REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From – To)*
28-02-2003	Final Technical Report	01-01-97 – 31-12-02

4. TITLE AND SUBTITLE
Receptors, Afferent Signaling and Central Integration in Compensated and Uncompensated Hemorrhagic Shock

5a. CONTRACT NUMBER

5b. GRANT NUMBER
N00014-97-1-0145

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Johnson, Alan Kim

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Iowa
Iowa City, IA 52242

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
University of Iowa
Iowa City, IA 52242

10. SPONSORING/MONITOR'S ACRONYM(S)

11. SPONSORING/MONITORING REPORT NUMBER

12. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

An understanding of the central and peripheral mechanisms which regulate sympathetic outflow during acute blood volume loss is critical for developing therapeutic treatments for hemorrhagic shock. Insight into the mechanisms by which melanopeptides, LV-HTS, and serotonergic drugs facilitate sympathetic outflow during hemorrhage may lead to the development of pharmacological interventions for hemorrhagic shock. Recent experiments conducted in our laboratory provide important new information about the saluretic effects and mechanisms of 3 potential therapeutic strategies to prevent or reverse phase II shock. We have 1) discovered that γ_2-MSH when administered systemically promotes survival, 2) confirmed that LV-HTS administered systemically improves survival and that this treatment is even more effective when administered directly to the brain cerebrospinal fluid, and 3) treatment with a frequently used serotonergic (5-HT) drug, the 5-HT selective reuptake inhibitor, fluoxetine, enhances baroreceptor reflex gain which may contribute to enhanced survival after severe hypotensive hemorrhage.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Johnson, Alan Kim
U	U	U	U	9	19b. TELEPHONE NUMBER *(Include area code)* 319-335-2423

FINAL TECHNICAL REPORT

GRANT #: N00014-97-1-0145

PRINCIPAL INVESTIGATOR: Dr. Alan Kim Johnson (alan-johnson@uiowa.edu)

INSTITUTION: University of Iowa

GRANT TITLE: Receptors, Afferent Signaling and Central Integration in Compensated and Uncompensated Hemorrhagic Shock

AWARD PERIOD: 1 January 1997 - 31 December 2002

OBJECTIVE: A comprehensive understanding of how the brain controls sympathetic and endocrine outflow during compensated (phase I) vs. early uncompensated (phase II) shock will lead to the development of better therapeutic treatments for severe hypotensive hypovolemia. Our research program has pursued two specific objectives that will increase basic knowledge about the neural control of the circulation in shock. The first was to define the hormonal and afferent nervous system signaling pathways over which systemic treatments with agents such as melanopeptides or low volume (LV) hypertonic saline (HTS) act to effect therapeutic resuscitation from hemorrhagic shock. The second was to define the critical central sites and brain neurochemical systems that control the pattern of autonomic and endocrine outflow mediating recovery and resuscitation from hemorrhagic shock.

APPROACH: This research used models of hemorrhage-induced shock in the rat combined with or without volemic resuscitation. The efficacy and mechanisms of melanopeptides, LV-HTS, and serotonergic drugs in reversing phase II hemorrhagic shock and promoting survival (i.e., 24 and 72 hr post-hemorrhage) were investigated by administering the putative therapeutic treatments either centrally or systemically.

ACCOMPLISHMENTS:

A. The Effects of Systemic γ_2-Melanocyte Stimulating Hormone (γ_2-MSH) and LV-HTS and on Regional Hemodynamics after Hypertensive Hemorrhage. Work conducted in our laboratory and elsewhere suggests that γ_2-MSH and LV-HTS, respectively both significantly increase arterial blood pressure to enhance survival following severe hypotensive hemorrhage. We have completed experiments to determine whether these treatments have their effects during hypotensive hemorrhage by activating similar efferent mechanisms. That is, we examined the effects of graded doses of γ_2-MSH and of LV-HTS in rats subjected to uncompensated hemorrhagic shock on hemodynamics in key vascular beds.

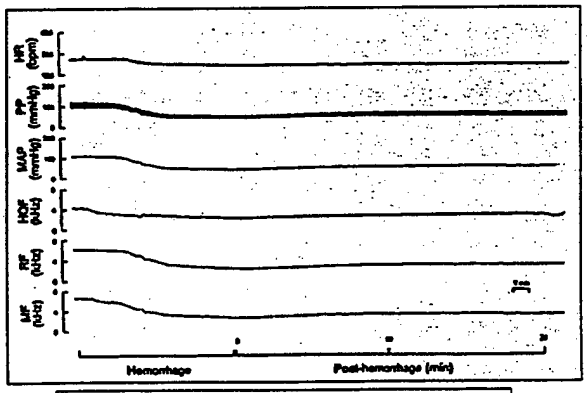


Fig. 1: Typical cardiovascular and hemodynamic changes accompanying blood withdrawal (1-1.5 ml/min) to induce phase II hypovolemic shock.

Male Sprague-Dawley rats (250-350 g) were anesthetized with pentobarbital (50 mg/kg) and implanted with femoral artery and venous catheters and Doppler flow probes on the mesenteric artery, one renal artery and the terminal aorta. Rats were then hemorrhaged (1-1.5 ml/min) via the femoral artery until arterial pressure was <40 mmHg. The mean arterial pressure (MAP) of control animals remained at this low level and there was a sustained bradycardia and marked reductions in blood flow in the hindquarter, renal and mesenteric beds throughout a 20 min post-hemorrhage period (Fig. 1). Animals receiving bolus intravenous injections of 10, 20 and 40 μg/kg of γ_2-MSH showed short-lived but significant increases in MAP (Fig. 2a,b). All doses of γ_2-MSH increased heart

rate (Fig. 2a,b). Increases in mesenteric (MR) and hindquarter (HQR) resistances (or decrease in flow) were seen with the two largest doses and renal resistance (RR) increased with the largest dose of the peptide (Fig. 2c). Interestingly, in contrast to γ_2-MSH, treatment with 0.75, 1.5, and 3.0 mmol/kg of HTS

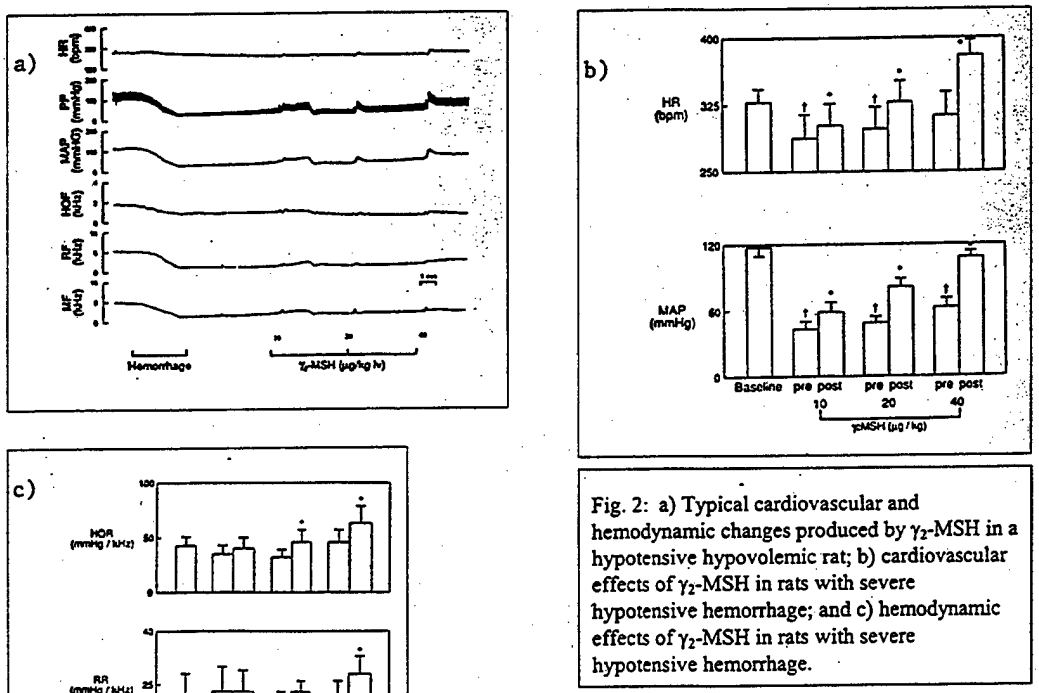


Fig. 2: a) Typical cardiovascular and hemodynamic changes produced by γ_2-MSH in a hypotensive hypovolemic rat; b) cardiovascular effects of γ_2-MSH in rats with severe hypotensive hemorrhage; and c) hemodynamic effects of γ_2-MSH in rats with severe hypotensive hemorrhage.

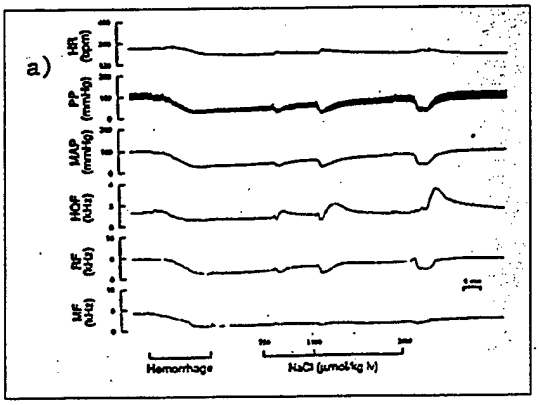
actually produced acute falls in arterial pressure (Fig. 3a,b.). Although there was an increase in heart rate with each LV-HTS injection, there were significant decreases in HQR, RR and MR following most of the LV-HTS treatments (Fig. 3c). In spite of LV-HTS failing to produce acute increases in MAP in one or more of the regional resistances, it is striking that there was a slow, progressive increase in MAP and heart rate and in HQR, RR and MR throughout the session, so that by the end of the test none of the parameters were significantly less than baseline values (Fig. 3b,c). In fact, MR was significantly increased at the conclusion of the period.

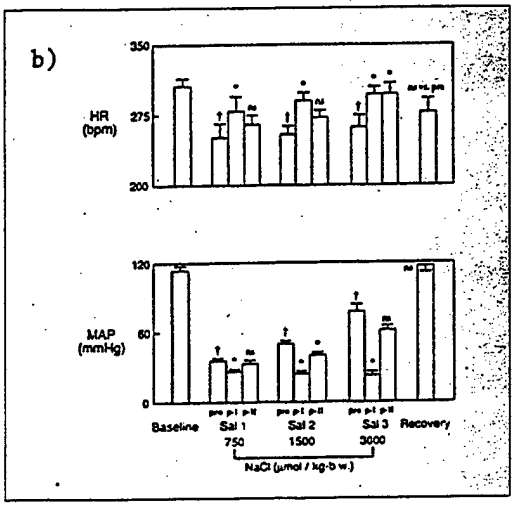
Taken together, the results of these experiments indicate that hemodynamic changes induced by treatments having putative therapeutic effects during phase II hemorrhagic shock produced markedly different changes in cardiovascular parameters and regional vascular resistances. This suggests that salutary actions of these agents involve different mechanisms. In light of this possibility, it is likely that combined administration of γ_2-MSH and LV-HTS will produce synergistic actions to restore the perfusion of key vascular beds and facilitate recovery from hemorrhagic shock.

In more recent work, we have focused on using lower concentrations of HTS. We have studied the hemodynamic effects of 5% NaCl because of its current FDA approval. In pentobarbital anesthetized rats instrumented with Doppler flow probes on the mesenteric and renal arteries and the terminal aorta, bolus (10% of the shed blood volume) administration of 5% NaCl produces a brief biphasic (minimal pressor) response and then a gradual, progressive rise in resistance in the mesenteric, renal and hindquarter vascular beds and a rise in MAP (Figure 4). The results are similar to those we previously observed and with highly concentrated HTS (~20%).

α-MSH is another melanocortin peptide structurally-related to γ_2-MSH and one which is approved for administration to humans. Male Sprague-Dawley rats (300-325 g) were instrumented with carotid and femoral arterial and femoral venous catheters 48 hrs prior to testing. At the time of testing, the animals were placed in hanging metabolic cages and vascular lines were connected to measure blood pressure, induce hemorrhage and deliver treatments. After adaptation and acquisition of baseline blood pressure

recordings, a 3.6 ml/100 g-body weight (bw) hemorrhage was initiated. Blood withdrawal typically took about 10 min and lowered MAP from approximately 120 to 50 mmHg. Immediately upon conclusion of the hemorrhage, experimental animals received 8.25, 16.5 or 27.5 μg of α-MSH administered intravenously; control animals received isotonic saline in comparable volumes to those administered to the





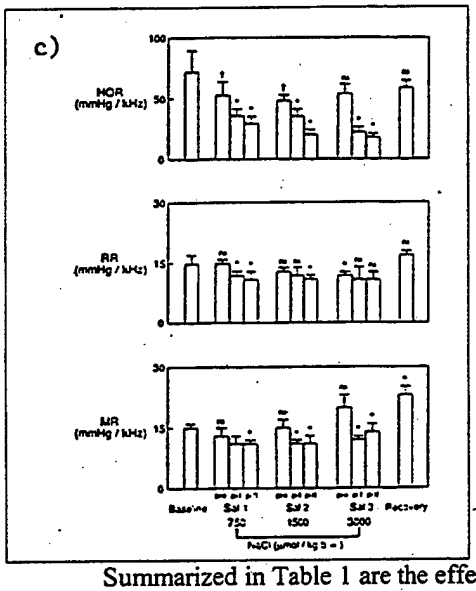


Fig. 3: a) Typical cardiovascular and hemodynamic changes produced by LV-HTS in a hypotensive hypovolemic rat; b) cardiovascular effects of low volume hypertonic saline (LV-HTS) in acute hypotensive hemorrhage; and c) hemodynamic effects of low volume hypertonic saline (LV-HTS) in acute hypotensive hemorrhage.

experimental animals. Fifty min after the conclusion of the hemorrhage, all animals received the equivalent of ½ their shed blood volume as a bolus of lactated Ringers and then 1½ times this volume as an intravenous infusion of lactated Ringers (~0.06 ml/min). At the conclusion of the infusion periods, animals were returned to their home cages and observed regularly over the next 5 days.

Summarized in Table 1 are the effects of acute isotonic saline (control) and α-MSH treatments on survival of hypotensive hemorrhage. Nearly all animals died following acute isotonic saline treatment (controls; 7% survival). In contrast, 8.25 and 16.5 μg intravenous treatments of α-MSH promoted survival in 4 out of 8 (50% survival) and 8 out of 14 (57% survival) animals, respectively. Treatment with 27.5 μg of α-MSH however appears to be on a declining limb of a dose response curve. The results of these experiments suggest that α-MSH should be explored further for its salutary effects in the treatment of severe hypotensive hemorrhage.

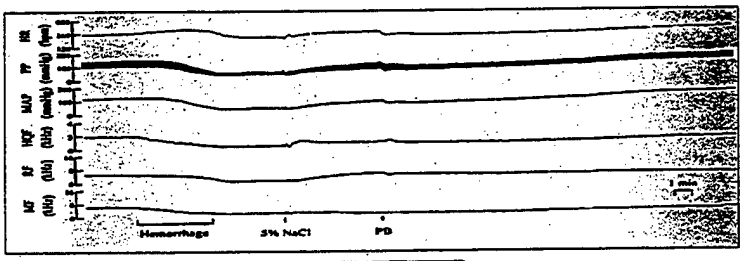


Fig. 4

Table 1: The effects of α-MSH treatment immediately post-hemorrhage (3.6 ml/100 g-bw) on hemorrhagic shock.

	Controls (Saline)	8.25 µg	16.5 µg	27.5 µg
Died prior to reperfusion	13	4	6	4
Survived 5 days post-resuscitation	1	4	8	0
TOTAL	14	8	14	4

We found that animals subjected to our severe hemorrhage shock model (3.6 ml/100 g-bw blood withdrawal over 10 min in 300-325 g-bw male rats) that were treated with 10 µl of 0.33 M NaCl into the lateral cerebral ventricle followed by a 3 µl/min for approximately 10-15 min of 0.33 M NaCl produced a profound increase in arterial pressure (i.e., an increase of 65-76 mmHg). The increase in pressure was markedly greater than the typical transient increase of 20-30 mmHg seen in control rats. Based on these observations, we decided to attempt to optimize the central infusion conditions. We tested a total of 24 hemorrhaged animals with lateral ventricle administration of isotonic saline (control) vs. 0.33 M NaCl at rates of 1 to 3 µl/min following a 10 µl lateral ventricle bolus for time periods of 15 to 50 min. Survival rates for experimental animals treated with lateral ventricle-infused 0.33 M NaCl for these longer periods were increased. However, we noted that many of the 0.33 M NaCl lateral ventricle infused animals show signs of seizures. Consequently, we conducted a subsequent study in which the concentration of NaCl in the experimental group was reduced to 0.27 M NaCl. Specifically, control (n=9) and experimental (n=10) rats received 3.6 ml/100 g-bw hemorrhages over approximately 10 min. At the conclusion of the hemorrhage, animals received a 10 µl bolus followed by a 50 min lateral ventricle infusion (3 µl/min) of either 0.15 M (control) or 0.27 M NaCl. Nine out of 10 experimental animals survived 1 hr post hemorrhage vs. only 3 out of 9 of the control animals ($\chi^2 = 13.07$; $P \leq 0.001$). At approximately 60 min after hemorrhage, half of the shed blood volume was administered as Ringer's lactate. All rats survived 1 hr after the reinfusion and 8 out of 9 experimental and 3 out of 3 controls survived 2 hr after reinfusion. These results suggest that activation of the sympathetic nervous system by a remarkably small amount of HTS administered directly to the brain reverses the acute effects of severe hypotensive hemorrhage to promote survival

In order to determine if lateral ventricular infused HTS is more effective than the same concentration and volume of HTS administered systemically in facilitating survival from severe hypotensive hemorrhage, a second experiment was conducted. Animals were prepared with catheters and hemorrhaged 2 days later as previously described. After removal of 3.6 ml/100 g-bw, a 10 µl bolus of 0.27 M NaCl was administered over 1 min and then at a rate of 3 µl/min for 50 min. At the conclusion of the 50 min infusion ½ the volume of the shed blood was administered as Ringers lactate. Control animals received the same procedure except they received isotonic saline. Five of 15 rats receiving intravenous HTS survived whereas all of the 12 animals receiving isotonic saline died within 1 hr after the start of hemorrhage. The survival rate for the intracerebroventricular infusions of HTS was 90% whereas the survival rate for intravenous infusions of HTS was only 33%. The results support the interpretation that LV-HTS therapy for hemorrhagic shock probably exerts its salutary effects through a CNS action to enhance the pattern of systemic hemodynamics.

B. Serotonergic Mechanisms and Protection Against Hypovolemic Hemorrhage. In previous studies (Scrogin, Veelken and Johnson, 1998)[1], we found that central administration of methysergide, a serotonergic drug, that acts primarily as a 5-$HT_{1/2}$ receptor antagonist but which also has some 5-HT_{1A} receptor agonist activity, significantly delays the withdrawal of renal sympathetic tone and bradycardia during non-compensated (phase II) hemorrhagic shock. We have completed work showing that intracerebroventricular administration of the highly selective 5-HT_{1A} receptor agonist, (±)-8-hydroxy-dipropylaminotetralin (8-OH-DPAT), also produces the same delay in onset of bradycardia and withdrawal of renal sympathetic nerve activity during hemorrhage. This finding suggests two interpretations: first,

[1] Scrogin, K. E., Veelken, R., & Johnson, A. K.: Central methysergide prevents renal sympathoinhibition and bradycardia during hypotensive hemorrhage. Am. J. Physiol., 1998, 274, H43-H51.

that these drugs are blocking 5-HT activity possibly through methysergide acting on post-synaptic receptors (perhaps in the raphe nuclei) and through 8-OH-DPAT's classic action on somatic/dendritic autoreceptors (i.e., in the hindbrain); or alternatively, that methysergide is exerting a 5-HT inhibitory action by acting primarily as an agonist on 5-HT_{1A} autoreceptors. In either case, it appears that acute pharmacological manipulations that reduce activity in central 5-HT pathways block the autonomic changes accompanying phase II of hemorrhagic shock.

These observations made with the 5-HT_{1A} receptor agonist prompted us to ask what types of systemically administered, clinically employed 5-HT-related drugs are likely to enhance cardiovascular function? We decided to test the effects of the widely used anti-depressant serotonin selective reuptake inhibitor, fluoxetine (Prozac®), to see if we could enhance the sympathetic baroreceptor reflex. We examined the effects of 4 days of fluoxetine administration (10 mg/kg, daily, i.p.) on baroreceptor reflex control of lumbar sympathetic nerve activity (LSNA). As compared to control (vehicle; 0.9% saline) administration, fluoxetine did not significantly change resting MAP or heart rate. However, it did significantly enhance the maximum baroreflex elicited increases in LSNA (VEH: 352 ± 26 vs. fluoxetine 454 ± 41; % baseline LSNA; $P<0.05$) and increased the maximum reflex gain (vehicle: -7.5 ± 1.0 vs. fluoxetine -12.8 ± 2.3; % LSNA/mmHg; $P<0.05$).

We also tested 4 day fluoxetine (10 mg/kg/day) treatment under a condition known to blunt cardiovascular baroreceptor reflexes, cardiovascular deconditioning. We used a hindlimb suspension paradigm which models the effects of cardiovascular deconditioning induced by bed rest or by exposure to microgravity. Fluoxetine treatment also enhanced LSNA baroreceptor reflexes in rats that had undergone 14 days of hindlimb unloading before testing.

	Hindlimb Unweighted	
	Control	*Fluoxetine Treated*
Maximum Reflex Gain % LSNA	252 ± 5.2	331 ± 6

CONCLUSIONS: Recent experiments conducted in our laboratory provide important new information about the saluretic effects and mechanisms of 3 potential therapeutic strategies to prevent or reverse phase II shock. We have 1) discovered that γ_2-MSH when administered systemically promotes survival, 2) confirmed that LV-HTS administered systemically improves survival and that this treatment is even more effective when administered directly to the brain cerebrospinal fluid, and 3) treatment with a frequently used serotonergic (5-HT) drug, the 5-HT selective reuptake inhibitor, fluoxetine, enhances baroreceptor reflex gain which may contribute to enhanced survival after severe hypotensive hemorrhage.

SIGNIFICANCE: An understanding of the central and peripheral mechanisms which regulate sympathetic outflow during acute blood volume loss is critical for developing therapeutic treatments for hemorrhagic shock. Insight into the mechanisms by which melanopeptides, LV-HTS, and serotonergic drugs facilitate sympathetic outflow during hemorrhage may lead to the development of pharmacological interventions for hemorrhagic shock.

PATENT INFORMATION: None

AWARD INFORMATION: Appointed as F. Wendell Miller Distinguished Professor

REFEREED PUBLICATIONS

1. Johnson, A. K., & Thunhorst, R. L.: The neuroendocrinology of thirst and salt appetite: Visceral sensory signals and mechanisms of central integration. Frontiers in Neuroendocrinology, 1997, 18, 292-353.

2. Scrogin, K. E., Veelken, R., & Johnson, A. K.: Central methysergide prevents renal sympathoinhibition and bradycardia during hypotensive hemorrhage. American Journal of Physiology, 1998, 274, H43-H51.

3. Menani, J. V., De Luca, Jr., L. A., & Johnson, A. K.: Lateral parabrachial nucleus serotonergic mechanisms and salt appetite induced by sodium depletion. American Journal of Physiology, 1998, 274, R555-R560.

4. Xu, Z., & Johnson, A. K.: Central renin injections: effects on drinking and expression of immediate early genes. Brain Research, 1998, 782, 24-35.

5. Thunhorst, R. L., Xu, Z., Cicha, M. Z., Zardetto-Smith, A. M., & Johnson, A. K.: Fos expression in rat brain during depletion-induced thirst and salt appetite. American Journal of Physiology, 1998, 274, R1807-R1814.

6. Menani, J. V., Colombari, D. S. A., Beltz, T. G., Thunhorst, R. L., & Johnson, A. K.: Salt appetite: interaction of forebrain angiotensinergic and hindbrain serotonergic mechanisms. Brain Research, 1998, 801, 29-35.

7. Menani, J. V., & Johnson, A. K.: Cholecystokinin actions in the parabrachial nucleus: effects on thirst and salt appetite. American Journal of Physiology, 1998, 275, R1431-R1437.

8. Xu, Z., & Johnson, A. K.: Non-NMDA receptor antagonist-induced drinking in rat. Brain Research, 1998, 808, 124-127.

9. Scrogin, K. E., Johnson, A. K., & Schmid, H. A.: Multiple receptor subtypes mediate the effects of serotonin on rat subfornical organ neurons. American Journal of Physiology, 1998, 275, R2035-R2042.

10. Vasquez, E. C., Johnson, R. F., Beltz, T. G., Haskell, R. E., Davidson, B. L., & Johnson, A. K.: Replication-deficient adenovirus vector transfer of *gfp* reporter gene into supraoptic nucleus and subfornical organ neurons. Experimental Neurology, 1998, 154, 353-365.

11. Whalen, E. J., Johnson, A. K., & Lewis, S. J.: Blockade of β-adrenoceptors enhances cAMP signal transduction *in vivo*. European Journal of Pharmacology, 1998, 355, 189-193.

12. Whalen, E. J., Johnson, A. K., & Lewis, S. J.: Hemodynamic actions of systemically-injected pituitary adenylate cyclase activating polypeptide-27 in the rat. European Journal of Pharmacology, 1999, 365, 205-215.

13. Whalen, E. J., Johnson, A. K., & Lewis, S. J.: Effects of nitric oxide synthase inhibition on sympathetically-mediated tachycardia. European Journal of Pharmacology, 1999, 365, 217-223.

14. Whalen, E. J., Beltz, T. G., Lewis, S. J., & Johnson, A. K.: AV3V lesions attenuate the cardiovascular responses produced by blood-borne excitatory amino acid analogs. American Journal of Physiology, 1999, 276, H1409-H1415.

15. Lewis, S. J., Whalen, E. J., Beltz, T. G., & Johnson, A. K.: Role of the anterior region of the third ventricle in the cardiovascular responses produced by systemic injection of a nitric oxide synthase inhibitor. Brain Research, 1999, 830, 191-194.

16. Whalen, E. J., Travis, M. D., Johnson, A. K., & Lewis, S. J.: Rapid tachyphylaxis to hemodynamic effects of PACAP-27 after inhibition of nitric oxide synthesis. American Journal of Physiology, 1999, 276, H2117-H2126.

17. Johnson, R. F., Beltz, T. G., Jurzak, M., Wachtel, R. E., & Johnson, A. K.: Characterization of ionic currents of cells of the subfornical organ that project to the supraoptic nuclei. Brain Research, 1999, 817, 226-231.

18. Thunhorst, R. L., Beltz, T. G., & Johnson, A. K.: Effects of subfornical organ lesions on acutely induced thirst and salt appetite. American Journal of Physiology, 1999, 277, R56-R65.

19. Johnson, A. K., de Olmos, J., Pastuskovas, C. V., Zardetto-Smith, A. M., & Vivas, L.: The extended amygdala and salt appetite. In: J.F. McGinty (Ed.), Advancing from the Ventral Striatum to the Extended Amygdala, Vol. 877, Annals of the New York Academy of Sciences, 1999, pp. 258-280.

20. Whalen, E. J., Beltz, T. G., Lewis, S. J., & Johnson, A. K.: Periventricular anteroventral third ventricle lesions diminish the pressor response produced by systemic injection of the *N*-methyl-D-aspartate receptor antagonist MK-801. Brain Research, 1999, 836, 210-212.

21. Lewis, S. J., Whalen, E. J., Beltz, T. G., & Johnson, A. K.: Effects of chronic lesions of the anteroventral third ventricle region on baroreceptor reflex function in conscious rats. Brain Research, 1999, 835, 330-333.

22. Rocha, M. J. A., Beltz, T. G., Dornelles, R. C. M., Johnson, A. K., & Franci, C. R.: Anteroventral third ventricle (AV3V) lesions alter c-*fos* expression induced by salt loading. Brain Research, 1999, 829, 197-200.

23. Robinson, M. M., McLennan, G. P., Thunhorst, R. L., & Johnson, A. K.: Interactions of the systemic and brain renin-angiotensin systems in the control of drinking and the central mediation of pressor responses. Brain Research, 1999, 842, 55-61.

24. Vasquez, E. C., Beltz, T. G., Meyrelles, S. S., & Johnson, A. K.: Adenovirus-mediated gene delivery to hypothalamic magnocellular neurons in mice. Hypertension, 1999, 34, 756-761.

25. Whalen, E. J., Johnson, A. K., & Lewis, S. J.: Tachyphylaxis to PACAP-27 after inhibition of NO synthesis: a loss of adenylate cyclase activation. American Journal of Physiology, 1999, 277, R1453-R1461.

26. Crippa G. E., Lewis, S. J., Johnson, A. K., & Corrêa, F. M. A.: Medial prefrontal cortex acetylcholine injection-induced hypotension: the role of hindlimb vasodilation. Journal of the Autonomic Nervous System, 2000, 79, 1-7.

27. Schoorlemmer, G. H. M., Johnson, A. K., & Thunhorst, R. L.: Effect of hyperosmotic solutions on salt excretion and thirst in rats. American Journal of Physiology, 2000, 278, R917-R923.

28. Menani, J. V., De Luca, Jr., L A., Thunhorst, R. L., & Johnson, A. K.: Hindbrain serotonin and the rapid induction of sodium appetite. American Journal of Physiology, 2000, 279, R126-R131.

29. Whalen, E. J., Johnson, A. K., & Lewis, S. J.: Functional evidence for the rapid desensitization of 5-HT_3 receptors on vagal afferents mediating the Bezold-Jarisch reflex. Brain Research, 2000, 873, 302-305.

30. Whalen, E. J., Johnson, A. K., & Lewis, S. J.: β-adrenoceptor dysfunction after inhibition of NO synthesis. Hypertension, 2000, 36, 376-382.

31. Scrogin, K. E., Johnson, A. K., & Brooks, V. L.: Methysergide delays the decompensatory responses to severe hemorrhage by activating 5-HT_{1A} receptors. American Journal of Physiology, 2000, 279, R1776-R1786.

32. Vasquez, E. C., Beltz, T. G., Haskell, R. E., Johnson, R. F., Meyrelles, S. S., Davidson, B. L., & Johnson, A. K.: Adenovirus-mediated gene delivery to cells of the magnocellular hypothalamo-neurohypophyseal system. Experimental Neurology, 2001, 167, 260-271.

33. Johnson, R. F., Beltz, T. G., Sharma, R. V., Xu, Z., Bhatty, R. A., & Johnson, A. K.: Agonist activation of cytosolic Ca^{2+} in subfornical organ cells projecting to the supraoptic nucleus. American Journal of Physiology, 2001, 280, R1592-R1599.

34. Xu, Z., Ross, M. G., & Johnson, A. K.: Intracerebroventricular carbachol induces FOS immunoreactivity in lamina terminalis neurons projecting to the supraoptic nucleus. Brain Research, 2001, 895, 104-110.

35. Vasquez, E. C., Meyrelles, S. S., Chapleau, M. W., & Johnson, A. K.: Approaches for gene delivery to the subfornical organ and magnocellular neurons. Clinical & Experimental Pharmacology & Physiology, 2001, 28, 602-609.

36. Schoorlemmer, G. H. M., Johnson, A. K., & Thunhorst, R. L.: Circulating angiotensin II mediates sodium appetite in adrenalectomized rats. American Journal of Physiology, 2001, 281, R723-R729.

37. Whalen, E. J., Schoorlemmer, G. H. M., Beltz, T. G., Johnson, A. K., & Lewis, S. J.: Effects of chronic lesions of the anteroventral region of the third ventricle on cardiac β-adrenoceptor function in conscious rats. Brain Research, 2001, 913, 82-85.

38. Franchini, L. F., Johnson, A. K., de Olmos, J., & Vivas, L.: Sodium appetite and fos activation in serotonergic neurons. American Journal of Physiology, 2002, 282, R235-R243.

39. De Luca, Jr., L A., Xu, Z., Schoorlemmer, G. H. M., Thunhorst, R. L., Beltz, T. G., Menani, J. V., & Johnson, A. K.: Water deprivation-induced sodium appetite: humoral and cardiovascular mediators and immediate early genes. American Journal of Physiology, 2002, 282, R552-R559.

40. Menani, J. V., Barbosa, S. P., De Luca Jr., L. A., De Gobbi, J. I. F., & Johnson, A. K.: Serotonergic mechanisms of the lateral parabrachial nucleus and cholinergic-induced sodium appetite. American Journal of Physiology, 2002, 282, R837-R841.

41. Grippo, A. J., Moffitt, J. A., & Johnson, A. K.: Cardiovascular alterations and autonomic imbalance in an experimental model of depression. American Journal of Physiology, 2002, 282, R1333-1341.

42. Whyte, D. G., & Johnson, A. K.: Lesions of periventricular tissue surrounding the anteroventral third ventricle (AV3V) attenuate salivation and thermal tolerance in response to a heat stress. Brain Research, 2002, 951, 146-149.

43. Moffitt, J. A., Grippo, A. J., Holmes, P. V., & Johnson, A. K.: Olfactory bulbectomy attenuates cardiovascular sympathoexcitatory reflexes in rats. American Journal of Physiology, 2002, 283, H2575-H2583.

44. Grippo, A. J., Francis, J., Weiss, R. M., Felder, R. B., & Johnson, A. K.: Cytokine mediation of experimental heart failure-induced anhedonia. American Journal of Physiology, (in press).

45. Pastuskovas, C. V., Cassell, M. D., Johnson, A. K., & Thunhorst, R. L.: Neuronal activity in insular cortex of rat after sodium ingestion induced by sodium depletion. American Journal of Physiology, (in press).

46. Grippo, A. J., & Johnson, A. K.: Biological mechanisms in the relationship between depression and heart disease. Neuroscience & Biobehavioral Reviews, (in press).

47. Grippo, A. J., Beltz, T. G., & Johnson, A. K.: Behavioral and cardiovascular changes in the chronic mild stress model of depression. Physiology & Behavior, (in press).

BOOK CHAPTERS, SUBMISSIONS, AND ABSTRACTS

1. Vasquez, E. C., & Johnson, A. K.: Green fluorescent protein as a reporter of adenovirus-mediated gene transfer and expression in the hypothalamic-neurohypophyseal system. In: B.W. Hicks (ed.), Methods in Molecular Biology, Vol. 183: Green Fluorescent Protein: Applications and Protocols, Totowa: Humana Press, 2002, 321-329.

2. Moffitt, J. A., & Johnson, A. K.: Short-term fluoxetine treatment enhances baroreflex control of sympathetic nervous system activity. (submitted).

3. Grippo, A. J., Santos, C. M., Johnson, R. F., Beltz, T. G., Martins, J. B., Felder, R. B., & Johnson, A. K.: Increased susceptibility to ventricular arrhythmias in an experimental rodent model of depression. (submitted).

4. Whyte, D. G., Thunhorst, R. L., & Johnson, A. K.: Reduced thirst in old, thermally dehydrated rats. (submitted).