 14 rabbits (6 female) to evaluate the effects of hypotensive hemorrhage with sham or air jet stress on respiratory rate and arterial blood gases. Both normotensive and hypotensive hemorrhage increased PaO_2 and decreased $PaCO_2$, consistent with an increase in alveolar ventilation. However, respiratory rate did not increase in males or females after normotensive hemorrhage and only increased in males (but not females) after hypotensive hemorrhage. Thus, the increase in alveolar ventilation during normotensive hemorrhage in males and females must be due to an increase in tidal volume (not measured). In addition, the ventilatory increase after hypotensive hemorrhage in female rabbits must also be due to an increase in tidal volume. However, in male rabbits the increased ventilation could be due to an increase in respiratory rate of a combination of increases in rate and tidal volume.

Opioid receptor blockade with naloxone acts centrally to increase arterial blood pressure after hypotensive hemorrhage in conscious rabbits. Although the central respiratory depressant effects of opioids are well documented, the respiratory effects of opioid receptor blockade after hypotensive hemorrhage are unknown. In 14 rabbits (8 male), we examined the effects of opioid receptor blockade with naloxone on respiratory rate and arterial blood gases after hypotensive hemorrhage. Naloxone increased MAP and respiratory rate, but did not change PaO_2 or $PaCO_2$. Naloxone's respiratory effects were independent of its effects on arterial pressure. Increasing arterial pressure a similar amount with phenylephrine decreased respiratory rate. In addition, blocking naloxone's pressor effect (alpha-adrenergic blockade with prazosin) enhanced naloxone's effects on respiratory rate. Thus, the increase in respiratory rate after naloxone was not due to naloxone's pressor effect. In fact, the naloxone-induced increase in arterial pressure likely limited the increase in respiratory rate perhaps through an effect of arterial baroreceptors. There were no sex differences in the respiratory response to naloxone. Thus, opioid peptides appear to inhibit respiratory rate during acute hemorrhagic hypotension.

Despite the close association in the real world between painful sensory stimuli and blood loss in traumatic injury, there is almost nothing known about the effects of painful sensory input on the response to blood loss. We performed what we believe are the first experiments (12 rabbits, 6 female) examining the effects of a noxious, visceral stimulus, colorectal distension (CRD) on the cardiorespiratory response to blood loss. While analysis of these studies is not complete, our preliminary analysis indicates that the presence of a painful sensory input alters cardiorespiratory control during blood loss. CRD increased mean arterial pressure and heart rate and decreased respiratory rate in conscious rabbits. While CRD did not affect the blood loss necessary to produce hypotension in males, CRD decreased this volume in female rabbits. Thus, a noxious visceral stimulus, CRD, differentially affected cardiovascular control during blood loss in male and female rabbits.

CONCLUSIONS: The local vasodilator compounds, NO and prostaglandins, participate in the vasodilation associated with phase 2 of the response to hemorrhage. This participation is also evident when blood loss occurs in the presence of a psychological stressor such as air jet. Vasopressin release influences the effects of nitric oxide during hemorrhage, and this influence is affected by sex. The renin-angiotensin system seems to

provide a platform or baseline influence on the effects of sympathetic activity during blood loss. This influence does not change during psychological stress such as air jet stress. In addition, central actions of angiotensin II do not appear to account for the effects of air jet stress on the response to hemorrhage. Thus, it appears that changes in the level of activity of either central or peripheral renin-angiotensin systems are not responsible for the effects of psychological stress (i.e. air jet) on the response to blood loss.

Respiratory control during blood loss appears to be altered. The alteration appears to be designed to maintain gas exchange in the face of decreased perfusion. In addition, some of the respiratory changes (e.g. increases in tidal volume during phase 1) that occur during blood loss may also aid cardiovascular function (e.g. increased venous return).

The presence of visceral pain (i.e. CRD) alters cardiovascular control and increases arterial blood pressure. However, unlike other sensory stressors such as air jet, painful sensory input does not increase the blood loss necessary to produce hypotension. CRD decreases the blood loss necessary to produce hypotension in female rabbits and does not affect the hypotensive blood loss in male rabbits.

SIGNIFICANCE: Our studies have provided important, new information about the role of neurohumoral systems as well as the interaction of these systems with local mechanisms in cardiovascular control during blood loss. Although, blood loss surely alters and compromises the highly integrated cardiovascular and respiratory control of oxygen delivery and removal of carbon dioxide, there is little if any published information related to respiratory control during blood loss in conscious animals. We have done experiments to define respiratory changes during a hypotensive hemorrhage in our conscious rabbit model. Finally, despite the presence of painful sensory stimuli during most traumatic blood loss, there does not appear to be any published information related to the effects of simultaneous painful sensory stimuli on cardiorespiratory control during blood loss. Our initial experiments in this area have provided a firm starting point for further studies evaluating the mechanisms involved in the effects of visceral pain on cardiovascular control in general and during hypotensive blood loss in particular.

PATENT INFORMATION: No patents were applied for or granted.

AWARD INFORMATION: Heidi Shafford, D.V.M. received a research recognition award from the Central Nervous System Section of the American Physiological Society for her abstract at the 2005 Experimental Biology Meeting (see below). This abstract was based on her Ph.D. dissertation project related to effects of a noxious visceral stimulus during hemorrhage.

REFEREED PUBLICATIONS (for total award period):

Schadt, J.C. and Hasser, E.M. The defense reaction alters the response to blood loss in the conscious rabbit. Am.J.Physiol. Regul. Integr. Comp. Physiol. 280:R985-R993, 2001.

Schadt, J.C. What is the role of serotonin during hemorrhage in conscious animals? Am.J.Physiol. Regul. Integr. Comp. Physiol. 284:R780-R781, 2003.

- Schadt, J.C. and Hasser, E.M. Hemodynamic response to blood loss during a passive response to a stressor in the conscious rabbit. Am.J.Physiol. Regul. Integr. Comp. Physiol. 286:R373-R380, 2004 (First published October 30, 2003; 10.1152/ajpregu.00351.2003).
- Schadt, J.C., Shafford, H.L. and McKown, M.D. Neuronal activity within the periaqueductal gray during simulated hemorrhage in conscious rabbits. Am.J.Physiol. Regul. Integr. Comp. Physiol. 290:R715-R725, 2006 (First published September 29, 2005; 10.1152/ajpregu.00374.2004).
- McKown, M.D. and Schadt, J.C. A modified Harper-McGinty microdrive for use in conscious rabbits. J. Neurosci. Meth. 153:239-242, 2006.
- Shafford, H.L., Strittmatter, R.R., and Schadt, J.C. A novel electrode for chronic recording of electromyographic activity. J. Neurosci. Meth., In press, 2006.

BOOK CHAPTERS, SUBMISSIONS, ABSTRACTS AND OTHER PUBLICATIONS (for total award period)

Book Chapters
None

Submitted Manuscripts and Manuscripts in Preparation

- Strittmatter, R.R. and Schadt, J.C. Respiratory response to hemorrhage in the conscious, New Zealand white rabbit. Am.J.Physiol. Regul. Integr. Comp. Physiol., Submitted, 2006.
- Shafford, H.L., Ivey, J.R., McKown, M.D., Scherff, E.J. and Schadt, J.C. Noxious visceral stimulation and the response to blood loss in conscious rabbits. In preparation, 2006.
- Rubino, R., Ivey, J.R., McKown, M.D., Scherff, E.J., and Schadt, J.C. Naloxone (NLX) increases respiratory rate (RR) as well as mean arterial pressure (MAP) after hypotensive hemorrhage (HH) in conscious rabbits. In preparation, 2006.

Published Abstracts (not yet submitted as full papers).

- Schadt, J.C. and M.D. McKown. Effects of mesenteric denervation and gender on the hemodynamic response to feeding in conscious rabbits. FASEB J., 15:A1152, 2001.
- Schadt, J.C. and M.D. McKown. Oscillation stress (OSC) enhances defense of mean arterial pressure (MAP) during blood loss in conscious rabbits. FASEB J. 16:A834, 2002.
- Schadt, J.C., J.R. Ivey, and M.D. McKown. Hemodynamic effects of nitric oxide (NO) synthase inhibition with L-NAME during simultaneous air jet stress (AIR) and hemorrhage (HEM) in male and female conscious rabbits. FASEB J. 17:A1233, 2003.

- Schadt, J.C. and M.D. McKown. Cranial mesenteric (CM) denervation (DEN) alters the response to hemorrhage (HEM) during oscillation stress (OSC) in the conscious rabbit. FASEB J. 17:A1295, 2003.
- Shafford, H.L., Scherff, E.J., McKown, M.D., and Schadt, J.C. Does the ventrolateral periaqueductal gray (VLPAG) trigger the decompensatory fall in arterial blood pressure during simulated hypotensive hemorrhage (HEM) in conscious rabbits? FASEB J. 18:A1072, 2004.
- Schadt, J.C., McKown, M.D., and Ivey, J.R. Angiotensin II (ANG II) enhances defense of arterial blood pressure during blood loss in the presence of air jet stress (AIR) in conscious rabbits. FASEB J. 18:A673, 2004.
- Rubino, R., Ivey, J.R., McKown, M.D., Scherff, E.J., and Schadt, J.C. Naloxone (NLX) increases respiratory rate (RR) as well as mean arterial pressure (MAP) after hypotensive hemorrhage (HH) in conscious rabbits. FASEB J. 19:A1285, 2005. (manuscript in preparation, see above)
- Shafford, H.L., Scherff, E.J., McKown, M.D., and Schadt, J.C. Periaqueductal gray neurons respond to both cardiovascular input and external sensory stimuli in conscious rabbits. FASEB J. 19:A607, 2005.
- Shafford, H.L., Ivey, J.R., McKown, M.D., Scherff, E.J. and Schadt, J.C. Noxious visceral stimulation and the response to blood loss in conscious rabbits. FASEB J. 20:A1383, 2006. (manuscript in preparation, see above)
- Rubino, R.R., Ivey, J.R., McKown, M.D., Scherff, E.J. and Schadt, J.C. Respiratory response to hypotensive hemorrhage (HH) in the presence of air jet stress (AIR). FASEB J. 20:A376, 2006.