Evaluation of pressor sensitivity to norepinephrine infusion in dogs with iatrogenic
hyperadrenocorticism


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(ABSTRACT)

Objective: To evaluate pressor sensitivity to catecholamines in dogs after induction of iatrogenic

hyperadrenocorticism (I-HAC) by serial arterial blood pressure measurements during infusions of

increasing dose rates of norepinephrine.

Animals: Eight dogs with I-HAC induced by administration of oral hydrocortisone at a mean dose

of 3.3 mg/kg PO TID for 42-49 days and 8 control dogs which received empty gelatin capsules PO

TID for 42-49 days.

Procedure: Systolic, diastolic, mean blood pressure and heart rate measurements were recorded

after sequential administration of increasing dose rates of norepinephrine (0.1, 0.125, 0.2, 0.3, 0.4,

0.6 and 0.8 µg/kg/min) for 10 minutes. The changes in systolic, diastolic, mean blood pressure and

heart rate were compared between control dogs and dogs with I-HAC.

Results: Dogs in the I-HAC group had a more pronounced pressor response to norepinephrine

infusions than control dogs. The infusions were not completed in 7 of the 8 dogs in the I-HAC

group versus 3 dogs in the control group due to severe elevations in systolic blood pressure. The

mean change in systolic blood pressure was consistently higher in dogs in the I-HAC group. The

difference was statistically significant at the 0.2 µg/kg/min norepinephrine dose rate. The mean

change in heart rate was consistently lower in the I-HAC group, a difference that was significant at

the 0.2 µg/kg/min norepinephrine dose rate.

Conclusions and clinical relevance: Increased pressor sensitivity or decreased baroreceptor response to norepinephrine was seen in dogs with I-HAC suggesting that this mechanism is involved in the development of hypertension in canine hyperadrenocorticism.

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| | |
|---|---|
| 11 β-HSD | 11 β-hydroxysteroid dehydrogenase |
| ACE | Angiotensin concerting enzyme |
| ACTH | Adrenocorticotropic hormone |
| ADH | Antidiuretic hormone |
| Ang II | Angiotensin II |
| ANF | Atrial natriuretic factor |
| BP | Blood pressure |
| CO | Cardiac output |
| cAMP | cyclic adenosine-3',5'-monophosphate |
| cGMP | cyclic guanosine-3',5'- monophosphate |
| CRH | Corticotropin-releasing hormone |
| CSF | Cerebrospinal fluid |
| DHEA | Dehydroepiandrosterone |
| DOC | Deoxycorticosterone |
| GC | Glucocorticoids |
| HAC | Hyperadrenocorticism |
| I-HAC | Iatrogenic hyperadrenocorticism |
| IP3 | Inositol-1,4,5-triphosphate |
| MC | Mineralocorticoids |
| NE | Norepinephrine |
| NO | Nitric oxide |
| NOS | Nitric oxide synthase |

| | |
|---|---|
| NPY | Neuropeptide Y |
| PDH | Pituitary-dependent hyperadrenocorticism |
| PGE2 | Prostaglandin E2 |
| PRA | Plasma renin activity |
| PVR | Peripheral vascular resistance |
| RAS | Reticular activating system |
| THE | Tetrahydrocortisone |
| THF | Tetrahydrocortisol |
| VSMCs | Vascular smooth muscle cells |

# INTRODUCTION

Hypertension has been found to occur in over 50% of cases of canine hyperadrenocorticism (HAC).[1,2] In the most extensive study performed in dogs, a prevalence of 86% was reported,[3] similar to that of human patients.[4-8] Up to 40 % of these dogs remain hypertensive even after successful treatment of the condition.[3] Arterial hypertension and its sequelae are a major cause of short and long term morbidity and mortality in human patients, often leading to lethal complications if left untreated.[8] Similarly, hypertension in dogs has been associated with development of ophthalmic changes such as blindness from intraocular hemorrhage and retinal detachment, glomerulosclerosis, chronic renal failure, left ventricular hypertrophy and congestive heart failure.[2,9,10] Hypertension may also predispose dogs to development of thromboembolism, which is a documented complication of canine HAC.[11-13]

The mechanisms involved in the development of hypertension in naturally occuring hyperadrenocorticism are not completely understood. The association between exogenous or synthetic glucocorticoids and hypertension has been recognized for many years.[14,15] Proposed mechanisms for the development of hypertension in hyperadrenocorticism include sodium retention, activation of the renin-angiotensin system and increased vascular reactivity to endogenous vasopressors.[16,17] Currently, increased vascular responsiveness to endogenous vasopressors is believed to be the major cause for glucocorticoid induced hypertension in humans.[5,6,15-19] The authors are unaware of studies of the pathogenesis of this condition in canine hyperadrenocorticism.

We hypothetized that HAC results in increased vascular responsiveness to catecholamines in the dog and that this is involved in the development of hypertension. The objective of this study

was to evaluate pressor sensitivity to catecholamines in dogs after induction of iatrogenic

hyperadrenocorticism by serial arterial blood pressure measurements during infusions of

increasing dose rates of norepinephrine.

**CHAPTER I**

Literature Review

I. Regulation of blood pressure

Introduction

The regulation of arterial blood pressure is mediated through complex, highly interactive and overlapping mechanisms that work to adjust blood pressure in response to changes in the body.[20] Both short and long term mechanisms are involved in blood pressure regulation. Short term responses are mediated by the autonomic nervous system and long term responses by a balance between extracellular fluid and blood volume on one hand and renal mechanisms controlling urinary output on the other.[21] Blood pressure is affected by cardiac output (CO) and peripheral vascular resistance (PVR), (BP = CO X PVR).[21] Cardiac output is altered by changes in heart rate and stroke volume. Factors affecting any of these variables will cause changes in blood pressure.[22] Blood pressure is reported as systolic pressure (highest pressure at the peak of the pulse wave), diastolic pressure (lowest pressure at the end of diastole), pulse pressure (difference between systolic and diastolic pressure) and mean arterial pressure (average pressure during the cardiac cycle, taking into account both the magnitude and the time that pressure is exerted).[23] Systolic blood pressure is affected by changes in stroke volume, aortic distensibility, and ejection velocity, whereas diastolic pressure is affected by changes in systolic pressure, aortic distensibility, heart rate, and peripheral resistance.[22] Increases in stroke volume are associated with increases in systolic, mean and pulse pressure. A decrease in aortic distensibility will cause an increase in systolic blood pressure. Increased ejection velocity will produce increased diastolic pressures. An increased peripheral vascular resistance will raise the mean arterial

pressure and diastolic pressure. Systolic pressure will also be raised depending on the degree of increase in peripheral resistance.[22] The responses in living organisms are more complicated than this, since changes in many of these variables usually coexists and are also affected by compensatory reflex mechanisms.[22]

Although the body is exposed to a variety of stimuli during the day, only minimal changes in blood pressure occur due to the fine control achieved by both the short and long term control mechanisms with the ultimate goal being maintenance of tissue perfusion during different physiologic conditions.[23]

A. *Neural control of blood pressure*

1. *Central nervous system*

Central nervous system control of blood pressure is primarily a function of the cardiovascular center in the brainstem.[23] This medullary center receives information from other areas in the brain such as the cortex, hypothalamus and bulbar sites and sensory information from the rest of the body. The information received is integrated by the spinal neurons and processed by the preganglionic fibers of the autonomic nervous system.[23]

The cardiovascular center of the brainstem can be divided into four functional areas: the vasoconstrictor and vasodilator areas which interact to cause changes in peripheral vascular resistance and blood flow, and the cardioexcitatory and cardioinhibitory areas which send sympathetic (excitatory) or parasympathetic (inhibitory) fibers to the heart and are responsible for control of the heart rate.[23]

Under normal conditions, the tone of the peripheral arterioles is maintained by tonic impulses that originate in the vasoconstrictor or pressor area of the cardiovascular center. The spontaneous activity from this area is maintained by stimulation from the reticular activating system (RAS) as

well as from other parts of the brain. The cardiovascular center also responds to the direct effects of the chemoreceptor cells located in the carotid and aortic bodies. These chemoreceptors are sensitive to decreases in blood pH and PO2 and their stimulation produce an increase in pulmonary ventilation and an increase in peripheral vascular resistance.[23] The basic rate of discharge of the vasoconstrictor area is also influenced by efferent impulses from other neural connections such as a negative feedback from the carotid and aortic baroreceptors.[23]

The cardioexcitatory and vasoconstrictor areas are closely related. Stimulation of the vasoconstrictor area causes an elevation of both heart rate and arterial pressure due to increased sympathetic discharge and reciprocal inhibition of the cardioinhibitory center.[23]

The vasodilator area exerts its major effects by sending inhibitory impulses to the pre-ganglionic sympathetic neurons in the spinal cord. The preganglionic cells receive both excitatory and inhibitory impulses from the vasoconstrictor and vasodilator centers, respectively. The vasodilator area also has connections with cardiac inhibitory cells in the dorsal vagal nucleus. The vagal center normally is tonically active and maintains control of heart rate. The activity of the vagal cells is altered in response to input from the carotid and aortic baroreceptors.[23]

2. *Peripheral nervous system*

All the sympathetic neural influences that control the heart and circulation converge at the pre-ganglionic sympathetic neurons located in the intermediolateral cell columns of the thoracic and lumbar spinal cord. Synapses between pre- and post-ganglionic neurons occur in the paravertebral ganglia or prevertebral ganglia. The post-ganglionic axons innervate the peripheral blood vessels, the heart and other organs.[24] Acetylcholine is the principal neurotransmitter released at the synapse between the pre-and post- ganglionic neurons. The main site of action of

acetylcholine is at the nicotinic receptors of the post-ganglionic cell membranes although there is some interaction with the muscarinic receptors at the post-ganglionic neurons, thus modulating synaptic transmission.[24]

There are two major types of adrenergic receptors involved in cardiovascular regulation. β-adrenergic receptors enhance contractility and heart rate. They can be further separated into those with a cardiac stimulatory effect (β 1- adrenergic receptors) and those found mainly in extracardiac sites such as the arterioles (β 2- adrenergic receptors) which cause vasodilation.[25] α-adrenergic receptors are present in the arteriolar walls and increase arteriolar tone. The pre- and post-synaptic receptors have been further classified as α 1 and α 2- adrenergic receptors based on their pharmacologic effects. α 1- adrenergic receptors are more sensitive to phenylephrine than to clonidine, and more susceptible to blockade by prazosin than by yohimbine. α 2- adrenoreceptors have a reverse order of effectiveness of these drugs. The pressor effects in dogs have been found to be mediated by both α 1- and α 2- vascular (post-synaptic) adrenoreceptors.[26] Both types of receptors have been found to produce increases in blood pressure and total peripheral resistance without significant inotropic or chronotropic effects in non-anesthetized dogs.[27] Stimulation of the β 1-adrenergic receptors causes increased cardiac contractility and heart rate. α-adrenergic receptors, on the other hand, have most of their effect on vascular smooth muscle, increasing arteriolar tone.[25] Norepinephrine is the principal neurotransmitter released at the effector junctions between the post-ganglionic neurons and the heart and blood vessels. Norepinephrine stimulates β adrenergic receptors in the heart and α adrenergic receptors in the vascular smooth muscle.[24]

The adrenal medulla is also under influence of pre-ganglionic sympathetic stimulation. Epinephrine, and to a lesser extent norepinephrine, are released to the blood stream after release

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of acetylcholine at the pre-ganglionic synapse. Control of the pre-ganglionic spinal neurons is by facilitatory or inhibitory impulses from the brain.[24] Epinephrine has greater β- adrenergic potency than norepinephrine, while norepinephrine has greater α- adrenergic potency. Systemic administration of epinephrine produces generalized vasodilation with a decrease in peripheral resistance associated with an increase in cardiac output and heart rate. Systolic pressure is increased following epinephrine administration due to an increase in cardiac output, but diastolic pressure remains unchanged or falls, with little change in mean arterial pressure. Norepinephrine causes increase in both systolic and diastolic pressures, due to generalized vasoconstriction. Although norepinephrine has β- sympathomimetic activity, and thus would be expected to increase heart rate, the increase in pressure causes reflex slowing of the heart rate, resulting in little change or a decrease in cardiac output.[28]

Many of the α- and β-adrenergic effects of these hormones are mediated through changes in the adenylate cyclase system. β-sympathomimetic effects are primarily induced by the stimulation of adenylate cyclase and increased cAMP generation.[29] The increased production of cAMP is mediated via the G s alpha subunit. CAMP then activates protein kinase A, which then phosphorylates a variety of other proteins, enzymes, ion channels and receptors.[30] Binding to α 1-adrenergic receptors causes the release of the alpha subunit of the guanylyl nucleotide binding protein G, which activates phospholipase C. Phospholipase C catalyzes the conversion of phosphatidyl inositol phosphate to 1,4,5-inositol triphosphate (IP$_3$) and diacylglycerol. IP$_3$ stimulates physiologic responses by the release of intracellular calcium. Diacylglycerol activates protein kinase C, which phosphorylates other proteins that are also involved in physiologic responses. Binding to α 2-adrenergic receptors causes the release of G I alpha, which inhibits adenylyl cyclase and reduces the formation of cAMP.[30] Vasomotor responses to sympathetic

stimulation cause increased resistance to blood flow mediated by stimulation of $\alpha$ -adrenergic receptors. However, increased sympathetic activity can also induce vasodilation by three different mechanisms: increased local tissue metabolism, stimulation of vascular $\beta$-adrenergic receptors and by the activity of specific postglanglionic sympathetic cholinergic fibers.[24]

Postganglionic sympathetic fibers innervate smooth muscle cells in large and small arteries and veins. The vasomotor changes elicited by sympathetic stimulation are mainly due to NE release, however, other neurotransmitters and neuromodulators are also released. Neuropeptide Y (NPY), a potent vasoconstrictor which acts on vascular smooth muscle, is present in storage vesicles of sympathetic nerves and is released in conjunction with NE.[24]

The major effects of the parasympathetic nervous system on the heart are bradycardia and a modest negative inotropic effect.[20] The two types of cholinergic receptors are the nicotinic receptors at the autonomic ganglia and the muscarinic receptor at the effector tissues. Acethylcholine is the neurotransmitter involved. The effects of the parasympathetic nervous system in general is the opposite effect to adrenergic stimulation.[20] Increased parasympathetic stimulation also releases kallikrein, which causes the release of bradykinin, a potent vasodilatory substance.[24]

3. *Baroreflexes:*

The vasomotor center in the brainstem receives afferent impulses from the baroreceptor in the walls of the aortic arch and at the origin of the internal carotid artery. These baroreceptors are considered high pressure receptors since they monitor the arterial side of the circulation. They are stretch receptors that respond to distension rather than to pressure, adjusting both vagal tone and sympathetic outflow against the level of receptor input. The baroreceptors send inhibitory impulses to the vasomotor center of the medulla oblongata via the vagus and glossopharyngeal

nerves. In response to an increase in blood pressure, there is an increased firing of the baroreceptor impulses, with inhibition of sympathetic outflow and increased vagal tone. These changes are responsible for a decrease in heart rate, contractility, cardiac output, and a decrease in peripheral vascular resistance. In the opposite situation, with a decrease in blood pressure, decreased distension of the baroreceptor results in a decreased frequency of discharge with decreased signals traveling to the vasomotor center, with an increase in sympathetic outflow and inhibition of vagal tone. This reflex is mediated by β-adrenergic stimulation, with increase heart rate and contractility, increasing cardiac output. The α-mediated increase in peripheral vascular resistance also helps to elevate blood pressure.[20]

In the venous side of the circulation, changes in distension volumes are sensed by low pressure stretch receptors (cardiopulmonary receptors) located in the atria, pulmonary arteries and ventricular endocardium. These receptors respond to changes in filling volumes on the venous circulation. An increase in blood volume sends signals to the brain that decrease sympathetic outflow and decrease the release of renin, decreasing peripheral vascular resistance and lessening the increase in blood pressure.[20]

Another reflex involved in the regulation of heart rate and blood pressure is the Bainbridge reflex. This reflex is mediated by stretch receptors in the atria and causes an increase in heart rate in response to an increase in atrial pressure.[20] Thus, increases in cardiac output associated with increased venous return are mediated not only by increased ventricular contraction by the Starling mechanism, but also due to increases in heart rate secondary to the Bainbridge reflex.[20] The Bainbridge reflex can lead to a less than expected decrease in heart rate during volume infusion since atrial distension is going to produce an increase in heart rate mediated by this reflex.[20]

The kidneys also respond indirectly to the baroreceptors and low pressure receptors. Decreased sympathetic outflow, such as when there is increased baroreceptor reflex activation due to an elevation in blood pressure, decreases renin release from the kidneys, decreasing the angiotensin mediated-vasoconstriction. Increased sympathetic outflow in response to hypotension increases renin production with subsequent increased production of angiotensin II (Ang II) causing increased peripheral vascular resistance and restoring blood pressure to the normal range.[20] Activation of the low pressure receptors in the venous circulation secondary to increased blood volume decreases sympathetic outflow and renin release from the kidneys, thus decreasing peripheral vascular resistance and lowering blood pressure.[20]

B. *Neurohumoral control of blood pressure*

1. *Renin-angiotensin system:*

The renin-angiotensin system plays a central role in the regulation of blood pressure, fluid and electrolyte balance and blood volume control. Renin is released from the juxtaglomerular apparatus in response to three major stimuli: increased β-1 adrenergic stimulation, decreased renal artery pressure, and decreased tubular reabsorption of sodium. Renin catalyzes the conversion of angiotensinogen to angiotensin I , which is then converted to Ang II by angiotensin-converting enzyme (ACE). Angiotensin II is a potent vasoconstrictor but also has other important effects. It stimulates the release of aldosterone from the adrenal cortex which increases sodium reabsorption in the kidney and decreases the release of renin. Angiotensin II stimulates constriction of the efferent renal arterioles, increasing intraglomerular pressure, thereby preserving renal filtration during hypotension. It also acts on the proximal tubules stimulating the sodium/hydrogen ion exchanger promoting sodium reabsorption and stimulates the thirst center in the hypothalamus resulting in increased water intake. Angiotensin II also has

an indirect permissive adrenergic effect, stimulating the sympathetic nervous system at several levels. It acts on the brainstem to promote central adrenergic activation, the autonomic ganglia to facilitate neurotransmission, and the presynaptic Ang II receptors of sympathetic nerve terminal neurons to stimulate the release of norepinephrine and to lessen its reuptake. In addition, Ang II acts on the endothelium to promote the release of endothelin.[20] Angiotensin II also resets the baroreceptors, lessening the anticipated bradycardia that occurs in response to a blood pressure increase.[31] Locally produced renin-angiotensin (heart and blood vessel walls) is also important, causing vasoconstriction and stimulating growth in myocardial tissue.[20]

2.      *Other humoral factors:*

Antidiuretic hormone (ADH) secretion from the posterior pituitary gland is stimulated by increased osmolality through osmoreceptors located in the anterior hypothalamus. Decreased blood pressure or blood volume activates baroreceptors and volume receptors which then stimulate ADH secretion. It increases the reabsorption of water by the kidneys and is a potent vasoconstrictor, producing an elevation in blood pressure.[20]

Atrial natriuretic peptide (ANP) is released from the atria in response to atrial distension. It promotes vasodilation via the cyclic guanosine monophosphate (cGMP) system and has also diuretic effects by both a direct renal effect and by inhibition of aldosterone secretion.[20]

C. *Control of peripheral vascular resistance*

Peripheral vascular resistance (PVR) is determined by the diameter of the arterioles and it can be defined as the inverse of the fourth power of the radius.[20] Peripheral vascular resistance is mediated by a combination of vasoconstrictive and vasodilatory systems. Norepinephrine released in response to adrenergic stimulation stimulates the $\alpha$-1 and $\alpha$-2 adrenoreceptors present in the walls of the arterioles and the myocardium producing vasoconstriction and increased

PVR.[27] Angiotensin II not only affects PVR directly by its potent vasoconstrictor effects but also indirectly by stimulation of the sympathetic nervous system.[20] Endothelin is another vasoconstrictory peptide released from damaged endothelium. Norepinephrine, Ang II and endothelin act through the formation of inositol-1,4,5-triphosphate (IP3).[20] IP3 leads to vasoconstriction by causing calcium ion release from intracellular stores.[30]

Vasodilation is mediated by inhibition of vascular contraction by cyclic adenosine-3',5'-monophosphate (cAMP) and cyclic guanosine-3'5'-monophosphate (cGMP). Stimulation of β-2 and to a lesser extent β-1 adrenoreceptors by epinephrine produces an increase in cAMP leading to vasodilation.[20] Cyclic guanosine-3'5'-monophosphate increases in response to stimulation of the nitric oxide (NO) messenger system which also causes vasodilation. Other vasodilators include prostacyclin (PGI2) and endothelium-derived hyperpolarizing factor. A wide range of other vasoactive substances act through endothelium-derived factors, including acethylcholine, bradykinin, arachidonic acid, histamine, 5-hydroxytryptamine (serotonin), substance P and vasopressin.[20]

II. *The role of glucocorticoids in blood pressure regulation*

*Introduction:*

Glucocorticoids are essential to the maintenance of blood pressure and in excess cause hypertension. The hypertension is considered to be salt-independent and of rapid onset and can be inhibited by glucocorticoid antagonists. Glucocorticoid antagonists blunt vascular reactivity to vasopressors in normal rats and induce a fall in blood pressure in normal rats on a low salt diet. Thus, glucocorticoids appear to be involved in the regulation of blood pressure by enhancing vascular reactivity.[32] The exact mechanism of action by which glucocorticoids influence blood pressure remains unknown. Possible mechanisms include stimulation of the renin-angiotensin

system, increasing plasma volume by promoting water movement from the intracellular to the extracellular space, interfering with catecholamine metabolism, enhancing vascular reactivity to catecholamines, inhibiting vascular synthesis of vasodilator prostaglandins such as prostacyclin and nitric oxide, or by a direct effect at vascular receptors.[33]

*A. Glucocorticoids and their effect in the vasculature*

Glucocorticoid (GC) and mineralocorticoid (MC) receptors have been found in the aorta, mesenteric arteries and vascular smooth muscle in culture.[34] These cytosolic receptors have been found to translocate to the cell nucleus complexed with GC or MC, where they stimulate protein synthesis which enhance sodium or calcium transmembrane transport.[34] Subsequent changes in intracellular electrolyte concentration in vascular smooth muscle cells (VSMCs) are believed to be involved in alterations of vascular responsiveness.[34] Two types of such cytosolic corticosteroid receptors have been identified. Type I, or mineralocorticoid (MC) receptors, bind with greatest affinity to aldosterone, deoxycorticosterone or corticosterone. Type II, or glucocorticoid (GC) receptors, bind with greatest affinity to glucocorticoids such as dexamethasone.[35] Both GC and MC can potentially bind to and activate both types of receptors and enhance the vasoconstrictive effects of catecholamines and angiotensin II.[35,36] The enzyme 11 β-hydroxysteroid dehydrogenase (11 β-HSD) metabolizes intracellular cortisol to the less active cortisone, serving as a protective mechanism against the action of glucocorticoids at mineralocorticoid receptors. This is important because circulating levels of glucocorticoids are higher than mineralocorticoids.[36]

Results of experiments by Kornel have provided evidence of increased sodium influx in cultured vascular smooth muscle mediated by both GC and MC receptors and in calcium influx mediated by glucocorticoid receptors. These effects were prevented by inhibitors of protein

synthesis, actinomycin D, and cycloheximide, revealing that the effects are mediated by induction of protein synthesis.[34] This is consistent with the mode of action of glucocorticoids, which bind to cytosolic receptors which then translocate to the nucleus and initiate gene transcription, ultimately leading to increased protein synthesis.[37] Activation of the GC and MC receptors is believed to induce the synthesis of proteins involved in the transmembrane transport of sodium and calcium.[34] Changes in the intracellular concentration of sodium and calcium mediated by stimulation of GC and MC receptors in VSMCs are believed to alter their reactivity to other agents such as catecholamines.[34]

Although both GC and MC receptors are involved in circulatory homeostasis and blood pressure control, glucocorticoids but not mineralocorticoids have been found to modulate receptors for vasoactive peptides in vascular smooth muscle cells.[38] The primary effect of GC on the vasculature appears to be to increase vascular reactivity to pressor agents.[14] In adrenalectomized rats, replacement with dexamethasone restores pressor responses to Ang II to normal whereas replacement with aldosterone did not.[35] Administration of a glucocorticoid antagonist to rats eliminated the pressor effects seen with administration of NE and Ang II, but did not alter responsiveness to ADH.[33] On the other hand, administration of a glucocorticoid agonist to rats produced an increase in blood pressure and pressor responses to norepinephrine administration.[33] Cardiac output did not appear to be affected, suggesting that the increased pressor responses are most likely due to changes in peripheral vascular resistance.[33]

One mechanism by which GC may augment vascular tone is by increasing vasoconstrictor substances (NE, Ang II) receptor numbers or affinity. Studies performed to evaluate α-adrenergic receptor numbers and affinity have revealed conflicting results, but in general do not support the concept of increased receptor number or affinity.[35] In contrast, Angiotensin II

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receptors numbers have been found to be elevated during corticosteroid administration in rats, with no change in receptor binding affinity.[35] GC have also been found to increase the formation of angiotensinogen and increase ACE activity, thus it is possible that increased vasoconstriction could also be mediated by non-receptor mechanisms.[35]

Reduced synthesis of vasodilator substances by the endothelium such as prostaglandins, kallikrein-kinin system, and nitric oxide could also be involved in regulation of vascular tone by GC.[35] Production of inducible nitric oxide synthase is decreased by dexamethasone administration.[35] Production of other agents such as endothelin, a potent vasoconstrictor, by endothelial cells is also likely to be involved in regulation of vascular tone. Increased concentrations of endothelin have resulted from exposure of vessels or cultured VSMCs from rat and rabbit to dexamethasone or cortisol.[35]

The production of ANF which induces natriuresis, diuresis, and vasodilation has been found to be modulated by GC. The formation of ANF-induced cGMP in renal artery VSMCs from rats treated with dexamethasone has been found to be lower than in controls. This suggests that decreased levels of this vasodilatory substance may be responsible for the alterations in blood pressure associated with GC administration.[39] An increase in cGMP and ANF receptor numbers have been seen after treatment of pulmonary artery endothelial cells with dexamethasone. This effect appears to be mediated by increased transcription of ANF receptor genes.[40] It is possible that the differences in responses of cGMP depend on the tissue involved. Since ANF produces vasodilation, alterations in its concentration or activity appear to be a compensatory response to increases in systemic vascular tone and not a primary mechanism involved in GC-induced hypertension.[40]

Lastly, GC may enhance vascular tone by trophic effects causing hypertrophy or hyperplasia of VSMCs. However, this is not likely to be involved in the mechanism, since short term exposure to corticosteroids, before there has been sufficient time for vascular hypertrophy to occur, results in enhanced vasoconstrictor responses to administration of pressor agents.[35] Morever, GC inhibit growth of VSMCs in culture.[35]

In conclusion, it is believed that GC increase vascular tone by potentiation of vasoconstrictor substances such as NE and Ang II and by direct effects on VSMC's independent of vasoconstrictors. The exact mechanisms for these changes, however, remain under study.

B. *Proposed mechanisms of glucocorticoid-induced hypertension*

Possible mechanisms by which glucocorticoids may induce hypertension include sodium retention, stimulation of the renin-angiotensin system, increased plasma volume with increased cardiac output, changes in catecholamine metabolism, enhancement of vascular reactivity to endogenous vasopressors and or inhibition of vasodilatory substances such as prostaglandins, kallikrein-kinin system, NO and atrial natriuretic factor.[33]

Cortisol appears to be the principal hormone involved in hypertension related to increased ACTH secretion. Administration of cortisol or ACTH to normal men produced similar responses, suggesting that the effects of ACTH are mediated by cortisol secretion.[41] Although cortisol administration to normal humans is associated with antinatriuresis and expansion of the extracellular fluid and plasma volume, sodium retention is not believed to be the primary mechanism by which GC produce hypertension.[41] Synthetic GC, such as prednisolone, methylprednisolone, triamcinolone and dexamethasone are associated with an increase in blood pressure but no urinary sodium retention or increase in plasma volume.[42] Similarly, administration of a type I (MC) receptor antagonist did not eliminate the rise in BP.[43] Reduced

dietary sodium intake ameliorated but did not eliminate the increase in blood pressure suggesting that although the hypertension associated with GC administration may be affected by sodium intake, it is not the primary mechanism involved.[43] Activation of the renin-angiotensin system is unlikely to be involved since plasma renin concentration and Ang II have been shown to be decreased in normal humans after administration of ACTH or cortisol.[41] However, increase in reactivity to Ang II is possible since GC administration was found to up-regulate Ang II receptors in rats and increase ACE activity and angiotensinogen production.[35] Plasma renin activity was found to be markedly depressed in normal dogs after administration of ACTH suggesting decreased activity of the renin-angiotensin-aldosterone system.[44,45] The contribution of these factors to hypertension in dogs is unclear, since cortisol administration was found to decrease blood pressure.[45] The reason for this discrepancy is not known, but it is possible that differences in sodium intake or duration of the ACTH infusion were responsible for the different findings.

Another possible explanation for the rise in blood pressure in response to cortisol administration is an increase in cardiac output due to volume expansion with a subsequent increase in stroke volume. The increase in cardiac output is a very reproducible finding in cortisol-treated subjects, but does not appear to be essential for the rise in blood pressure.[42] In studies in normal men administered hydrocortisone, a rise in systolic pressure, increased cardiac output and evidence of MC effects (hypernatremia, hypokalemia and plasma volume expansion) were seen.[46] Pretreatment with atenolol (β 1-adrenergic blocker) prevented the increase in cardiac output but not the elevation in arterial pressure or the increase in plasma volume. The rise in pressure in the atenolol-treated group was associated with a rise in total peripheral resistance consistent with vasoconstriction.[46] Further evidence that the rise in cardiac output is

not essential for the development of hypertension comes from studies that demonstrate an increase in total peripheral vascular resistance without an increase in plasma volume or cardiac output after dexamethasone administration.[19]

Increased cardiac output is also associated with an increased in heart rate or increased contractility as seen with stimulation of β 1-adrenergic receptors. An increase in cardiac output by these mechanisms could also lead to increased blood pressure. These mechanisms are not likely to be involved in glucocorticoid-induced hypertension since pre-treatment with atenolol did not eliminate the hypertensive effects of hydrocortisone when administered to normal human subjects.[46]

The role of vascular resistance in the development of cortisol-induced hypertension was examined by administration of the calcium channel blocker felodipine. In normal humans, felodipine administration is associated with decreased peripheral vascular resistance due to vasodilation. No difference was seen in the blood pressure rise after cortisol administration between the felodipine and placebo treated groups.[42] Since cortisol administration increased blood pressure even in the absence of an increase in peripheral vascular resistance mediated by increased intracellular calcium, other mechanisms must be involved. Cortisol may produce an increase in cardiac output by a centrally mediated increase in sympathetic activity (increasing heart rate or stroke volume). However, if this rise in cardiac output is prevented, by for example, administration of a calcium channel blocker, then the central signal could be translated into an increase in vascular resistance.[43] The possibility that centrally-mediated effects play a role in hypertension is supported by studies in dogs with deoxycorticosterone (DOC)-induced hypertension.[47] In these dogs, DOC administration was associated with an increased cardiac output but no changes in heart rate or total peripheral resistance. Although a decline in plasma

Ang II was seen, no change in the concentration of Ang II was found in the cerebrospinal fluid (CSF) despite an increase in CSF sodium.[47] In the DOC treated dogs, the normal responses associated with the carotid occlusion reflex (increase in heart rate and cardiac output) were absent.[47] The carotid occlusion reflex is initiated by a decrease in blood pressure in the carotid sinus resulting in loss of the normal reflex inhibition of the cardioinhibitory center from the carotid baroreceptors. The lack of inhibition leads to an increase in sympathetic drive from the hypothalamic-medullary-spinal control centers to the resistance vessels leading to an increase in blood pressure and heart rate. The lack of stimulation from the baroreceptors also produces decreased vagal stimulation to the heart.[48] The absence of these normal responses in DOC-treated dogs is consistent with an alteration of the normal central responses to the baroreceptor reflex mechanism, possibly by imposing an abnormal inhibitory effect on the central control of blood pressure.[47] The lack of suppression of Ang II in the CSF and altered cardiac reflexes support the possibility of centrally mediated changes being responsible for the hypertension associated with DOC administration. Angiotensin II is believed to have actions in the brain on the baroreflex control of heart rate by resetting the threshold of the reflex in response to elevations in blood pressure to a higher pressure.[47,49] Similar studies are needed with the administration of GC to evaluate the possibility that similar mechanisms are involved in the GC-induced hypertension.

Increased sympathetic nervous system activity does not occur in response to cortisol administration. No significant differences in the concentrations of epinephrine or norepinephrine were found in normal humans treated with hydrocortisone.[14,41] In addition, plasma neuropeptide Y (NPY) concentration, a sympathetic co-transmitter, is not elevated in normal humans after cortisol administration.[50]

Increased pressor responsiveness to vasoactive substances is believed to be a major factor in GC-induced hypertension. Increased pressor sensitivity to infusions of NE and Ang II has been seen in normal humans after treatment with dexamethasone[19] and cortisol.[14] Administration of oral hydrocortisone to normal humans produced a rise in blood pressure associated with an increase in cardiac output, decreased total peripheral vascular resistance, increased vascular responses with cold pressor stimulation (forearm blood flow measured after application of ice to the base of the neck of the ipsilateral side for 60 seconds), and increased forearm vascular responsiveness to reflexly induced constrictor effects and exogenous norepinephrine. The increases in pressor responsiveness were not found to be related to changes in plasma norepinephrine levels.[14] Administration of dexamethasone was associated with increases in blood pressure due to increased peripheral vascular resistance without associated changes in cardiac output.[19] The different responses in cardiac output and total peripheral vascular resistance obtained with hydrocortisone and dexamethasone are probably due to MC effects of the former. As discussed before, centrally mediated changes altering cardiovascular reflexes are possible since the increase in cardiac output or changes in total peripheral resistance are not always associated with the elevations in blood pressure.

Depression of vasodilatory substances has also been implicated in the pathophysiology of GC-induced hypertension. Glucocorticoids downregulate the expression of the inducible form of NO synthase within vascular smooth muscle cells, attenuate guanylate cyclase activity induced by ANP, and reduce the biosynthesis of prostacyclin. By suppressing vasodilatory processes that serve to attenuate other vasoconstrictive hormones, hypertension may occur.[36] Glucocorticoids decrease prostaglandin production by inhibition of phospholipase A2 activity.[33] Decreased production of prostacyclin ($PGI_2$), a vasodilator, could be responsible for the GC-induced

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hypertension. However, increased $PGF_1$ has been found in rats pretreated with a glucocorticoid antagonist even in the presence of increased pressor responses to norepinephrine infusions.[33] Since prostaglandins produce vasodilation, increased levels would be associated with lowering of blood pressure instead of hypertension. Moreover, indomethacin, a prostaglandin inhibitor, did not modify pressor responses to phenylephrine or Ang II in cortisol treated subjects.[15] Although prostaglandin production is believed to be already suppressed by cortisol administration, larger increases in blood pressure would have been expected in the cortisol treated subjects due to further prostaglandin inhibition. Thus, decreased prostaglandin production does not appear to be responsible for GC-induced hypertension.

There is evidence that suppression of the NO system is involved in GC-induced hypertension. Nitric oxide is a physiologic vasodilator produced by endothelial cells. Pharmacologic inhibition of NO synthase is associated with an increase in blood pressure.[51] Oxidation products of NO, which are indicative of NO production, have been found to be decreased in rats treated with dexamethasone.[51] Dexamethasone was also shown to decrease NOS mRNA levels in the aorta and other tissues.[51] Thus, decrease production of NO is likely to be involved in the development of GC-induced hypertension.

Although dexamethasone has been found to suppress ANF mediated responses in cultured VSMCs,[39] ANF levels actually have been found to be increased in human subjects treated with cortisol[41] despite elevations in blood pressure. Thus, the elevation in ANF is probably compensatory and does not play a role in GC-induced hypertension.

A last possible mechanism for GC-induced hypertension is by direct effects of glucocorticoids on the vasculature. Chronic treatment of VSMCs with GC results in elevated sodium and calcium influx.[36] Glucocorticoids appear to downregulate the expression of the

sodium/calcium exchanger in VSMC.[36] This exchanger is particularly important in regulating the cytosolic concentration of calcium ions. Its inhibition promotes vasoconstriction.[14] The expression of calcium dependent potassium channels is also downregulated by GC administration. These channels allow for potassium efflux from the cells, resulting in vasodilation.[36]

In summary, GC-induced hypertension appears to be independent of sodium retention or volume expansion. Although it is associated with an increase in cardiac output, this is not essential for the development of hypertension. Increased vascular responsiveness to vasopressors in the absence of changes in the plasma levels of catecholamines has been demonstrated in multiple studies and is believed to be a major factor. Depression of vasodilatory substances such as NO is likely contributory to development of hypertension. Finally, direct effects of GC at the VSMCs, producing changes in intracellular electrolyte composition leading to changes in vascular tone are possible.

III. *Hypertension in hyperadrenocorticism*

*Introduction*

Hyperadrenocorticism refers to the effects of chronic excesses of systemic cortisol. The excess cortisol can be caused by a pituitary tumor or pituitary hyperplasia secreting excessive levels of ACTH resulting in adrenocortical hyperplasia, functional adrenocortical adenomas or carcinomas, or from exogenous glucocorticoid administration. Other causes reported in humans but not in dogs include adrenocortical hyperplasia from a hypothalamic disorder producing excessive corticotropin-releasing hormone and ectopic ACTH production by non-pituitary neoplasia.[1]

The term Cushing's disease is reserved for those cases of hypercortisolism due to inappropriate secretion of ACTH by the pituitary gland. The term Cushing's syndrome refers to the clinical signs and clinicopathologic abnormalities caused by chronic exposure to elevated glucocorticoid levels. HAC is a common condition in dogs, usually affecting middle age to older dogs.[1]

The adrenal gland cortex has three distinct zones, the zona glomerulosa, fasciculata and reticularis. The outermost layer produces aldosterone under regulation of the renin-angiotensin system and potassium. This zona lacks the enxyme 17-α hydroxylase, thus can not produce 1-α hydroxyprogesterone and 17-α hydroxypregnenolone which are the precursors of cortisol and the adrenal androgens. The zona fasciculata and reticularis produce glucocorticoids under ACTH regulation. They cannot convert DOC to aldosterone since the cells lack the necessary gene. These zones also produce DOC, 18-hydroxydeoxycorticosterone and corticosterone. Dehydroepiandrosterone (DHEA) and androstenedione constitute the adrenal androgens and are produced both by the zona fasciculata and reticularis. Cholesterol serves as the precursor for the synthesis of all adrenal steroids.[52] The conversion of cholesterol to pregnenolone is the rate limiting step in adrenal steroidogenesis and the major site of ACTH action on the adrenal gland.[52] Once in the circulation, cortisol is bound to corticosteroid-binding globulin (CBG) and albumin. The liver is the major site of steroid catabolism and conjugation, while the majority of steroid hormones are excreted by the kidneys.[52] Cortisol is also inactivated in the kidney by the action of 11 β-hydroxysteroid dehydrogenase. This mechanism is particularly important since it prevents occupation of the mineralocorticoid receptors by cortisol.[52] Cortisol exerts a negative feedback both at the pituitary and hypothalamic levels by inhibiting the secretion of CRH and ACTH.[53]

*A. Pathophysiology of hyperadrenocorticism in dogs*

Pituitary dependent hyperadrenocortism (PDH) is the most common form of HAC in dogs, accounting for 85% of the cases. Pituitary dependent hyperadrenocorticism usually results from a tumor of the pars intermedia or pars distalis, but hyperplasia may also be found. It may also result from a primary central nervous system abnormality such as excessive stimulation by CRH or neurotransmitters such as serotonin, norepinephrine and epinephrine. Increased levels of ACTH cause adrenal gland hyperplasia with excessive cortisol production. Adrenal tumors constitute 10 to 15% of cases of HAC in dogs. They may be benign adenomas or malignant adenocarcinomas and are usually unilateral. Adrenal tumors secrete cortisol autonomously and are not under regulation of ACTH.[53]

*B. Hypertension in hyperadrenocorticism*

The reported prevalence of hypertension in human patients with PDH is 70 to 90%.[4,54,,55] The reported prevalence in canine hyperadrenocorticism is over 50%,[1,2] however, the most extensive study of hypertension in canine HAC found a prevalence of 86%.[3] Hypertension was found to be present in 64% of humans with PDH and in 70% of those with adrenal adenomas and bilateral nodular hyperplasia.[55] In the same study, all patients with adrenal carcinomas or ectopic ACTH production were found to be hypertensive.[55] A lower prevalence (20%) has been found associated with exogenous glucocorticoid administration.[54] In dogs, systemic hypertension was found to be most prevalent, and blood pressure was the highest in dogs with untreated adrenocortical tumors. However, the differences were not significant compared to dogs with untreated PDH or inadequately controlled PDH.[3] The mean systolic, diastolic, and mean arterial blood pressure in dogs with HAC have been found to be 162 mmHg, 116 mmHg and 135 mmHg, respectively.[3]

The hypertension associated with HAC is usually mild, but can be severe. In man, it is characterized by alterations in the blood pressure circadian rhythm, with loss of the normal nocturnal decrease.[54] Although in most instances the hypertension resolves after resolution or adequate control of HAC, systolic and diastolic hypertension persisted in one third and one fourth of human patients, respectively.[54] In the canine study, all dogs with adrenocortical tumors had complete resolution of hypertension after surgery. The prevalence of hypertension in dogs with "well controlled" PDH was 40%.[3]

Hypertension is associated with life threatening complications in both human and canine patients. Cardiovascular complications such as myocardial infarction, congestive heart failure and stroke account for a high proportion of death in humans with untreated HAC. Clinical evidence of end-organ damage, including retinopathies, cardiomegaly, and myocardial ischemia and left ventricular hypertrophy are also common.[54] Hypertension can also lead to glomerulopathies causing protein loss, which in turn can predispose to other problems such as thromboembolism.[3] Thromboembolic disease is associated with HAC even in the absence of hypertension.[11-13]

Proposed mechanisms involved in the development of hypertension in HAC include elevation in the renin substrate, activation of the renin-angiotensin system, enhanced vascular sensitivity to endogenous vasopressors, glucocorticoids acting as mineralocorticoids, direct vascular effects, and reduced activity of the depressor system such as the prostaglandins, NO, and kallikrein-kinin system.[4,54,55] Increased pressor sensitivity to endogenous vasopressors appears to be the major cause for glucocorticoid induced hypertension; however, the exact mechanism remains to be elucidated.[5,6,15-19]

*C. Studies on the pathogenesis of hypertension in human spontaneous*

*hyperadrenocorticism*

The hypertension in humans with HAC appears to be independent of sodium status and activation of the RAS. Studies of humans with both pituitary dependent and adrenal dependent HAC have found no evidence of mineralocorticoid excess or sodium retention. Serum potassium, sodium and aldosterone concentrations have been found to be normal.[5,16-18] Evaluation of DOC and corticosterone have shown conflicting results. In a study of humans with pituitary adenoma, DOC and corticosterone concentrations were found to be significantly elevated when compared to control subjects[16,17] whereas they were found to be normal in a group with adrenocortical adenomas.[18] While the reason for this discrepancy is not know, it is possible that the mechanism of hypertension might be different between different etiologies of HAC. DOC has significant MC activity whereas corticosterone has relative little MC activity in humans.[56] Despite the elevated DOC and corticosterone concentrations, total exchangeable sodium and serum potassium concentrations were normal and no correlation was found between blood pressure and total exchangeable sodium.[16,17] Results of plasma renin measurements are also conflicting. In one study of adrenal dependent HAC, plasma renin concentration was found to be lower and plasma renin substrate was higher than in controls.[5] In another study, plasma renin activity (PRA) was found to be within the normal range.[18] If the increased plasma renin substrate level was the cause for the hypertension, acting through the renin-angiotensin system, plasma renin activity and plasma aldosterone concentration would be expected to be increased.[18] Studies in dogs with HAC have shown both decreased[57] and increased plasma aldosterone concentrations.[58]

Oral administration of captopril (ACE inhibitor) was found to reduce the blood pressure in humans with adrenal adenoma despite apparently normal plasma renin activity. However, an

infusion of an angiotensin II analog during norepinephrine infusions did not cause a change in the pressor responses in these subjects. The fact that an Ang II analog did not produce a change in the pressor response supports the concept that changes in Ang II concentrations are not primarily involved in the pathogenesis of hypertension.[5] However, administration of captopril did cause a significant reduction in blood pressure in these patients.[5] Increased pressor responses to exogenous Ang II was also found in another group of humans with adrenal adenoma.[18] One possible explanation for this discrepancy is that captopril can also cause attenuation of the kallikrein-kinin system and prostaglandins in patients with normal renin.[5] However, the captopril-induced hypotensive effects were preserved even with pretreatment with indomethacin, a prostaglandin synthesis inhibitor.[18] One possible explanation of this phenomenon is that angiotensin II modulates norepinephrine release from the sympathetic nervous system or augments the action of norepinephrine. Thus blocking of angiotensin II formation is able to induce a fall in blood pressure.[5]

There is no evidence that cortisol acting as a mineralocorticoid is involved in hypertension associated with HAC. Increased intrarenal cortisol concentration can exert a potent mineralocorticoid effect in the presence of abnormal 11β-hydroxysteroid dehydrogenase (11 β-HSD) as in the syndrome of apparent MC excess. Cortisol can act upon MC receptors in the renal vasculature when in elevated concentrations. 11 β-hydroxysteroid dehydrogenase converts cortisol to the less active form, cortisone, which has decreased affinity for MC receptors. When 11 β-HSD activity is abnormal, more cortisol is present at the MC receptors, producing changes consistent with excessive MC production.[59] The syndrome of apparent MC excess is characterized by increased ratio of urinary tetrahydrocortisol (THF) to tetrahydrocortisone (THE). No significant difference was found between hypertensive and normotensive humans

with HAC in regards to THF/THE ratio and THF excretion. No correlation was found with blood pressure, indicating that it is probably not a mechanism involved in the hypertension of HAC.[59]

Increased vascular reactivity to catecholamines is currently believed to be the major factor contributing to the hypertension in humans with HAC. Increased pressor responsiveness to infusions of NE and Ang II have been found to be present in patients with both pituitary and adrenal dependent HAC.[5,6,16-18] This is not caused by increased catecholamine concentrations or receptor number or activity since plasma and urinary concentrations of catecholamines as well as α-2 adrenoreceptor density and affinity, and β-2 adrenoreceptor density have been found to be normal. β-adrenoreceptor affinity, however, was found to be decreased.[16,17] Increased chronotropic cardiac sensitivity was also found in this group of humans. Increased cardiac sensitivity to both the chronotropic and the inotropic effects of catecholamines, resulting in an increased cardiac output, may be one of the factors involved in the hypertension of HAC, which appears to be characterized by increases in both cardiac output and total peripheral resistance. It is possible that the changes produced by GC may be mediated by actions at post receptor sites in VSMCs to modulate the effects of catecholamines.[17] Another explanation for the exaggeration of pressor responses in patients with HAC is the direct effect of cortisol on the VSMCs that could potentiate the effects of angiotensin II.[18]

Although the importance of increased pressor responsiveness is generally accepted, some studies have shown contradictory results. For example, increased pressor sensitivity to the α-adrenergic agonist phenylephrine was not found in a group of ten humans with HAC of various etiologies. One possible explanation for this difference is that pressor sensitivity differs between specific causes of HAC.[7]

Neuropeptide Y, released in response to sympathetic stimulation, could be involved in the enhanced sensitivity to norepinephrine because it induces direct vasoconstriction and potentiates the action of norepinephrine. Plasma levels of NPY were found to have no correlation with mean blood pressure in humans with HAC, so it is unlikely to contribute to hypertension in these patients.[60]

Plasma atrial natriuretic peptide concentration has been found to be elevated in humans with HAC[61] but it does not appear to be directly related to hypertension seen in HAC since no significant correlation has been found between plasma ANF concentration and PRA, plasma aldosterone concentration or mean blood pressure.[61,62] The elevations seen are more likely part of a compensatory mechanism since elevations of ANF are associated with natriuresis, diuresis, and vasodilation.[39]

Reduced activity of the depressor systems such as the prostaglandin, NO, and kallikrein-kinin system has also been implicated in the hypertension of HAC. In a study of 12 humans with HAC due to adrenal adenoma, urinary kallikrein and prostaglandin $E_2$ (PG $E_2$) were found to be markedly decreased when compared to normal subjects, suggesting that a reduction in PG E2 and kallikrein production contributes to high blood pressure in patients with HAC.[5]

In summary, current literature mostly agrees that the hypertension in human HAC is not sodium-dependent or mediated by changes in ANG II, but instead is related to increased vascular reactivity to vasoactive substances and suppression of the depressor systems. The exact mechanism for the increased sensitivity to pressor agents has not been clearly defined. No studies have been performed in canine HAC to evaluate the possible mechanisms leading to hypertension.

In the following study, we hypothesized that increased pressor sensitivity to catecholamines is present in canine HAC. Pressor sensitivity to norepinephrine was evaluated in dogs after induction of iatrogenic HAC (I-HAC) by serial arterial blood pressure measurements during infusions of increasing dose rates of norepinephrine. Other factors such as the activity of the depressor systems and the exact mechanism for the increased pressor sensitivity were not evaluated in this study. This was a first step in the evaluation of the pathophysiology for the development of hypertension in canine HAC; further studies to evaluate other factors should follow.

## Chapter II

Evaluation of pressor sensitivity to norepinephrine infusion in dogs with iatrogenic

hyperadrenocorticism

A. Abstract

Objective**:** To evaluate pressor sensitivity to catecholamines in dogs after induction of iatrogenic

hyperadrenocorticism (I-HAC) by serial arterial blood pressure measurements during infusions of

increasing dose rates of norepinephrine.

Animals**:** Eight dogs with I-HAC induced by administration of oral hydrocortisone at a mean dose

of 3.3 mg/kg PO TID for 42-49 days and 8 control dogs which received empty gelatin capsules PO

TID for 42-49 days.

Procedure**:** Systolic, diastolic, mean blood pressure and heart rate measurements were recorded

after sequential administration of increasing dose rates of norepinephrine (0.1, 0.15, 0.2, 0.3, 0.4,

0.6 and 0.8 µg/kg/min) for 10 minutes. The changes in systolic, diastolic, mean blood pressure and

heart rate were compared between control dogs and dogs with I-HAC.

Results**:** Dogs in the I-HAC group had a more pronounced pressor response to norepinephrine

infusions than control dogs. The infusions were not completed in 7 of the 8 dogs in the I-HAC

group versus 3 dogs in the control group due to severe elevations in systolic blood pressure. The

mean change in systolic blood pressure was consistently higher in dogs in the I-HAC group. The

difference was statistically significant at the 0.2 µg/kg/min norepinephrine dose rate. The mean

change in heart rate was consistently lower in the I-HAC group, a difference that was significant at the 0.2 µg/kg/min norepinephrine dose rate.

Conclusions and Clinical Relevance: Increased pressor sensitivity to norepinephrine was seen in dogs with I-HAC. Based on the blood pressure changes, the mechanism appeared to be related to an increase in cardiac output and not due to increased peripheral vascular resistance.

B. Introduction

Hypertension has been demonstrated in over 50% of cases of canine hyperadrenocorticism (HAC).[1,2] In the most extensive study performed in dogs, a prevalence of 85% was reported,[3] similar to that of humans.[4-8] In one study, up to 40 % of dogs remained hypertensive even after successful treatment of HAC.[3] Arterial hypertension and its sequelae are a major cause of short and long term morbidity and mortality in human patients, often leading to lethal complications if left untreated.[8] Similarly, hypertension in dogs has been associated with development of ophthalmic changes such as blindness from intraocular hemorrhage and retinal detachment, glomerulosclerosis, chronic renal failure, left ventricular hypertrophy and congestive heart failure.[2,9,10] Hypertension may also predispose dogs to development of thromboembolism, which is a documented complication of canine HAC.[11-13]

The mechanisms involved in the development of hypertension in naturally occurring (HAC) are not completely understood. The association between exogenous or synthetic glucocorticoids and hypertension has been recognized for many years.[14,15] Proposed mechanisms for the development of hypertension in hyperadrenocorticism include sodium retention, activation of the renin-angiotensin system and increased vascular reactivity to endogenous vasopressors.[16,17] Currently, increased vascular responsiveness to endogenous vasopressors is believed to be the

major cause for glucocorticoid induced hypertension in humans.[5,6,15-19] The authors are unaware of studies of the pathogenesis of this condition in canine hyperadrenocorticism.

We hypothesized that canine HAC results in increased vascular responsiveness to catecholamines and that this increased vascular responsiveness is involved in the development of hypertension. The objective of this study was to evaluate pressor sensitivity to catecholamines in dogs after induction of iatrogenic hyperadrenocorticism (I-HAC) by serial arterial blood pressure measurements during infusions of increasing dose rates of norepinephrine.

C. Materials and Methods

*Animals:* Twenty young adult mixed breed dogs (exact age unknown), 12 intact females, 6 intact males, and 2 neutered males, with body weights ranging from 14.8-21.4 kg (mean 17.9 kg) were studied. Dogs were determined to be healthy based on physical examination, fundic examination, complete blood count, serum chemistry profile, urinalysis, urine culture, urine protein/creatinine ratio, heartworm antigen test, and zinc sulfate fecal flotations. Indirect systolic arterial blood pressure determinations by Doppler ultrasonography[a] were performed to rule hypertension. Adrenal gland function was evaluated prior to the initiation of the study by measurement of plasma cortisol concentration at a commercial laboratory[b] before and one hour after intravenous administration of 5 μg/kg cosyntropin[c]. An electrocardiogram for evaluation of cardiac rhythm and echocardiogram that included Doppler studies of valvular function were performed prior to initiation of the study to rule out pre-existing cardiac dysfunction that could affect the responses to norepinephrine infusions.

*Procedures:* The dogs were acclimated for 2 weeks prior to the initiation of the study. During this time, dogs were restrained in lateral recumbency for 10 minutes five days per week to mimic restraint required during the study. During the remainder of the study, dogs were restrained in

lateral recumbency for 10 minutes twice per week. The dogs were then randomly assigned to two groups of 10 dogs. Dogs in I-HAC group were administered oral hydrocortisone[d] at a mean dose of 3.3 mg/kg (range 3.0 to 3.5 mg/kg) PO every 8 hours for 42-49 days for induction of iatrogenic hyperadrenocorticism. Dogs in the control group were administered a placebo (gelatin capsule) PO every 8 hours for 42-49 days. The investigators were not blinded to the treatment groups since all measurements were to be recorded objectively by a pressure transducer and heart rate monitor. Each group was further sub-divided into groups of five and allocated into two blocks with hydrocortisone treatment beginning one week apart. The groups were sub-divided to enable experiments to be carried out after 42 to 49 days of treatment since only two infusions could be carried out each day. All the dogs received the same maintenance diet and treats throughout the study period[e]. Body weight was monitored weekly and the amount of food given was adjusted for maintenance of body weight. The dogs were monitored daily for development of signs suggestive of HAC (polyuria, polydipsia, polyphagia, hair coat and skin changes, abdominal distention).

*Infusions*: At the end of the induction period (42-49 days), a 20 ga teflon venous catheter[f] was placed in the cephalic vein for infusion of norepinephrine. A 22 ga teflon catheter[f] was placed in the dorsal pedal artery for measurement of direct arterial blood pressure. The dogs were returned to the holding cages for 30 minutes after catheter placement for relaxation. The dogs were then restrained in lateral recumbency. A lead II electrocardiogram was recorded continuously during norepinephrine infusion to detect arrhythmias and to record heart rate. A transducer[g] for direct arterial blood pressure measurement was connected to the dorsal pedal artery catheter. Direct systolic, diastolic, and mean arterial pressure measurements[h] and heart rate[h] were recorded after 10 minutes of restraint before drug infusion and at the end of each 10 minute norepinephrine

infusion period. The measurements were taken at 3 points in time twenty seconds apart.

Norepinephrine bitartrate[i] was administered as a continuous rate intravenous infusion at 0.1

µg/kg/min, 0.15 µg/kg/min, 0.2 µg/kg/min, 0.3 µg/kg/min, 0.4 µg/kg/min, 0.6 µg/kg/min, and 0.8

µg/kg/min in 5% dextrose at a norepinephrine concentration of 0.02 mg/ml delivered by an

infusion pump.[j] Each dose was administered for 10 minutes sequentially, beginning with the

lowest dose. The infusion was stopped if systolic blood pressure reached 240 mmHg, diastolic

blood pressure reached 120 mmHg, or heart rate reached 180 beats/min for over one minute.

ACTH stimulation tests and serum alkaline phosphatase activity were performed at the end of the

study as previously described to confirm the presence of I-HAC.

*Statistical analysis*: The mean change in direct systolic, diastolic, mean blood pressure and heart

rate in response to the administration of increasing dose rates of norepinephrine were compared

between the two groups by a two-way analysis of variance (generalized randomized complete

block design). Indirect arterial blood pressure measurements by Doppler ultrasonography,

baseline direct systolic, diastolic, mean blood pressure and heart rate before initiation of

norepinephrine infusions, plasma cortisol concentrations before and after ACTH administration,

and serum alkaline phosphatase concentration at the end of the study were also compared by a

two-way analysis of variance. Differences with *p*-values <0.05 were considered significant. All

statistical analyses were performed with a commercial software program.[k] Data is presented as

mean +/- standard error except when noted otherwise. Dogs were cared for in accordance with

the Federal Animal Welfare Act and PHS policies. The study was approved by the Virginia Tech

Animal Care Committee.

Sixteen dogs completed the study, 8 each in the control group and the I-HAC group. There
were five intact females and three males per group, one of the males in each group being
neutered. The mean +/- SD body weight was 19 +/- 2.28 kg in the I-HAC group and 17.5 +/-
1.75 kg in the control group. One dog in the control group had a positive urine culture with
*Enterobacter faecalis*. It was treated with antimicrobial therapy
(trimethoprim/sulfamethoxazole)[1] for 2 weeks. Recheck urine culture was negative before
initiation of the induction phase of the study. Three dogs were excluded from the study because
of echocardiographic abnormalities (one dog had mitral valvular insufficiency, two dogs had
abnormal fractional shortening). Echocardiographic studies and electrocardiograms were normal
for the remaining 17 dogs. One dog was considered untrainable and was excluded from the
study. Indirect systolic arterial blood pressure measurements by Doppler ultrasonography were
considered normal for all the dogs included in the study (mean of 129 mmHg +/- 8 and 122
mmHg +/- 5 in I-HAC and control dogs, respectively). The differences were not significant. One
of the dogs (I-HAC group) had an indirect systolic arterial blood pressure of 170 mmHg upon
initial evaluation. This dog was very agitated and was difficult to restrain at the beginning of the
study. Its disposition improved as acclimation proceeded. This dog's indirect systolic arterial
blood pressure was re-evaluated prior to instituting treatment and it was normal (mean of 142
mmHg).

The dogs in the I-HAC group were observed to develop typical clinical signs of
hyperadrenocorticism including polyuria, polydipsia, distended abdomen, and thinning of the

ventral abdominal skin with prominent subcutaneous veins. One dog developed lesions consistent with mild superficial pyoderma (epidermal collarettes).

*ACTH stimulation test.* Prior to hydrocortisone administration, mean plasma cortisol concentrations before and after ACTH administration in the control dogs (35 +/- 6 and 327 +/- 27 nmol/L, respectively) and I-HAC (58 +/- 10 and 344 +/- 14 nmol/L, respectively) were normal. The difference between the two groups was not significant. ACTH stimulation test was repeated in both groups 36 hours after discontinuation of hydrocortisone and placebo administration. After induction of hyperadrenocorticism, there was a significant difference in mean plasma cortisol concentrations both before and after ACTH stimulation between control (36 +/- 8 and 366 +/- 37 nmol/L, respectively) and I-HAC dogs (1 +/- 0.3 and 9 +/- 3 nmol/L, respectively; $p= 0.003$ and $p< 0.001$).

Serum alkaline phosphatase was within the reference interval (14-105 U/L) in all control and I-HAC dogs before hydrocortisone treatment. Mean serum alkaline phosphatase was 44 +/- 7 U/L in the control group and 232 +/- 55 U/L in the I-HAC group after treatment, a difference that was significant ($p < 0.05$).

*Baseline direct systolic, diastolic, mean blood pressure and heart rate prior to initiation of norepinephrine infusions.* Mean baseline direct systolic blood pressure was 156 +/- 7 mmHg in the control group and 171 +/- 8 mmHg in the I-HAC group. Mean baseline direct diastolic blood pressure was 70 +/- 2 mmHg in the control dogs and 70 +/- 3 mmHg in the I-HAC. Mean baseline direct mean blood pressure was 92 +/- 5 mmHg in the control group and 93 +/- 4 mmHg in the I-HAC group. Mean baseline heart rate was 101 +/- 7 beats/min in the control group and 81 +/- 8 beats/min in the I-HAC group. The differences between controls and I-HAC dogs were not significant for any of the variables.

*Norepinephrine infusions*

 *Control dogs.* In three of the control dogs, the infusions were stopped because of severe hypertension during the 0.8 μg/kg/min infusion rate after 1 minute, 4 minutes and 30 seconds, and 6 minutes and 10 seconds of infusion. The systolic blood pressure was above 240 mmHg and the diastolic blood pressure was above 120 mmHg. The dogs also vocalized and acted agitated at that time. The infusions were completed in the remaining control dogs. Occasional ventricular premature complexes were seen after the 0.15 μg/kg/min infusion rate in two dogs and the 0.2 μg/kg/min infusion rate in one dog.

 *I-HAC group.* Infusions were stopped because of severe hypertension in 7 of the I-HAC group dogs during the 0.15 μg/kg/min infusion rate in one dog, 0.2 μg/kg/min infusion rate in two dogs, 0.3 μg/kg/min infusion rate in one dog, 0.6 μg/kg/min infusion rate in one dog, and after 5 minutes and 37 seconds, and 9 minutes and 50 seconds during the 0.8 μg/kg/min infusion rate in the two remaining dogs. In only one dog in the I-HAC group was infusion completed. The dogs had an increased respiratory rate, vocalized and acted agitated when the direct systolic arterial blood pressure was above 240 mmHg and the diastolic arterial blood pressure was above 120 mmHg. The infusions were discontinued as soon as blood pressure elevations over the pre-establish limits remained for over 3 minutes. Blood pressure normalized within two minutes after discontinuation of the infusions. One dog in the I-HAC group developed occasional premature ventricular depolarizations after administration of the 0.15 μg/kg/min dose rate.

 The mean change in systolic blood pressure in response to norepinephrine was consistently higher in the I-HAC group versus the control group (Figure 1). The difference became

statistically significant at 0.2 ug/kg/min ($p = 0.0267$), but not at higher dose rates. No significant

difference was observed in diastolic or mean blood pressure at any dose rate of norepinephrine

when the mean change was compared between the two groups (Figures 2 and 3). The change in

heart rate during norepinephrine infusion was lower in the I-HAC group when compared to the

control group, with the difference being significant ($p = 0.0110$) at a norepinephrine dose rate of

0.2 μg/kg/min. (Figure 4)

E. Discussion

Age, breed, sex, temperament, disease state, exercise routine and diet have been reported to

produce variations in blood pressure in dogs.[63] We attempted to minimize these factors by

including dogs within a limited age range (young adults) and size range and by providing the

same diet and same amount of exercise to the dogs in both groups. All the dogs that completed

the study were well acclimated and required minimal restraint during the infusions. Thus, any

difference in the response seen in the two groups should be attributed to the effects of

hydrocortisone administration in the I-HAC group or individual dog variation. Results of ACTH

stimulation tests, elevation of serum alkaline phosphatase and development of clinical signs

consistent with hyperadrenocorticism support successful creation of I-HAC. Previous studies of

the effects of long term corticosteroid administration in dogs have used prednisone for 42 days[64]

or cortisone acetate for 30 days.[65] We selected hydrocortisone in an attempt to mimic as closely

as possible naturally occurring canine hyperadrenocorticism. In humans, 95% of the

glucocorticoid activity of adrenocortical secretions results from the secretion of cortisol

(hydrocortisone).[66] Canine hyperadrenocorticism is associated with excessive cortisol secretion

from the adrenal gland in response to excessive ACTH secretion from the pituitary gland or from

a functional adrenal tumor,[1] although overproduction of other hormones has also been

reported.[67-69] Therefore, despite the use of hydrocortisone, our model may not replicate all the hormonal changes of all cases of spontaneous hyperadrenocorticism.

Norepinephrine was selected in our study as the pressor agent since it has been shown that pressor effects in dogs are mediated by both $\alpha$-1 and $\alpha$-2 adrenoreceptor activity.[6] Ideally, a dose response curve should have been performed to assess changes in sensitivity to norepinephrine, but due to the potential for life threatening hypertension, the infusions could not be continued in all dogs to achieve a maximum response.

No significant differences were found when baseline direct blood pressure measurements were compared between control and I-HAC dogs. The values in both groups were considered to be normal since they were within normal published ranges.[70] Studies in humans with hyperadrenocorticism have revealed variable responses of systolic, diastolic and mean blood pressure in response to exogenous vasopressors. Significant differences have been found in systolic and diastolic pressure[5] and in diastolic and mean blood pressure[6] in humans with pituitary and adrenal dependent HAC. These differences have been observed in both hypertensive and normotensive human patients with HAC,[5,6] suggesting the presence of increased sensitivity to vasopressors even when patients are normotensive. Significant elevations of systolic blood pressure with minimal changes in diastolic blood pressure have also been seen in normal humans after short term administration of oral hydrocortisone.[14]

Mean changes in systolic blood pressure in response to norepinephrine were consistently higher in the I-HAC group dogs when compared to control dogs, but the difference was only significant at the norepinephrine dose rate of 0.2 $\mu$g/kg/min. The lack of significance at higher infusions could have been due to a reduced number of dogs in the I-HAC group since there were 5 or fewer dogs in the I-HAC group at infusion rates higher than 0.2 $\mu$g/kg/min. Thus, the dogs

in the I-HAC group appeared much more sensitive to norepinephrine infusions because their systolic blood pressures reached 240 mmHg at lower concentrations compared to the control dogs.

No significant differences were found in the responses of diastolic and mean blood pressure to norepinephrine infusions when compared between groups. Studies in humans with hyperadrenocorticism have revealed variable responses of systolic, diastolic and mean blood pressure in response to exogenous vasopressors. Significant differences have been found in systolic and diastolic pressure[5] and in diastolic and mean blood pressure[6] in humans with pituitary and adrenal dependent HAC. These differences have been observed in both hypertensive and normotensive human patients with hyperadrenocorticism,[5,6] suggesting the presence of increased sensitivity to vasopressors even when patients are normotensive. Significant elevations of systolic blood pressure with minimal changes in diastolic blood pressure have also been seen in normal human subjects after short term administration of oral hydrocortisone.[14,46]

The mean change in heart rate was significantly less in the I-HAC group than in the control group. In a normal subject, the expected response to an elevation in blood pressure is a reflex decrease in the heart rate mediated by the baroreceptor reflex mechanism.[66] Elevation of systolic blood pressure with a smaller decrease in the heart rate could indicate a decreased sensitivity of the baroreceptor reflex mechanism in the I-HAC dogs. The net effect of baroreceptor activation is inhibition of the vasoconstrictor center in the medulla and excitation of the vagal center causing vasodilation of veins and arterioles through the peripheral circulatory system and decreased heart rate and inotropism.[66] Administration of the mineralocorticoid agent deoxycorticosterone (DOC) to dogs has been shown to increase stroke volume and cardiac

output with a resultant increase in blood pressure without producing the decrease in heart rate that would be expected in normal dogs due to activation of the baroreceptor reflexes.[47] A similar mechanism could explain why the dogs in the I-HAC had a lesser decrease in heart rate in response to increasing systolic blood pressure when compared to control dogs. However, the mechanism of cortisol induced hypertension appears to differ from that of DOC hypertension, as was demonstrated in a study in humans in whom an elevation of cardiac output was not found to be essential for the rise in systolic and mean blood pressure when treated with oral hydrocortisone for 10 days.[46] When the increase in cardiac output was prevented in these subjects by the administration of atenolol, a rise in blood pressure and increase in plasma volume still occurred.[46] The rise in blood pressure in this instance was associated with an increase in total peripheral resistance. Moreover, administration of other glucocorticoids such as dexamethasone, a pure glucocorticoid, to normal human subjects has been shown to increase mean blood pressure without an associated increase in cardiac output.[19] It is likely that naturally occurring HAC involves a combination of mechanisms since cortisol has both mineralocorticoid and glucocorticoid effects.

Although current literature proposes increased pressor sensitivity to endogenous vasopressors as the major cause for the hypertension seen in human HAC,[6,16,17] it cannot be concluded from the results of the present study that the changes in systolic pressure were due solely due to enhanced vasoconstriction. An increase in arteriolar resistance is expected to produce a higher elevation in diastolic blood pressure compared with systolic blood pressure.[23] The dogs in the I-HAC had similar or lesser elevations in diastolic blood pressure than control dogs despite higher elevations in systolic blood pressure and higher heart rates. It is possible that the dogs in our study had elevations in stroke volume and cardiac output or increased aortic stiffness leading to

larger increases in systolic blood pressure. Another possibility is increased cardiac β-1

adrenergic sensitivity causing elevations in heart rate. The changes seen in the control dogs are

consistent with increased vasoconstriction since elevations in diastolic blood pressure were seen

despite reflex slowing of the heart. The role of increased cardiac output in dogs affected with

HAC deserves more attention. It is possible, in contrast to humans with HAC, the hypertension

in canine HAC is dependent on an increase in cardiac output.

Another possible explanation for the rise in blood pressure without a reflex decrease in heart

rate in the I-HAC is the central effects of angiotensin II. Angiotensin II has been found to

enhance sympathetic outflow and to control baroreceptor reflex sensitivity.[49] It increases blood

pressure without decreasing heart rate because it resets the baroreceptor reflex control of heart

rate to a higher pressure.[31] Brain angiotensin II could be involved in the regulation of cardiac

reflexes since cerebrospinal fluid angiotensin II concentration remained unchanged despite

undetectable plasma angiotensin II concentrations in dogs with DOC induced hypertension.[47]

Since the objective of our study was only to assess changes in blood pressure and heart rate

responses to norepinephrine administration, other factors implicated in the development of

hypertension associated with HAC were not evaluated. The renin-angiotensin-aldosterone

system was not evaluated in our model of I-HAC, but based on human studies it is unlikely to be

involved in the pathogenesis of hypertension in the presence of hypercortisolemia.[16-18] Similarly,

the sodium content was not evaluated in the dogs in the study, but no evidence of sodium

retention has been found associated with glucocorticoid hypertension.[16] Increased plasma

aldosterone concentration does not occur in dogs with naturally occurring or I-HAC.[57] Other

factors that may contribute to glucocorticoid-induced hypertension but were not evaluated in our

study include depression of vasodilatory substances such as nitric oxide, bradykinin and

prostacyclin.[36]

## CHAPTER III

<u>Conclusions</u>

This study demonstrated that, similar to humans with HAC and exogenous glucocorticoid therapy, dogs with I-HAC appear to have increased elevations in blood pressure in response to norepinephrine administration. However, based on the pattern of blood pressure and heart rate changes, the increases in blood pressure may be related to increases in cardiac output or decreased aortic compliance and not due to increased vasoconstriction. Thus, the mechanism for development of hypertension in canine HAC appears to differ from that of humans in which changes in cardiac output are not essential for the elevations in blood pressure. Further studies are necessary to elucidate the importance of increases in cardiac output and changes in peripheral vascular resistance in canine HAC. Measurement of cardiac output and administration of $\alpha$- and $\beta$- adrenergic blockers can be performed to assess the importance of these variables in dogs with HAC. Measurement of vasodilatory substances such as NO and kallikrein-kinin system should also be evaluated since they appeared to be decreased in human patients affected with HAC. In vitro studies of intracellular electrolytes concentrations in VSMCs of dogs with HAC should also be conducted to assess changes in electrolyte composition that could be responsible for difference in responses.

## FOOTNOTES

[a]Ultrasonographic Doppler Flow Detector, model 811-AL, Parks Medical, Inc., Aloha OK

[b]Michigan State University Animal Health Diagnostic Laboratory, Lansing, MI

[c]Cortrosyn®, Organon Inc., West Orange, NJ

[d]Hydrocortisone tablets, Global Pharmaceutical Corporation, Philadelphia, PA

[e]Hills Science Diet Maintenance® dry and chicken treats, Hills Pet Nutrition Inc., Topeka KS

[f]Angiocath™, Becton Dickinson, Infusion Therapy Systems Inc, Sandy, Utah

[g]Deltran® IV Disposable pressure transducer, Utah Medical Products, Inc., Midvale Utah,

[h]Datascope Passport 5L, Datascope Corp., 580 Winters Avenue, P.O. Box 5, Paramus, NJ

[i]Norepinephrine bitartrate, Abbott Laboratories, North Chicago, IL

[j]Auto Syringe, Model AS50 Infusion pump, 105/125 VAC, 60 Hz, Baxter Health Care Corporation, I.V. Systems Division, Deerfield, IL

[k]SAS, Version 8.1, SAS Institute Inc. Cary, NC

[l]SMZ-TMP, Danbury Pharmaceutical Inc., Flohrham Park, NJ

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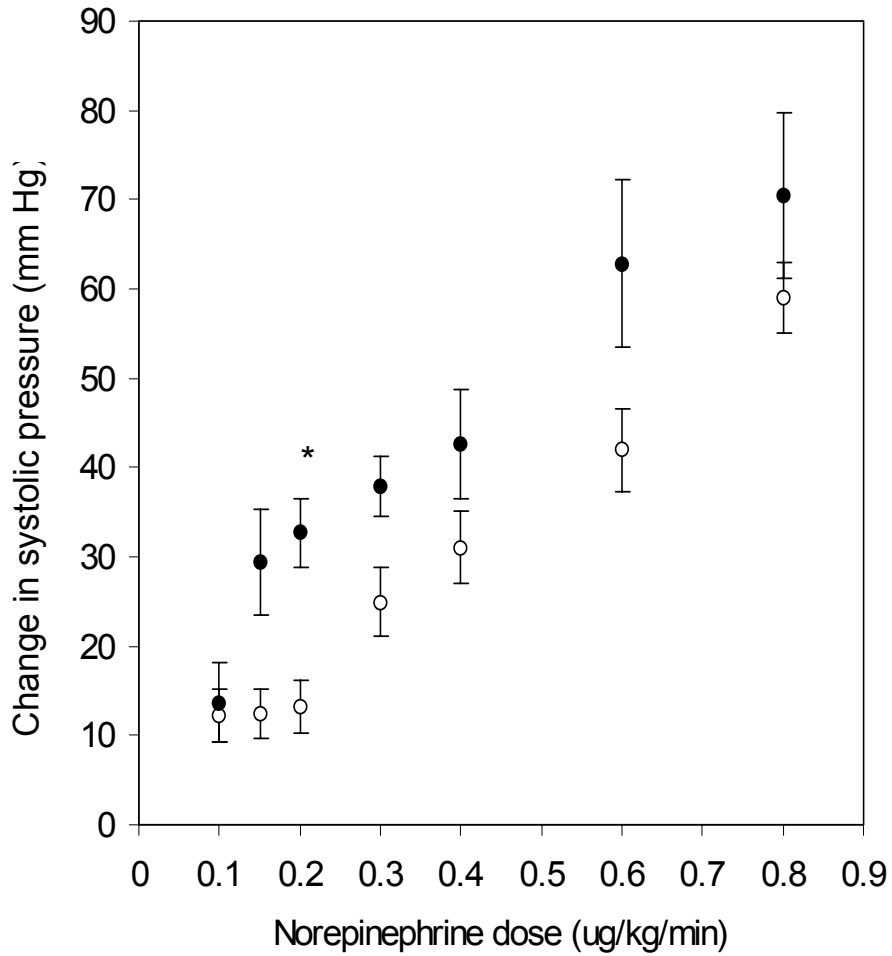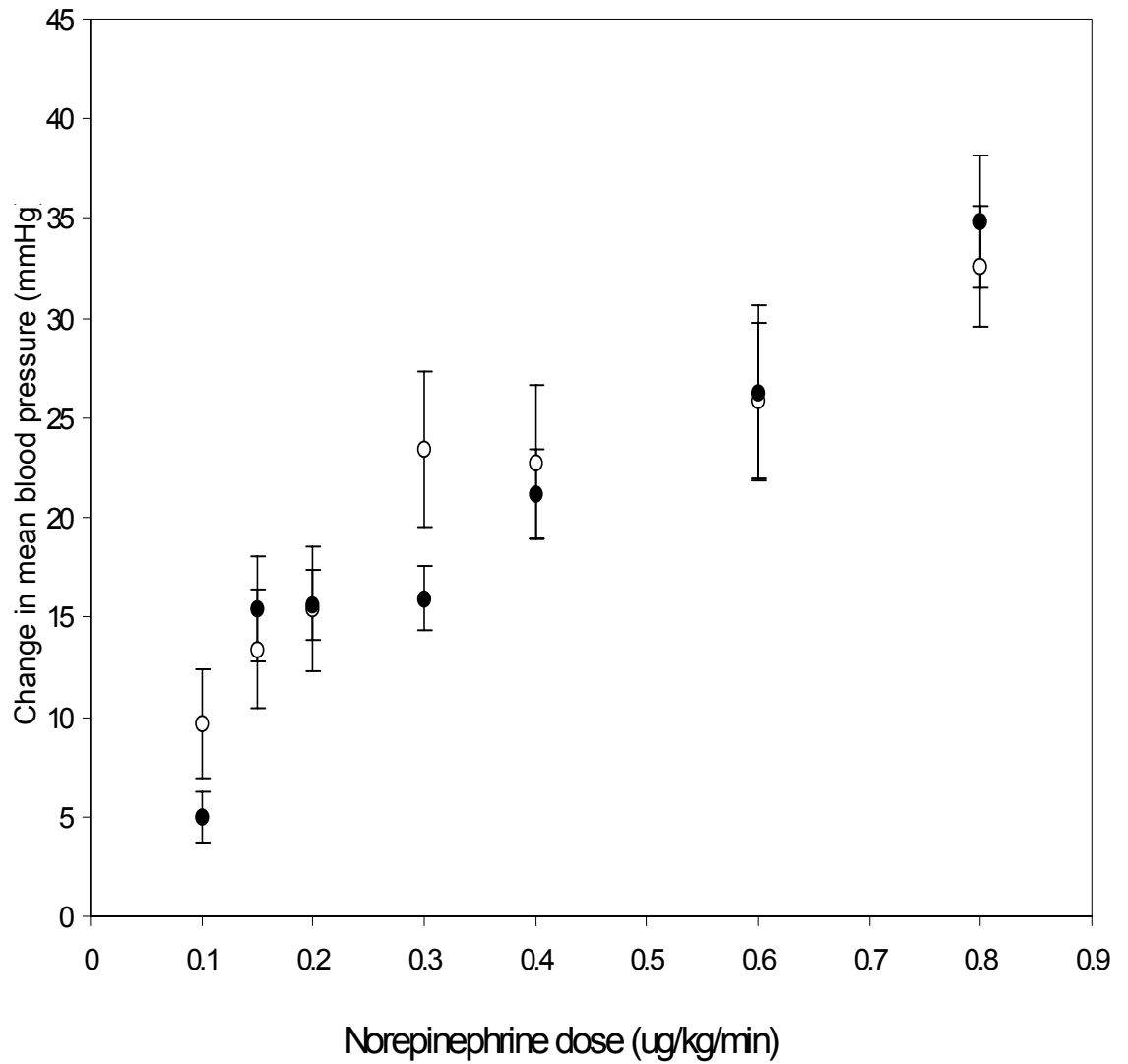
Figures



**Figure 1.** Mean change +/- SE in systolic blood pressure in response to increasing concentrations of norepinephrine in control dogs (open circles) and in dogs with iatrogenic hyperadrenocorticism (closed circles). n= 8 for each point in the control group and 8,8,7,5,4,4, and 3 respectively for increasing concentrations in the I-HAC group. Significant differences are marked with an asterisk (*).
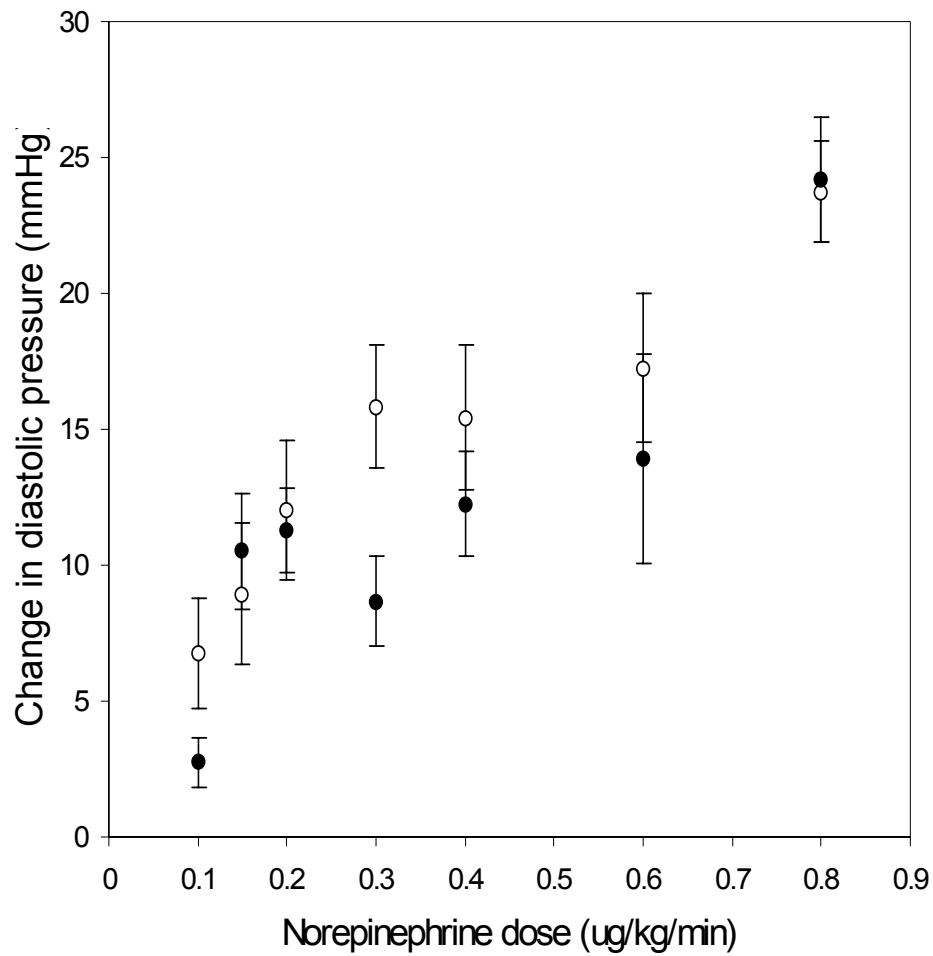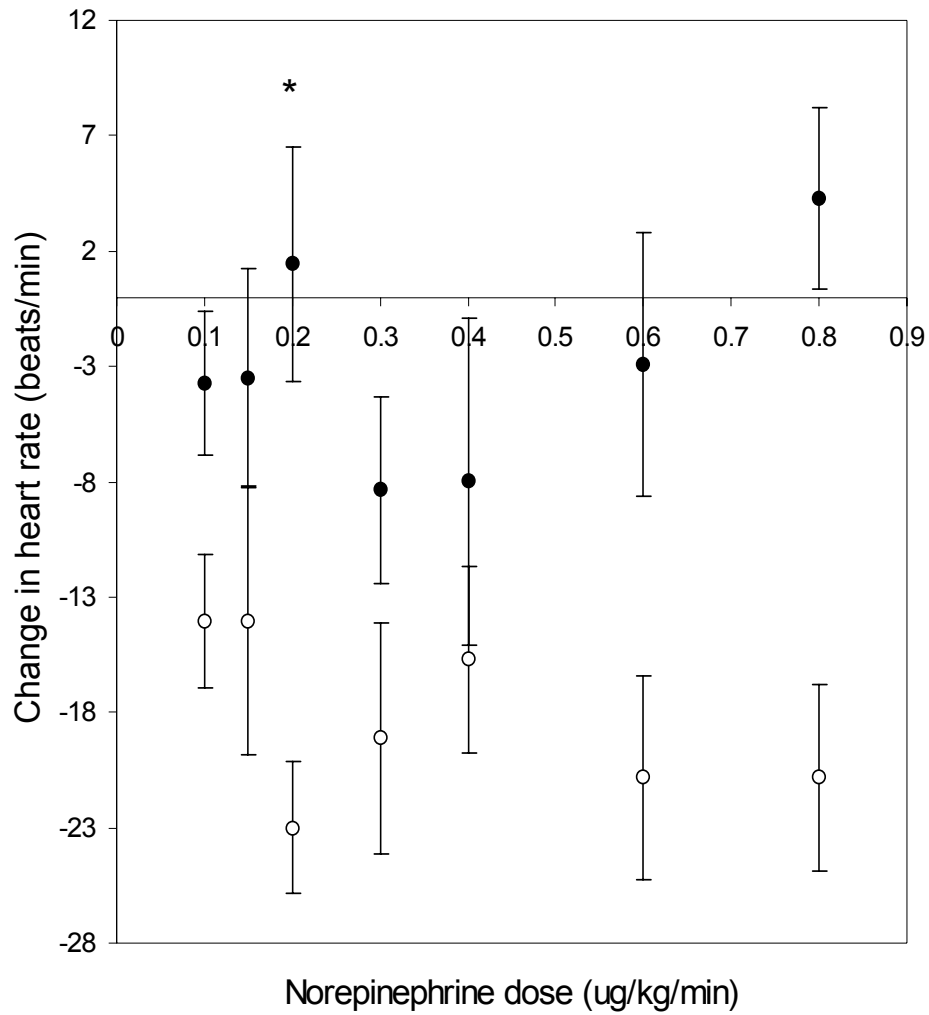
**Figure 2**. Mean change +/- SE in diastolic blood pressure in response to increasing concentrations of norepinephrine in control dogs (open circle) and dogs with iatrogenic hyperadrenocorticism (closed circle). n= 8 for each point in the control group and 8,8,7,5,4,4, and 3 respectively for increasing concentrations in the I-HAC group. Significant differences are marked with an asterisk (*).

**Figure 3**. Mean change +/- SE in the mean blood pressure in response to increasing concentrations of norepinephrine in control dogs (open circles) and in dogs with iatrogenic hyperadrenocorticism (closed circles). n= 8 for each point in the control group and 8,8,7,5,4,4, and 3 respectively for increasing concentrations in the I-HAC group. Significant differences are marked with an asterisk (*).

**Figure 4.** Mean change +/- SE in heart rate in response to increasing concentrations of norepinephrine in control dogs (open circles) and dogs with iatrogenic hyperadrenocorticism (closed circles). n= 8 for each point in the control group and 8,8,7,5,4,4, and 3 respectively for increasing concentrations in the I-HAC group. Significant differences are marked with an asterisk (*).

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## RESEARCH

Evaluation of pressor sensitivity to norepinephrine infusion in dogs with iatrogenic hyperadrenocortism, supported by a grant from the Virginia Veterinary Medical Association

## PUBLICATIONS

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Martínez NI, Panciera DL, Ward DL, Evaluation of pressor sensitivity to norepinephrine in dogs with iatrogenic hyperadrenocorticism. (In preparation)

Forrester SD, Martínez NI, Panciera DL, et al. Absence of urinary tract infection in dogs with experimentally induced hyperadrenocorticism. Res Vet Sci (Submitted)

## PRESENTATIONS

Martínez, NI. Hypertension in canine hyperadrenocortism. Graduate Seminar, VA-MD Regional College of Veterinary Medicine

Martínez, NI. Evaluation of pressor sensitivity to norepinephrine infusion in dogs with iatrogenic hyperadrenocorticism, Graduate Seminar, VA-MD Regional College of Veterinary Medicine

Martínez, NI. Ciliary Dyskinesia in a West Highland White Terrier. Graduate Seminar, VA-MD Regional College of Veterinary Medicine


Martínez, NI, Panciera DL, Ward D, Evaluation of pressor sensitivity to norepinephrine infusion in dogs with iatrogenic hyperadrenocorticism, 20th Annual ACVIM Forum, Dallas, TX, 2002

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