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Cardiovascular and hormonal responses to hypotension during hypoxia in the conscious goat

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University of Hawaii, 1993
CARDIOVASCULAR AND HORMONAL RESPONSES TO HYPOTENSION DURING HYPOXIA IN THE CONSCIOUS GOAT

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BIOMEDICAL SCIENCES (PHYSIOLOGY)

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Finally, I would like to recognize the financial support provided by The U.S. Army Health Services and Medical Research and Development Commands.
The cardiovascular and hormonal responses to hemorrhage and chemically-induced hypotension in conscious goats were assessed in the present study. A progressive hemorrhage (0.5 ml/kg/min for 30 min) under hypoxic (FiO₂ = 0.10) conditions (HH, n = 4) reduced mean arterial blood pressure (MABP) after only 20 min of blood loss. An identical hemorrhage under normoxic conditions (NH, n = 4) did not reduce MABP until after the blood loss was complete. Heart rate (HR) was significantly increased with hemorrhage only during normoxia. Arginine vasopressin (AVP) responses followed MABP changes, with HH levels being greater at an earlier time point. Final AVP values were not different between NH and HH. Plasma renin activity (PRA) responded in near identical fashion between the two settings, despite the earlier reduction in MABP with HH. Atrial natriuretic factor (ANF) was reduced during hemorrhage with both exposures.

Sodium nitroprusside (SNP) infusions sufficient in reducing MABP 20% increased HR only during normoxia (NH, n = 4). HR was not changed with hypotension during hypoxia (HH, n = 4). AVP levels were increased with SNP infusions during both exposures, with HH values greater than NH. PRA and epinephrine (EPI) increased in similar fashion during NH and HH. Norepinephrine (NE) and ANF were unchanged with SNP induced hypotension.

Finally, an acute 60 minute hypoxic exposure increased HR within the first
10 minutes. No transient increases in MABP, plasma NE or EPI were detected over the 60 min period of hypoxia.

Thus, hemorrhage during hypoxia poses a greater challenge to the cardiovascular system than does an equal blood loss during normoxia. Further, it appears that hypoxia attenuates the PRA response to hemorrhage. However, when MABP is reduced over an identical time frame during normoxia and hypoxia, no differences in the PRA responses can be observed, while a hypoxic augmentation of the AVP response to hypotension becomes apparent. Finally, the data suggest a hypoxic attenuation of the arterial baroreflex tachycardia with hypotension in conscious goats.
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CHAPTER I

INTRODUCTION

Integrated Cardiovascular Responses To Hemorrhage and Hypotension

Uncontrolled blood loss leads to hypotension and death. The effects of hypovolemia and hypotension are offset primarily through cardiovascular reflexes and three hormonal systems. These three hormonal systems are the circulating catecholamines, the renin-angiotensin axis, and arginine vasopressin (AVP). Obviously, if blood loss of a severe magnitude cannot be corrected for by these mechanisms, death will result. However, since hypoxia has been shown to alter the release mechanisms of these hormones (Chalmers et al., 1965; Critchley et al., 1980; Rose et al., 1983; Share and Levy, 1966) as well as cardiovascular reflexes (Bagshaw et al., 1986; Heistad and Wheeler, 1970), it follows that the responses to a cardiovascular challenge such as hypovolemia or hypotension would differ between the normoxic and hypoxic states.

The cardiovascular and hormonal responses to hemorrhage are well documented (Claybaugh and Share, 1973; Fejes-Toth et al., 1988; Larsson et al., 1978). Mild hypovolemia (generally less than a 20% reduction in blood
volume) elicits a reflex tachycardia with little or no decline in arterial pressure. Pressor hormones often increase during this "nonhypotensive" phase of blood loss, but generally only slightly. Sympathetic nerve activity is greatly enhanced, and total peripheral resistance is increased. If the blood loss persists, however, general circulatory "collapse" is observed; heart rate declines, cardiac output is reduced, and total peripheral resistance may greatly decrease. It is during this hypotensive phase of hemorrhage that pressor hormones are measured at their highest plasma levels. Seemingly paradoxical, however, it is also a time when overall sympathetic nerve activity may decrease (Hasser and Schadt, 1992; Schadt and Ludbrook, 1991).

While many of the above general responses to hemorrhage were observed in anesthetized preparations, more recent studies have been conducted in conscious animal models. The cardiovascular response to slow hemorrhage in conscious animals can be divided into two phases (Schadt and Ludbrook, 1991). The first phase, often termed the "nonhypotensive" phase of hemorrhage, is characterized by an increase in total peripheral resistance and heart rate in almost all conscious animals studied. The increase in heart rate, coupled with the increased total peripheral resistance, is most often adequate in preserving blood pressure at or near normal levels, despite a decreasing cardiac output. The increase in heart rate is due both to a reduction in parasympathetic tone and increase in sympathetic activity. The relative degree of each contribution varies with the species studied (Sander-Jensen et al.,
The increase in total peripheral resistance is almost certainly sympathetically mediated. Not surprising, then, is the common observation that plasma norepinephrine levels are greatly increased at this time (see below).

The second phase of blood loss is characterized by an inability to maintain blood pressure with the continuation of hemorrhage. In general, this hypotensive phase of hemorrhage is initiated when the accumulated blood loss reaches or exceeds 25-30% of total blood volume (Korner et al., 1990; Schadt and Ludbrook, 1991). Total peripheral resistance is greatly reduced during this period, and most species demonstrate a profound bradycardia as well (Schadt and Ludbrook, 1991). The bradycardia appears to be simultaneous to the sudden and dramatic reduction in arterial pressure. Further, many species show a dramatic reduction in sympathetic nerve activity. For instance, renal sympathetic nerve activity (RSNA) has been recorded in conscious rabbits during progressive blood loss (Hesser and Schadt, 1992). Early stages of blood loss were associated with maintained arterial pressure, increased heart rate and RSNA. However, when blood loss exceeded 14 ml/kg, arterial pressure, heart rate and RSNA were all greatly reduced. The reduction in RSNA was maintained for at least 5 min after the induction of hypotension, suggesting that the sympathoinhibition associated with hypotensive hemorrhage is not of a transient nature. Additionally, RSNA remained depressed when arterial pressure was returned to control levels through
phenylephrine infusions. Likewise, Skoog and Thoren (1985) reduced arterial pressure in rats to 50 mm Hg by rapid bleeding and noted a sustained bradycardia and reduction in RSNA and splanchnic nerve activity. Further, the bradycardia and reduction in RSNA were both reversed in rats that were subjected to a bilateral vagotomy either before or after blood loss, suggesting an important role for vagal innervation in both the bradycardia associated with hypotension and the reflex decrease in RSNA. Similar findings are reported from conscious dog studies (Schadt and Ludbrook, 1991). Thus, the cardiovascular responses to hemorrhage are far more complex than a general massive sympathoexcitation struggling to maintain blood pressure and heart rate in the face of a declining blood volume.

Vasoactive Hormone Systems and Responses to Hemorrhage

Catecholamines. The circulating catecholamines norepinephrine and epinephrine provide rapid changes in blood pressure and cardiovascular function. Acting predominantly through alpha-1 receptors, norepinephrine (NE) is a potent peripheral vasoconstrictor with strong effects in the skin, renal and splanchnic beds. Circulating norepinephrine may be derived from neural spill-over and the adrenal medullae (Goldstein, 1987). In contrast, circulating epinephrine (EPI) is derived almost exclusively from the adrenal medullae and acts primarily through beta-1 receptors to increase heart rate and conduction.
velocity. In addition, epinephrine may elicit vasodilation by binding to beta-2 receptors (The Adrenal Glands, 1988).

The role of endogenous circulating catecholamines in blood pressure regulation is not easily interpreted. Many researchers contend that under normal circumstances blood borne catecholamines do little to affect vascular tone and pressure. Instead, some feel it is the sympathetic fibers directly synapsing with smooth muscle of the resistance vessels that play the major role in blood pressure regulation. For instance, Silverberg and colleagues (1978) contend that both norepinephrine and epinephrine act as true hormones in humans only during times of stress, such as hemorrhage or hypoxia, when adrenal release and synaptic spill-over increase plasma concentrations. However, the measurement of plasma norepinephrine, during resting or basal conditions, has been shown to correlate with muscle sympathetic activity in humans (Wallin et al., 1980). Thus, the assessment of plasma catecholamines during both rest and stress may provide some indication of both sympathetic nerve activity and hormone levels capable of affecting target organs.

While it may not be easy to ascertain a quantitative role for circulating catecholamines on regulation of blood pressure under normal conditions, they have been shown to be of great importance during various forms of vascular compromise. It is widely agreed that hemorrhage is a powerful stimulus for the secretion of catecholamines (Hall and Hodge, 1971; Schadt and Gaddis, 1988; Schadt and Ludbrook, 1991; Zerbe et al., 1982), and Gardiner et al. (1989),
have demonstrated that the sympathoadrenal system is capable of maintaining blood pressure in hypovolemic rats even in the absence of other pressor systems. In the study by Gardiner et al. (1989), sympathoadrenal blockade with pentolinium abolished the maintenance of blood pressure when the vasopressin and renin-angiotensin systems were previously blocked. Thus, there is difficulty in arguing that any particular hormone system can directly regulate blood pressure as other systems are capable of increasing their level of participation. Regardless, studies have clearly shown that sympathoadrenal blockade lowers resting blood pressure in many species (Hesser and Bishop, 1988; Gardiner et al., 1989), thus directly implicating the sympathetic nervous system and the catecholamines in blood pressure regulation.

As mentioned above, it is now generally recognized that progressive blood loss elicits a biphasic cardiovascular response in many animal models studied. Similarly, the catecholaminergic response to progressive blood loss can be subdivided. The early nonhypotensive stage of blood loss is characterized by increased sympathetic nerve activity and associated increases in plasma norepinephrine concentrations (Schadt and Gaddis, 1988; Schadt and Ludbrook, 1991). During this early period of hemorrhage, plasma epinephrine levels are also increased. The onset of hypotension with blood loss greatly decreases overall sympathetic nerve activity (Schadt and Ludbrook, 1991), and presumably spill-over of norepinephrine into the bloodstream. Plasma concentrations of norepinephrine have been reported to not change during
periods of arterial hypotension (Brooks and Hatton, 1991; Fejes-Toth et al., 1988; Schadt and Gaddis, 1988; Schadt and Ludbrook, 1991). Studies have confirmed that the maintained plasma concentrations of norepinephrine during the hypotensive phase of hemorrhage are due to increased adrenal release of the hormone. Thus, adrenal denervated animals show a decrease in circulating NE levels with hypotension (Schadt and Gaddis, 1988). While plasma concentrations of NE are likely to remain unchanged during the hypotensive phase of blood loss, epinephrine levels have been consistently reported to increase with significant reductions in arterial pressure (Brooks and Hatton, 1991; Fejes-Toth et al., 1988; Schadt and Gaddis, 1988; Schadt and Ludbrook, 1991). The relative significance of the elevated EPI concentrations on cardiovascular function during hypovolemia is questionable however. Schadt and Gaddis (1988) prevented a rise in epinephrine following hemorrhage through adrenal denervation, and noted no differences in cardiovascular responses. In actuality, then, both the nonhypotensive and hypotensive stages of hemorrhage are characterized by increases in sympathetic nerve activity. As blood loss continues, however, hypotension ensues, and the sympathetic nerve activity increase becomes more selective as peripheral activity is reduced and adrenal nerve activity is greatly increased.

Renin-Angiotensin System. The enzyme renin, produced by the juxtaglomerular cells of the renal afferent arterioles, acts to cleave angiotensinogen to the decapeptide angiotensin I (AI). AI is further reduced to
the vasoactive octapeptide angiotensin II (All) by epithelial bound converting enzymes, especially in the lungs. Since the substrate angiotensinogen is present in great quantities in the blood, renin regulation becomes the most important limiting step in the renin-angiotensin axis. Thus, an _in vitro_ estimate of Al production, termed plasma renin activity (PRA), becomes a crucial hormone assay for the study of cardiovascular-endocrine interactions.

There are four, well characterized stimuli for renin release; a decreased renal sodium delivery, decreased renal perfusion pressure, increased renal sympathetic nerve activity (RSNA), and beta adrenergic stimulation from circulating catecholamines (Davis and Freeman, 1976; Keeton and Campbell, 1981). Renal sympathetic nerve activity may be increased by a number of different physiological conditions, including decreased atrial and arterial blood pressures (Davis and Freeman, 1976; Skoog et al., 1985; Toretti, 1982). A decrease in these pressures "unloads" the atrial and arterial baroreceptors, leading to an increase in RSNA from the central nervous system. The same conditions responsible for an increase in RSNA may also decrease renal perfusion pressure and increase plasma catecholamine concentrations (Davis and Freeman, 1976; Hall and Hodge, 1971). Thus, in the case of a hemorrhage, many stimuli may be responsible for an increase in plasma renin activity.

Conclusive evidence for a role of the renin-angiotensin system in blood pressure regulation was provided by Cowley and co-workers (1980a). These
researchers demonstrated that increasing rates of All infusions into dogs caused increases in arterial blood pressure. Further, increases in blood pressure were greatest in the absence of baroreceptor reflexes. Cowley and colleagues (1971) were also among the first to demonstrate a role of the endogenous renin-angiotensin-axis (RAS) in blood pressure control in dogs. Renin secretion was stimulated in areflexic dogs through renal artery occlusion. Blood pressure and PRA were observed to increase during the period of occlusion. Following All antibody administration, renal artery occlusion was found to cause only a minor increase in arterial blood pressure. Cowley et al. (1971) have determined that an intact dog (two kidney) can compensate for a decreased arterial blood pressure by 65%, utilizing the RAS alone.

During the same time period as the studies by Cowley and colleagues (1971), Hall and Hodge (1971) demonstrated an increased angiotensin generation in anesthetized cats and dogs with hemorrhage. Further, the increase in angiotensin generation was found to be independent of the rate of hemorrhage. Hemorrhage is undoubtedly a strong stimulus for renin release, and PRA has been shown to increase in nearly all hemorrhage studies in which it has been measured. However, the mechanisms for the increased PRA with hemorrhage are both numerous and complex.

PRA is increased with both nonhypotensive and hypotensive hemorrhage. Nonhypotensive hemorrhages have been shown to increase renin secretion in intact, but not vagotomized animals suggesting that vagal afferents are
important in the renin response to hypovolemia (Keeton and Campbell, 1981). Further, researchers (reported in Davis and Freeman, 1976) have demonstrated that interruption of renal nerve traffic during nonhypotensive hemorrhage prevents a rise in PRA. Presently, it is understood that a reduction in atrial pressure with mild blood loss reduces tonic baroreceptor inhibition of vagal afferent nerve traffic to the central nervous system and elicits a reflex increase in renal sympathetic nerve efference. As mentioned earlier, the increase in RSNA leads directly to an increase in renin secretion by the juxtaglomerular cells of the renal glomerular afferent arterioles (Davis and Freeman, 1976; Keeton and Campbell, 1981).

The continuation of hemorrhage from a state of maintained arterial pressure to one of systemic hypotension brings into play additional regulators of renin secretion. As mentioned above, the hypotensive stage of hemorrhage is characterized by a reduction in sympathetic nerve activity, most often measured as renal sympathetic nerve activity. Skoog and Thoren (1985) documented a reduction in RSNA with hemorrhage in anesthetized rats. In their experiments, arterial pressure was quickly reduced to 50 mm Hg, and a brief increase in RSNA was noted. However, within minutes a significant and sustained reduction in RSNA of 65% was noted along with a profound bradycardia. Following bilateral cervical vagotomy, RSNA was returned to baseline levels in the hemorrhaged rats. Thus, vagal afferent activity is important in adjustments of RSNA during both the early, nonhypotensive and
late, hypotensive stages of blood loss.

Despite the reduction in RSNA, PRA often continues to increase during the hypotensive stages of hemorrhage. The continued increase in PRA is most certainly due, in part, to a reduction in renal perfusion pressure, a powerful stimulus for renin release (Davis and Freeman, 1976; Keeton and Campbell, 1981). It seems most likely that renal afferent arteriolar "strain" or wall "tension" is inversely related to the release of renin (Keeton and Campbell, 1981). A reduction in vascular sympathetic tone is believed to increase the afferent arteriolar diameter (reduce "strain") ; likewise, a reduction in renal perfusion pressure as with hypovolemia unloads the renal baroreceptors. Studies from isolated kidneys have revealed that two primary renal baroreceptors can act to control renin release independent of renal sympathetic nerves (Davis and Freeman, 1976; Keeton and Campbell, 1981). Presently, it is understood that a renal vascular baroreceptor, located on the renal afferent arteriole, and a separate macula densa receptor, function to control renin release independent of extrinsic regulation. The macula densa receptor senses changes in filtered sodium load, and there is evidence that this receptor is important in regulating renin release during hemorrhage when blood pressure drops below the level compatible with autoregulatory function by the kidney (Davis and Freeman, 1976).

As with many other endocrine systems, the renin-angiotensin system is greatly influenced by other hormones. As mentioned earlier, the renal
sympathetic nerves play a direct role in regulating renin release through direct innervation of the juxtaglomerular cells. An increase in synaptic-derived norepinephrine stimulates renin release through alpha-adrenergic pathways (Winer et al., 1971). However, these same juxtaglomerular cells are richly innervated with beta adrenergic receptors (Hackenthal and Taugner, 1986; Vander, 1965; Keeton and Campbell, 1981; Winer et al., 1971), and an increase in synaptic derived norepinephrine may mediate renin release through this mechanism also. Owing to its strong beta adrenergic activity, epinephrine is the strongest endogenous catecholaminergic regulator of renin. Thus, increased epinephrine secretion from the adrenal medullae with hemorrhage may directly stimulate renin release (Johnson et al., 1979a; Johnson et al., 1979b; Schadt and Ludbrook, 1991). Lastly, brief mention must be made about arginine vasopressin (AVP) control of renin release. Several studies have indicated a direct suppression of renin release by AVP in intact rats and dogs (Malayan et al., 1980; Reid, 1983). An earlier study by Shade et al. (1973) demonstrated an AVP supression of renin secretion in isolated, nonfiltering kidneys. More recently, Peuler and colleagues (1990) have suggested that the increased levels of AVP associated with hemorrhage in rats may, in part, be responsible for the reduction in RSNA seen during periods of hypotension. Lastly, Malayan and co-workers (1980) provided evidence that levels of AVP seen during nonhypotensive hemorrhage (approx. 47 pg/ml) are effective in decreasing renin secretion. Thus, the potential role of other humoral agents
in the regulation of renin must be considered when documenting the renin response to hemorrhage or hypotension.

**Arginine Vasopressin (AVP).** Arginine vasopressin (AVP) is both a water conserving and vasoactive nonapeptide hormone released from the posterior pituitary. Through its role on water reabsorption in the kidney, AVP (or antidiuretic hormone) is an important regulator of fluid balance and thus long term blood pressure regulation. However, AVP is also an important regulator of short term blood pressure through its strong vasopressor action. Low dose infusions of the hormone are capable of affecting blood flow distribution (Montani et al., 1980) while larger doses increase total peripheral resistance and decrease cardiac output (Cowley and Liard, 1987; Cowley et al., 1974).

Many studies have implicated a direct role for AVP in blood pressure regulation. Cowley and co-workers (1980b) bled spinal destroyed nephrectomized dogs and reduced blood pressure from 100 mm Hg to 51 mm Hg. These dogs were capable of a 71% compensation in blood pressure. However, following a selective AVP antagonist, pressure was returned to the post-hemorrhage level. The blood pressure responses to hemorrhage in Brattleboro (ie., AVP deficient) rats, and Long Evans (ie., normal) rats was compared by Laycock et al. (1979). Following blood loss, Brattleboro rats were found to have blood pressures significantly lower than Long Evans rats; the differences were corrected with AVP infusions. The role of AVP on blood pressure regulation during hemorrhage in intact conscious dogs was
demonstrated by Schwartz and Reid (1981). A hemorrhage of 1 ml/kg/min for 15 min elicited no significant change in arterial pressure or heart rate. However, the same hemorrhage performed in dogs pretreated with a vasopressin antagonist reduced MABP by over 30 mm Hg and increased heart rate from 71 to 130 bpm (Schwartz and Reid, 1981).

Undoubtedly, hypotensive hemorrhage is a potent stimulus for AVP release (Claybaugh and Share, 1973; Goetz and Wang, 1988; Larsson et al., 1978; Wang et al., 1983), and additional evidence suggests that the increased AVP levels are important in maintaining blood pressure following blood loss (Schwartz and Reid, 1981; Zerbe et al., 1982).

While hemorrhage has been shown to increase plasma AVP levels in many animal models, increases in hormone concentrations are often detected before reductions in arterial pressure are observed. The early rises in AVP concentrations with hemorrhage are due to unloading of the atrial baroreceptors, while reductions in MABP unload the carotid sinus and aortic baroreceptors, stimulating greater release of AVP from the posterior pituitary (Cowley and Liard, 1987; Goetz and Wang, 1985). Cardiac denervated dogs (with intact aortic and carotid sinus baroreceptors) show significantly lower AVP levels than intact or sinoaortic denervated animals following either vena caval constriction or hemorrhage, suggesting that unloading atrial baroreceptors is a primary mechanism for AVP release (Goetz and Wang, 1988; Quillen et al., 1988; Wang et al., 1983). Moreover, the AVP response to either maneuver
is not different between sham-operated and sinoaortic denervated dogs, further supporting this notion. Yet, others have demonstrated only modest increases in AVP with hemorrhage until arterial blood pressure was significantly reduced. A study by Arnauld and colleagues (1977) in conscious monkeys concluded that the volume of blood removed had little influence on plasma AVP levels provided arterial pressure was unaffected. By varying the rate of removal of a constant blood volume, the authors were able to conclude that plasma AVP concentrations following hemorrhage in monkeys were closely correlated to arterial pressure changes and not hypovolemia, per se. Similarly, Larsson and co-workers (1978) concluded that the increases in plasma AVP with hemorrhage in the conscious goat were associated with changes in arterial but not venous pressures. Finally, at least one study in humans (Goetz et al., 1974) has shown that a mild "hemorrhage" which did not reduce arterial pressure was ineffective in producing significant increases in plasma AVP levels. Thus, while hemorrhage and the subsequent reduction in MABP undeniably increases plasma AVP levels, the exact contribution of the low and high pressure systems in the control of AVP during and following blood loss is unclear, and may vary significantly with the species being investigated.

Atrial Natriuretic Factor. Relatively speaking, the atrial natriuretic factor (ANF) is a new candidate for the list of hormones that regulate blood pressure. The peptide hormone is contained within granules of both the atria and ventricles of the heart, and is released into the blood stream upon distension
of the cardiac tissue. Therefore, any condition which leads to increased filling of the heart’s chambers can be expected to increase plasma ANF levels (Goetz, 1988). Early research of ANF was concerned with its natriuretic properties; that is, its ability to increase urinary sodium excretion (Cantin and Genest, 1985; Goetz, 1988). More recently, however, attention has been focused on the potential direct vasoactions of ANF, particularly its ability to elicit vasodilation.

Oshima et al. (1984) determined that infusions of atrial extracts into the renal arteries of rats caused a vasodilation of the renal vasculature, but the vasodilation was without any changes in arterial blood pressure. In contrast to those findings, at least two studies have shown that ANF can elicit blood pressure changes in conscious humans.

Richards et al. (1985b) administered ANF in bolus form to healthy male subjects. They found that administration of 100 ug of human ANF decreased mean arterial blood pressure, and that the decrease was sustained over a period of several minutes. Further, heart rate was increased after ANF administration and remained increased for the duration of the study. In addition, the researchers also noted significant increases in urine volume, sodium, calcium, magnesium, and phospherous excretion. Plasma AVP, PRA, NE and aldosterone were found to remain unchanged.

In a second study, Richards and co-workers (1985a) studied the effects of ANF infusion in hypertensive subjects. In contrast to the healthy subjects
observed in the above study, the hypertensive individuals were unable to sustain the decrease in arterial blood pressure, although heart rate was significantly elevated for the duration of the study.

From these studies, it would seem possible that ANF is capable of influencing blood pressure in human beings. However, interpretation of studies like those mentioned above is difficult when comparisons of the normal circulating levels of ANF are made to the concentrations administered to the subjects in the experimental settings. Also, it results from animal studies have not been as conclusive for a direct role of ANF in blood pressure regulation (Cantin and Genest, 1985; Goetz, 1988; Lappe et al., 1985).

The interpretation of ANF responses to to hemorrhage is difficult, as investigators have shown both decreases (Edwards et al., 1988) and increases (Carlson et al., 1989) following blood loss. Presumably, the decrease in ANF seen with hypovolemia results from decreased atrial stretch secondary to a reduced central blood volume. The increase in plasma ANF seen with hemorrhage in conscious pigs (Carlson et al., 1989) is more difficult to explain. Carlson and co-workers (1989) noted no significant decreases in ANF in one group of pigs and increases in a second group of animals bled 14 ml/kg over a 5 minute period. No significant correlations were found between the changes in plasma ANF concentrations and the changes in right atrial volume. Instead, significant correlations were noted between ANF and plasma lysine vasopressin (circulating form of vasopressin in the pig), renin and NE. In
addition, the authors noted greater atrial rates in the group with increased ANF levels after blood loss. Thus, the authors concluded that part of the increase in plasma ANF observed after hemorrhage may have been due to briefly increased atrial pressures resulting from contractions of the atria against closed valves during ventricular systole.

Hormonal Responses to Hypoxia

Catecholamines. Hypoxic exposure has long been associated with increased plasma catecholamine levels. An increase in the plasma catecholamines norepinephrine and epinephrine following hypoxemic perfusion of the carotid chemoreceptors was demonstrated by Critchley and co-workers (1980) in anesthetized dogs and cats. Importantly, the authors concluded that the increase in circulating levels of the hormones was due in part to increased secretory rates from the adrenal medullae. Likewise, Rose and colleagues (1983) have shown that a reduction of arterial oxygen tension from 78 to 33 mm Hg was effective in increasing plasma norepinephrine and epinephrine in conscious dogs. Interestingly, an increase in norepinephrine levels were detected during the first 80 min of hypoxia, while an increase in epinephrine was not detected until after 200 min of hypoxic exposure (Rose et al., 1983). Further, the authors demonstrated that a combination of hypoxia and hypercapnia produced a greater increase in circulating catecholamines than
would be predicted from the responses observed to the individual stimuli. While such findings may fit the notion of the "classic" response to hypoxic exposure, other studies have not demonstrated an effect of hypoxemia on catecholamine release. Nearly twenty years ago, Singh and colleagues (1974) were unable to demonstrate increased urinary norepinephrine concentrations in human subjects stationed at 3500 meters. The study by Singh et al. (1974) however, may not have approached the severity of hypoxia incorporated in most animal studies. However, another study with human subjects which incorporated a more severe level of hypoxia was unable to demonstrate an increase in plasma norepinephrine. Ashack and co-workers (1985) were unable to demonstrate an increase in plasma norepinephrine in human subjects acutely exposed to 10.5% oxygen. In their study, two subjects experienced profound nausea during hypoxic exposure, while one of the two subjects also experienced a dramatic reduction in blood pressure. No change in plasma norepinephrine could be detected in even these two "symptomatic" subjects.

Renin-Angiotensin System. The hypoxic moderation of both the catecholamines and AVP (below) has been investigated in some detail. In contrast, relatively little is known about the control of renin secretion during hypoxic exposure. In fact, little or no attention to chemoreceptor control of renin release is given in comprehensive and authoritative reviews of renin regulation (e.g., Davis and Freeman, 1976; Keeton and Campbell, 1981). Studies that have measured PRA during hypoxic exposure most often
demonstrate no change (Ashack et al., 1985; Colice and Ramirez, 1985; Curran-Everett et al., 1988; Heyes et al., 1982) or a decrease (Hogan et al., 1973; Maher et al., 1975a). Apparently, renal hypoxia or anoxia is also ineffective in increasing PRA. A reduction in oxygen saturation from 96 to 56% had no effect on renin release; nor did perfusion of isolated canine kidneys with venous blood or exposure of dogs to 8% oxygen (reviewed in Davis and Freeman, 1976).

Studies in human subjects have indicated an apparent decrease in PRA with hypoxic exposure. Hogan et al (1973), subjected humans to a simulated altitude of 12,000 ft and noticed a significant decrease in standing and recumbent PRA values. Further, the standing PRA values were depressed for three days of simulated altitude. Similarly, Maher and colleagues (1975a) noted decreased resting plasma renin levels in human subjects at 4300 m; the decreases were maintained over a 10 day period. However, other researchers have demonstrated no change in PRA with hypoxia in human subjects. Colice and Ramirez (1985) decreased hemoglobin saturation to 90 and 80% in human subjects through breathing of hypoxic gas and noted no significant changes in PRA. Lawrence and co-workers (1990) exposed human subjects to a more severe level of hypoxia (12%, hemoglobin saturation reduced to 68%) and likewise noted no significant change in PRA.

While the apparent lack of hypoxic stimulation of renin release appears to be widely accepted, at least two studies provide especially compelling
information on blood pressure regulation of PRA during hypoxia. Heyes and colleagues (1982) exposed human subjects to both hypobaric hypoxia or normobaric hypoxia to reduce inspired oxygen to approximately 10%. Four of the eight subjects in the hypobaric hypoxia group, and two of four subjects in the normoxic group experienced "acute mountain sickness" with profound nausea and hypotension. Despite the significant reduction in blood pressure in all of the ill subjects, PRA remained unchanged. In a similar manner, two subjects experienced profound hypotension (16 and 21% reduction in MABP) while breathing 10.5% oxygen in a study conducted by Ashack et. al (1985). While AVP was significantly increased in these two subjects, PRA remained unchanged from control values. Thus, while hypoxic exposure is generally regarded to be ineffective on PRA, evidence exists for an impairment of renin release with hypotension during hypoxia. Unfortunately, studies designed to specifically address the hypoxic moderation of renin responses to blood pressure changes are lacking.

Arginine Vasopressin. Share and Levy (1966) were the first investigators to demonstrate a chemoreceptor stimulation of vasopressin. In their experiment, isolated carotid chemoreceptors of vagotomized, anesthetized dogs were perfused with hypoxemic blood. A significant increase in plasma vasopressin was noted. The authors further noted that a greater rise in plasma vasopressin was possible with chemoreceptor stimulation if increases in blood pressure were prevented by simultaneous bleeding. In the 25 years since the
work of Share and Levy (1966), researchers have demonstrated that direct hypoxic stimulation of the carotid chemoreceptors leads to an increase in hypothalamic neuron activity and neurohypophyseal blood flow (Hanley et al., 1988; Harris, 1979; Wilson et al., 1987). Thus, there is little doubt that hypoxic exposure represents a potential stimulus for an increased release of vasopressin. However, findings from studies in intact animals, as well as in humans, have not been as conclusive for a hypoxic stimulation of vasopressin.

Urinary and plasma vasopressin concentrations have been shown to increase (Claybaugh et al., 1982; Forsling et al., 1980; Forsling and Ullman, 1977), not change (Curran-Everett et al., 1988; Harber et al., 1981), or decrease (Porchet et al., 1984) with hypoxic exposure in a variety of animal models. Studies with dogs have consistently demonstrated a hypoxic stimulation of AVP (Raff et al., 1983a; Raff et al., 1983b; Walker 1983). At least one study has determined the arterial oxygen partial pressure "threshold" for increased plasma vasopressin in anesthetized, paralyzed, artificially ventilated dogs. In their study, Raff and colleagues (1983b) were able to vary the level of arterial oxygen partial pressure from approximately 83-88 mm Hg to 26-29 mm Hg. Plasma AVP was found to be significantly elevated at about 35 mm Hg during normocapnic conditions. Also, the increases in plasma AVP concentrations were greater at any level of arterial oxygen partial pressure (PaO₂) if the animals were simultaneously made hypercapnic, further suggesting that the increases in hormone concentrations were chemoreceptor mediated.
In a similar study, Raff and co-workers (1983a) decreased the arterial oxygen partial pressure at varying rates and examined the AVP responses. It was concluded that the rate of reduction in $\text{PaO}_2$ did not have an effect on plasma AVP concentration. Instead, the authors concluded, the final steady state $\text{PaO}_2$ was the determining factor on AVP stimulation.

However, few studies in humans have shown increases in plasma vasopressin during hypoxia without concurrent complications (Claybaugh et al., 1982; Hackett et al., 1978; Heyes et al., 1982; Wang et al., 1984). Such complications include acute mountain sickness, nausea, hypocapnia, and hypotension. For example, Heyes et al. (1982) exposed subjects to hypobaric hypoxia (simulated 5100 m) or normobaric hypoxia (10.5% $\text{O}_2$) for a one hour period. Both forms of hypoxia were found to increase plasma AVP levels in approximately half the subjects. However, these same subjects were also made hypotensive with hypoxic exposure. Similar increases in plasma AVP concentrations have been observed in subjects exhibiting nausea, dizziness, dyspnea and other clinical signs of acute mountain sickness. Finally, two studies have demonstrated a decrease in plasma AVP with exposure to mild hypoxia. It has been shown, for instance, that breathing 13.9% oxygen for 20 minutes will decrease plasma AVP (Claybaugh, et al., 1977). Similarly, Porchet et al. (1984), demonstrated a reduction in AVP in healthy subjects following a rapid ascent of more than 2000 m. Blood pressure and plasma osmolality were unchanged with the increase in altitude, and AVP levels
returned to pre-ascent values following return to the low altitude environs. Thus, the influence of hypoxia on plasma AVP levels varies with the animal model studied. A review of the literature suggests the anesthetized and conscious dog (Raff et al., 1983a; Walker 1983), and pig (Forsling et al., 1980) demonstrate increases in AVP levels with hypoxic exposure, while conscious rats (Raff et al., 1991), sheep (Curran-Everett et al., 1988) and humans (Claybaugh et al., 1989) most often do not.

Evidence also exists that AVP plays a role in the cardiovascular responses of the conscious rat to hypoxia. Walker (1986) has shown that administration of a specific vasopressin V1 antagonist to conscious, iso- and hypercapnic hypoxic rats decreased arterial blood pressure and increased stroke volume and cardiac output.

**Atrial Natriuretic Factor.** To date, the available evidence supports a hypoxic stimulation of ANF. Baertschi and colleagues (1988) have demonstrated increased levels of plasma ANF in anesthetized, spontaneously breathing rabbits after only 10 minutes of hypoxic exposure ($P_{aO_2} = 22.3-44.3$ mm Hg). Additionally, no significant correlations were found between increased ANF levels and central venous pressures, suggesting a direct effect of hypoxia on the release of ANF. An in vitro experiment one year later by Lew and Baertschi (1989), investigated the ANF response to hypoxia in isolated, perfused rat hearts. Reductions in perfusate oxygen concentrations were associated with increasing releases of ANF. Phentolamine and
propranolol were both effective in reducing peak ANF concentrations, and hearts from rats pre-treated with a catecholamine depleting agent also had reduced ANF release associated with hypoxia. Thus, part of the hypoxic stimulation of ANF release in rats is thought to be due to alpha- and beta-adrenergic pathways.

Increased ANF release has been reported in human studies as well. Lawrence et al. (1990) exposed human subjects to 12% oxygen for a 60 minute period. Heart rate was elevated in the subjects and plasma ANF concentrations were found to be significantly increased by 50%, although no measurements of plasma catecholamines, central blood volume or central blood pressures were made. Contradictory to this report, Bartsch and colleagues (1988) failed to demonstrate an increase in human subjects exposed to altitude. Measurements of plasma ANF from climbers advancing to 4,559 meters were significantly greater only in those individuals displaying multiple signs of acute mountain sickness. In that group, plasma ANF levels more than doubled, while concentrations of the hormones remained unchanged in the "mildly" ill and non-symptomatic subjects. The two studies vary greatly in duration of hypoxic exposure and the variables associated with laboratory and field studies, making direct comparisons of the results difficult. Due to its potential role in regulating blood pressure as well documented reports of hypoxic stimulation of hormone concentration, the measurement of plasma ANF is important in a study investigating cardiovascular responses to
hypoxemia. Further, responses of plasma ANF to hemorrhage have been reported, although it is difficult to hypothesize how the hypoxic exposure and blood loss may interact to control the hormone’s release. For this reason, too, the measurement of plasma ANF concentrations in a conscious animal model exposed to hemorrhage and hypoxia would prove useful.

**Blood Pressure Regulation During Hypoxia**

As mentioned earlier, arterial blood pressure is maintained near normal levels in conscious animals acutely exposed to hypoxia. Also, heart rate and cardiac output are increased. Apparently, then, hypoxia alone does not present a great challenge for the conscious animal in maintaining cardiac function. However, several cardiovascular adjustments are made during hypoxia that may limit the ability of the cardiovascular system to adjust for an imposed "challenge" such as hemorrhage or hypotension.

In a comprehensive review, Heistad and Abboud (1980) discuss primary circulatory adjustments to hypoxia. The first adjustment is due to local vascular effects of hypoxia. Work by Heistad et al. (1975), and Daugherty et al. (1967), indicate that isolated vascular beds perfused with hypoxemic blood respond with a marked vasodilation. This dilation occurs despite systemic normoxemia, suggesting that the response is truly local in nature. However, vasodilation was not observed in all vascular beds studied, and was strongest
in coronary and cerebral blood vessels. Vasodilation was nonexistent in the renal vascular beds down to a PaO$_2$ of 20 mm Hg.

A second local adjustment to hypoxia has been demonstrated with the vascular responsiveness to vasoconstrictor agents. Heistad et al. (1975), perfused isolated vascular beds at a constant flow and measured changes in perfusion pressures (an indicator of vascular resistance) following administration of increasing doses of angiotensin. Angiotensin increased perfusion pressure in a dose dependent fashion during normoxemic (PaO$_2$ = 99 mm Hg) perfusion of coronary and gracilis arteries. However, angiotensin had no vasoconstrictor effect at any dose on coronary arteries perfused with hypoxemic (PaO$_2$ = 40 mm Hg) blood. In contrast, gracilis arteries maintained vasoconstrictor responses to angiotensin during hypoxemic perfusion, indicating the inhibition of angiotensin vasoconstriction during hypoxia is selective. Similar findings have been made with other vasoactive agents. For instance, Doyle and Walker (1991) have shown that both acute and chronic hypoxic exposure eliminated the pressor response to administered phenylephrine, vasopressin and angiotensin in whole, conscious rats. Further, in the same experiments, contractility of rat abdominal aortic rings to vasopressin was reduced with hypoxic perfusion of the vessel segments.

The second class of cardiovascular adjustments to hypoxia, as outlined by Heistad and Abboud (1980), involves the effects arising from chemoreceptor activation. While local vascular hypoxemia may elicit vasodilation and prevent
full expression of vasoactive hormones, evidence suggests that activation of carotid chemoreceptors prevents system wide hypotension. Pelletier and Shepherd (1972) measured perfusion pressures in anesthetized dogs during hypoxemic stimulation of isolated carotid chemoreceptors. Hypoxic stimulation of the chemoreceptor regions increased perfusion pressures in the aorta and hindlimbs. Additionally, hepatic venous pressure was increased while perfusion pressure of the saphenous vein was reduced. The authors concluded that chemoreceptor activation induced selective vasoconstriction and dilation in the anesthetized dog. Perhaps the strongest argument for chemoreceptor control of blood pressure comes from an interesting study by Lugliani et al. (as referenced in Heistad and Abboud, 1980). In their study, patients who had undergone surgical removal of the carotid bodies, and normal subjects, breathed a hypoxic gas mixture (FiO₂ = 0.10). The normal subjects maintained or slightly increased arterial pressure during hypoxia, while the patient group showed a significant reduction in arterial pressure. Thus, the inclusion of the carotid bodies in the integrated effects of hypoxia allows for the maintenance of blood pressure.

Finally, changes in cardiovascular reflexes have been documented during hypoxia with most of the changes indicative of impairments. Bagshaw and co-workers (1986) have provided evidence that mild hypoxia maximizes the ability of the carotid sinus reflexes to maintain arterial pressure. Similarly, Attinger and Attinger (1982) have demonstrated an increased baroreceptor sensitivity
with hypoxia. Thus it appears that hypoxic exposure may increase the ability of the cardiovascular system to reflexively maintain cardiovascular function. However, more than twenty years ago, Heistad and Wheeler (1970) demonstrated an inability in maintaining blood pressure during simulated hemorrhage with hypoxia in human subjects. In their study, human subjects were exposed to lower body negative pressure (LBNP) while breathing either normoxic gas or a hypoxic gas mixture (10% oxygen). A level of LBNP ineffective in altering arterial blood pressure with normoxia, produced significant hypotension while the subjects breathed the hypoxic gas mixture. Further, hypoxia prevented the reflexive increase in forearm vascular resistance with LBNP observed in normoxic subjects. A similar study by Heistad and colleagues (1971) showed that the reflexive impairment was present after 36 hr of hypoxia, and that the heart rate response to a reduction in arterial pressure was similarly reduced. Thus, while it is apparent that hypoxia impairs the cardiovascular reflexes and prevents complete adjustment to stresses such as hypotension, it is also apparent that changes in cardiovascular function are made during hypoxic exposure allowing the intact organism to maintain normal or near normal blood pressure.

Statement of Problem

Considerable evidence has been presented to suggest that hypoxic
exposure may result in the regulation of three pressor hormone systems - the circulating catecholamines, the renin-angiotensin axis, and arginine vasopressin. Further, it is evident that hypoxia presents a challenge to the cardiovascular system, requiring selective changes for the maintenance of blood pressure. Also, evidence exists to suggest that hypoxia impairs the cardiovascular constrictive reflex in humans exposed to lower body negative pressure. With this knowledge it would seem a logical extension to hypothesize that a hemorrhage during hypoxia would produce an earlier and greater development of hypotension than would an identical hemorrhage performed under normoxic conditions. Further, the vasoactive hormone systems, themselves greatly affected by changes in blood pressure, would thus also show altered responses to blood loss or hypotension with hypoxia. Surprisingly, however, little is known about either the cardiovascular or hormonal responses to hemorrhage during hypoxia.

Only two studies have investigated hemorrhage combined with hypoxia in conscious animals (Raft et al., 1991; Raft et al., 1986). However, both studies utilized an exposure of hypoxia of at least 24 hr, and neither study followed the cardiovascular or hormonal responses to blood loss during hemorrhage. The combination of hypoxia and lower body negative pressure (Heistad and Wheeler, 1970), and hypoxia and central hypovolemia (Rowell and Seals, 1990) have been addressed in human subjects. However, hormonal measurements were lacking in the studies, and there is difficulty in
extrapolating the findings to systemic hypotensive hemorrhage. Therefore, the aims of the present study become:

a). To investigate the cardiovascular and hormonal responses during a controlled and progressive hemorrhage with hypoxic exposure in a conscious animal model.

b). To investigate the effects of hypoxia on the cardiovascular and hormonal responses to arterial hypotension, _per se_, in a conscious animal model.

c). Lastly, to carefully investigate the cardiovascular and hormonal adjustments to acute hypoxic exposure in a conscious animal model through close and continuous monitoring over a 60 min period.
CHAPTER II

METHODS

Selection of Animal Model

Due to the invasive nature of these studies, an animal model was employed. The use of all animals in this study was approved by the Tripler Army Medical Center Institute Animal Use and Care Committee.

Many studies involving hemorrhage and the measurement of cardiovascular and hormonal parameters in conscious animals have utilized the dog (Claybaugh and Share, 1973; Cowley et al., 1971, 1974, 1980b; RAff et al., 1983a, 1983b). However, due to the sensitive nature of using "pet" animals at government research hospitals, it was not feasible for me to pursue this option. Also, there already existed an established goat colony at Tripler Army Medical Center, the site where all research was conducted.

The adult goat has previously been employed in hypoxia (Claybaugh et al., 1987) and hemorrhage studies (Larsson et al., 1978). Due to its size, the adult goat allows for serial blood sampling for hormonal measurements. Also, surgical preparation of the animals (see below) was accomplished without microscopic work. Finally, many cardiovascular and hormonal responses to blood loss and hypoxia in goats appear to be similar to those observed in humans (unpublished observations), making the interpretation and application
of findings quite appropriate. Thus, for all experiments outlined herein, conscious, adult (1-3 yrs.) , nonpregnant female goats were used. The goats were housed at Tripler Army Medical Center and were fed standard feed (Purina Goat Chow) and Timothy hay free choice.

**Surgical Preparation**

All animals underwent surgical preparation for construction of an exteriorized carotid artery loop at least three weeks prior to use in experimental procedures. Pre-anaesthetic administration consisted of Ketamine (10 mg/kg) and xylazine (0.2 mg/kg). During surgery, goats were maintained on halothane (0.5-1.5%) and oxygen (3 l/min). An incision, 10 cm in length, was made midline about the midcervical area of the ventral side of the neck. A second parallel incision, 5 cm in length and with the same midpoint, was made 2.5 cm to the right of the first. The right common carotid artery was isolated and freed of its sheath. The vagus nerve was carefully teased away from the artery and left intact in its original location. The strip of skin created by the two incisions was stripped of excess fat and connective tissue. Lastly, the artery was enclosed in the skin flap and closed with suture to form an external loop. No internal suturing was required, and all loops remained patent for the duration of the studies. All surgeries were performed by M. Eichinger,
with assistance from Dr. J. R. Claybaugh, Dr. A. McCullen, or Dr. C. Eisenhauer.

**Hemorrhage Series**

**Experiments**

The cardiovascular and hormonal responses to hemorrhage and hemorrhage during hypoxia were studied in conscious, conditioned female goats (wt = 30-47 kg). Goats were placed in a stanchion the night prior to experimentation and allowed water *ad libitum*. At approximately 8:00 am a catheter (Novalon, 20 gauge, Deseret Medical) was inserted into the carotid artery loop to allow for measurement of arterial blood pressure (MABP) and heart rate (HR), as well as removal of blood for sampling and the hemorrhage procedure. Additionally, an intravenous catheter was placed in the saphenous vein for an infusion site. Goats remained standing for the duration of the study, and were fitted about the head with a large box to allow for gas administration. Gases were delivered through the box from compressed gas cylinders; compressed medical grade air was delivered for the control and normoxia portions of the study and compressed medical grade air diluted with compressed nitrogen was delivered for the hypoxic periods. Fractional inspired gases were continuously monitored with a Horizon Metabolic Cart.
Arterial blood gases were measured utilizing standard electrodes (Corning Blood Gas Analyzer, model 168) and were corrected for body temperature. MABP and HR were monitored using a Hewlett-Packard pressure transducer and monitor (model 66).

Animals were randomly assigned to four experimental protocols (see Fig. 1). In a non-hemorrhage control series, goats were exposed to normoxic air for a period of 60 min. Then, the animals either continued with normoxic exposure or were exposed to hypoxia (FiO₂ = 0.10) for an additional 120 min. These studies served as the normoxic control (NC) and hypoxic control (HC) experiments, respectively. The other two protocols employed a 0.5 ml/kg/min hemorrhage initiated 60 min after control period and terminated 90 min after control period. These experiments thus served as the normoxic hemorrhages (NH) and hypoxic hemorrhages (HH). Each animal (n = 5) participated in all four protocols, performed on different days. One animal was unable to complete the HH protocol in two attempts and so is not included in the report of the data for the HH (i.e., for HH, n = 4). At least two weeks were allowed between NC and HC studies, and at least three weeks after a hemorrhage study.

During the hemorrhage, arterial blood was removed with a peristaltic pump (Sage model 375A) and collected in sterile blood donor bags. The hemorrhaged blood was filtered (Travenol Laboratories), and reinfused upon completion of the study period. Immediately prior to blood sampling, HR and MABP were noted and 4 ml of blood were withdrawn from the arterial line and
Figure 1. Top: Time line for hemorrhage series time control experiments. Time is in minutes. CATHS = catheterization period. GAS = induction of normoxia or hypoxia. b1, b2, b3 = blood sample, blood pressure and heart rate, and blood gas measurement period. Bottom: Time line for hemorrhage series hemorrhage experiments. <HEMORRHAGE> = blood removal period. All other abbreviations as above.
discarded. Next, 1 ml of blood was drawn into a heparinized syringe and inserted into the blood gas machine. Thirty-milliliter blood samples were withdrawn after the control period and at 60 and 120 min of gas exposure in the NC and HC protocols. An infusion of 30 ml isotonic lactated Ringer's solution was made simultaneous to the blood sampling to minimize plasma volume changes. Blood samples were also taken at 10, 20 and 30 min of hemorrhage in the NH and HH groups. The volume of these samples was incorporated into the hemorrhage and was removed at the predetermined hemorrhage rate. Twenty-milliliters of each blood sample was placed in iced heparinized tubes for later determination of plasma AVP and osmolality (OSM). The remainder of the blood was placed in iced Na-EDTA tubes for later determination of PRA and ANF concentrations. Four-milliliter aliquots of plasma for AVP were acidified with 0.4 ml of 0.1 N HCl. Two-milliliter aliquots for ANF were treated with 50 ul aprotinin (10,000 kIU/ml).

**Blood Measurements**

**Arginine Vasopressin Assay.** Plasma vasopressin concentration was measured from 4 ml of acidified plasma. Plasma was kept frozen until the assay date (within 1 wk of experiment). Samples were slowly thawed in ice water immediately prior to extraction. Octadecysilane cartridges (Waters) were pre-treated with 5 ml of 100% methanol, 5 ml of 8 molar urea, and 10
ml of distilled water before plasma samples were applied. Each successive volume of liquid was passed through the cartridge with the aide of a vacuum. Next, each cartridge was washed with 10 ml distilled water and 10 ml of 4% acetic acid. The AVP was eluted with 5 ml of 40% ethanol-4% acetic acid in water. In addition to the experiment samples, three other acidified goat plasma samples were extracted with each batch; one control plasma sample and two "spike" samples to which 10 ul of AVP (1 mU/ml) had been added. These control and "spike" samples allowed for calculation of recovery percentage of extracted hormone.

A 0.2 molar phosphate assay buffer solution was prepared by combining a 0.2M NaH$_2$PO$_4$-dibasic solution (14.197g/500 ml H$_2$O) with a 0.2M NaH$_2$PO$_4$-monobasic solution (6.895g/250 ml H$_2$O) until the resulting solution reached a pH of 7.2. Next, 3.3 g of NaCl and 1.0 g of bovine serum albumin (BSA) were added to 500 ml of the pH 7.2 solution. The final solution was adjusted to a volume of 1000 ml through the addition of 500 ml distilled water. The day following extraction, eluted samples were dried under slight heat and vacuum. The resulting dried residues were resuspended with 0.5 ml phosphate assay buffer in preparation for the radioimmunoassay procedure. AVP standards in phosphate buffer were used to generate a standard curve for each assay. Standards in doses of 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, and 32.0 uU/tube were prepared in duplicate. Samples were assayed at several doses and were added to phosphate buffer to produce an assay volume of 400
Additionally, 50 uL of a 1:30,000 dilution of an antiserum prepared against lysine-vasopressin was added to the standards and samples. This antiserum was prepared at the Department of Clinical Investigation, Tripler Army Medical Center in rabbits and has been shown to have a crossreactivity of 100% with AVP and < 0.05% with oxytocin (CU dissertation).

Standards and samples were kept at 4°C for 72 hours in the presence of antisera before adding 50 uL (approx. 3000 CPM) 125I-AVP (New England Nuclear). The labeled AVP was then allowed 48 hours to compete with antiserum binding sites. After 48 hours, 300 uL BSA coated carbon was added to every tube in the assay and the tubes were spun at 2000XG for 20 min. All assay tubes were decanted and both the carbon (unbound 125I-AVP) and supernatant (bound 125I-AVP) were counted for one minute on a Tracor Analytic gamma counter (model 1285). A standard curve for each assay was constructed by plotting the logit of the percent bound 125I-AVP added to each standard against the log of the standard dose. A within-assay coefficient of variability (CV) was calculated from the measurement of six known controls assayed simultaneously in one assay. The within-assay coefficient of variability was 9%. Likewise, a between-assay CV was determined from the measurement of controls from each assay. Between-assay CV was 6%. Due to low plasma AVP concentrations, control period and time control experiment samples were extracted from 4 ml of plasma, diluted with 400 uL assay buffer, and assayed entirely. Theoretically, this would allow for a minimum sensitivity
of 0.06 uU/ml. The within-assay CV for these samples was 4%; between-assay CV was 6%.

**Plasma Renin Activity and Atrial Natriuretic Factor Assays.** PRA and ANF were measured using commercially available radioimmunoassay kits (New England Nuclear and Peninsula Laboratories, respectively). The PRA assay assessed the concentration of angiotensin I generated in plasma in a one hour period while incubated at 37 degrees centigrade. EDTA, dimercaprol and 8-hydroxyquinoline were added to the samples to block converting enzyme and angiotensinase activities present in the plasma. Thus, the accumulation of angiotensin I under these controlled conditions reflected the renin enzyme activity.

Plasma ANF extraction was accomplished by passing heparinized plasma through octadecylsilane cartridges pretreated with methanol and water. ANF was eluted from the cartridges with a solution containing 80% methanol, 0.1% trifluoroacetic acid and 19.9% water. Standards were prepared from human ANF supplied by the manufacturer. Recovery of extracted ANF averaged 51%. Within- and between-assay CVs for PRA were 11 and 8%, respectively. Within- and between-assay CVs for ANF were 3 and 15%, respectively.

**Plasma Osmolality.** Plasma osmolality was measured from 250 uL samples by a freezing point depression osmometer (Advanced DigiMatic model 3D2).
Hematocrit. Hematocrit (Hct) was determined in duplicate by microcapillary centrifugation.

Statistical Analyses.

The original statistical design was to employ a two-way analysis of variance (ANOVA) for repeated measures on all data. However, I was unable to achieve an equal number of subjects in the hemorrhage groups. Because the difference in group size was due to the treatment effect per se (i.e., the hemorrhage with hypoxia), I was unjustified in utilizing the original statistical design (Winer, 1971). Therefore, each group was analyzed with a separate one-way ANOVA. A Duncan's multiple range test was performed to establish significance between means within each group. Corresponding time points between groups were compared with a two-tailed, unpaired \( t \) test. Time control data were handled in an identical manner to maintain consistency. Due to the heterogeneity of variance in the hemorrhage AVP data, values were converted to \( \log_{10} \) before statistical comparisons were made. The level of significance was set at \( P < 0.05 \). Unless otherwise indicated, all data are presented as means ± standard error of the means (± SEM).
Sodium Nitroprusside (SNP) Infusion Series.

Experiments

To more specifically address the issue of hypotension and hypoxia, a series of experiments were run in which the vasodilator sodium nitroprusside (SNP) was infused into conscious goats. Plasma concentrations of AVP, ANF, PRA and the catecholamines norepinephrine (NE) and epinephrine (EPI) were measured during ten minutes of SNP induced hypotension. In addition, heart rate and blood pressure were also measured. Each animal underwent two brief periods of hypotension in one experimental day, once during hypoxia and once during normoxia. The order of gas mixture exposure was randomized.

Prior to use in this set of experiments, goats were conditioned to a stanchion and a fitted face mask. This conditioning insured the animals would present basal heart rate and blood pressure before the start of all experimental procedures. After carotid artery and saphenous vein catheterization, animals underwent a 60 min equilibration period breathing room air (see Time-line in Fig. 2). Normoxia or hypoxia ($\text{FiO}_2=0.10$) was then administered for a period of 70 min. Normoxic air was delivered from compressed medical grade air cylinders. Hypoxic air was administered from a mixture of compressed medical grade air diluted with nitrogen. Gases were mixed and humidified before delivery to the animals via a fitted mask and two-way non-rebreathing valve.
Figure 2. Top: Time line for sodium nitroprusside (SNP) time control experiments. Time is in minutes. CATHS = catheterization period. GAS = induction of either normoxia or hypoxia. RAC = period of room air control. b1, b2, b3,... = blood sample, blood pressure and heart rate, and blood gas measurement period. Bottom: Time line for SNP infusion experiments. <SNP> = infusion period of sodium nitroprusside. All other abbreviations as above.
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Fractional inspired gases were continuously monitored with a Horizon Metabolic Cart (Sensormedics).

Immediately prior to each blood sample, HR and MABP were noted. Then, 4 ml of blood were withdrawn from the arterial pressure line and discarded. One-milliliter of blood was then drawn into heparinized syringes for blood gas measurements Corning Blood Gas Analyzer, model 168). This procedure was repeated before every blood sample. A 20 ml blood sample was drawn after the 60 min period of normoxia or hypoxia. Immediately following this blood sample, SNP infusion was begun for a period of 10 min. Additional 20 ml blood samples were drawn at 5 and 10 min of SNP infusion. Simultaneous to the blood sampling was an infusion of 20 ml isotonic lactated Ringer's solution to minimize plasma volume changes. After completion of infusion and blood sampling, animals returned to breathing room air (with the mask) for a period of 60 min. After this 60 min period, blood gases were measured and a 20 ml blood sample was drawn for plasma hormone measurements to assure the animals had returned to basal conditions; this measurement served as a room air control (RAC). Next, the gas mixture not utilized in the first half was administered, and after a 60 min period of exposure SNP infusion was begun again for 10 min. HR and MABP continuously recorded, and blood gases were assessed at 5 and 10 min of SNP infusion; Twenty-milliliter blood samples were also drawn at those times. A portion of each blood sample (15 ml) was placed in iced heparinized tubes for later determination of plasma AVP,
catecholamines and OSM. The remainder of the blood was placed in iced Na-EDTA tubes for later determination of PRA and ANF concentrations. Four-milliliter aliquots of plasma AVP were acidified with 0.4 ml of 0.1 N HCl. Two-milliliter aliquots for ANF were treated with 50 uL aprotinin (10,000 kIU/ml).

In addition to the SNP trials, a time control series of experiments was completed. All gas exposure and blood sampling followed the time course outlined above. However, during these trials SNP was not infused.

The selection of SNP to induce hypotension was made with careful thought. As mentioned earlier, SNP is a cyclic GMP stimulator. Renin release is coupled to increased intracellular levels of cyclic AMP (Davis and Freeman, 1976; Keeton and Campbell, 1981). However, recent reports suggest that cyclic GMP may act as a secondary messenger for in vitro inhibition of renin release (Henrich et al., 1988). Indeed, SNP has been shown to inhibit cyclic AMP stimulated renin release from rat renal cortical slices, but not basal renin release (Henrich et al., 1988). While in vitro studies suggested that SNP induced hypotension was inappropriate for the study of renin responses to blood pressure changes, a review of whole animal experiments did not support this. Brooks (1989) demonstrated that iv infusions of SNP (0.3-1.0 ug/kg/min) in conscious dogs decreased blood pressure only at the highest concentration used. While PRA tended to increase with increasing SNP doses, the lower doses of SNP employed did not reduce PRA values when no
reduction in blood pressure (i.e., resting or unstimulated conditions for renin release). In addition, Gorman and Chen (1989) have also shown that SNP induced hypotension elevated PRA in conscious dogs. In another set of experiments, left ventricular pressure was increased by aortic constriction during SNP induced peripheral hypotension; left atrial pressure was not altered from baseline (no apparent PRA stimulation from cardiac baroreceptor unloading). PRA was not different from control values, suggesting that SNP alone (without simultaneous atrial baroreceptor stimulation) had no effect on PRA. Thus the findings of Brooks (1989) and Gorman and Chen (1989) suggest no direct role of SNP on PRA in intact conscious dogs. I therefore chose SNP to induce hypotension in conscious goats based on those previous findings.

The aim of the SNP infusion was to reduce mean arterial blood pressure by approximately 20% of control. The dose of SNP required to induce such hypotension was determined in this study based on previous reports (Brooks, 1989; Fritsch et al., 1989; Raff et al., 1990). Through preliminary trials, it was found that a range of doses from 1.5 to 3.5 ug/kg/min would be sufficient in reducing MABP approximately 20%. To achieve this range of doses a stock solution (200 ug/ml) was made by dissolving 20 mg of SNP (Sigma) in 100 ml normal, sterile saline. On the day of experimentation, the animal was weighed, and the pump flow rate was adjusted to deliver the preset dose. On occasion, it became necessary to stop and restart the pump to assure the reduction in
pressure did not exceed 20%. A range of tolerance of 5 mmHg was established a priori; that is, the goal was to reduce MABP 20% ± 5 mmHg. Additionally, it was anticipated that the dose of SNP required to reduce MABP 20% during hypoxic exposure would be less than that required for the normoxic animals. Thus, the dosages for the hypoxic animals were always scheduled to be at the low end of the range (1.5-2.5 ug/kg/min).

Blood Measurements

Arginine Vasopressin Assay. Plasma vasopressin was measured from four ml of acidified plasma. The extraction and assay procedures were completed as described in detail above. Within assay CV for this series averaged 9%; between assay CV was 11%. Assay recovery of extracted plasma was 92%.

Plasma Catecholamine Assays. Plasma norepinephrine and epinephrine were assayed from extracted plasma by high pressure liquid chromatography with electrochemical detection (HPLC-EC). The method of extraction and detection were modified from previous reports (Goldstein, 1986; Goldstein et al., 1981; Jiang and Machek, 1987).

One-milliliter aliquots of heparinized plasma were stored at -70°C until extracted (no more than 2 weeks). On the day of extraction, plasma was slowly thawed in an ice water bath. The plasma was transferred to glass extraction vials containing 50 mg acid washed alumina (BAS, Inc.). Next, 10
uL of an EDTA-Na$_2$S$_2$O$_5$ solution (50 mg EDTA, 50 mg Na$_2$S$_2$O$_5$ in 1 ml water) and 500 uL of a 0.5 M Tris-HCl/Trizma base solution (pH = 8.4) was added. All samples were immediately vortexed and shaken for 5 min. The alumina slurry was allowed to settle and the supernatant was aspirated. The alumina was then washed three times with 0.5 ml ice cold distilled water and aspirated each time. An additional 1 ml of ice cold distilled water was added to each vial and the alumina slurry was transferred to a microfilter apparatus (BAS, Inc.) fitted with a 0.2 um pore-sized filter disk. The alumina was centrifuged to dryness at 1000XG for one min. A new receiver tube was placed on each microfilter unit and 100 ul of 2% acetic acid was added to each sample. The microfilter units were vortexed briefly and allowed to stand for 5 min. The microfilter units were spun at 1000XG for three minutes and the 100 ul extracts were collected and stored frozen at -70°C until HPLC-EC measurement (within one week). In addition to the experiment samples, two more aliquots of plasma were processed with each batch extraction. The additional samples consisted of 1 ml of "control" goat plasma, and a second 1 ml sample of goat plasma to which 10 ul of NE and EPI (75 and 25 ng/ml, respectively) had been added. These additional samples allowed for the determination of extracted hormone recovery.

The HPLC-EC apparatus consisted of an ODS 3 um stationary phase column (100 x 3.2 mm) and an amperometric detector comprised of a thin layer cell, glassy carbon working electrode with a silver/silver chloride gel reference cell.
An applied potential of +720 millivolts was maintained across the electrode. Current changes across the working electrode and reference cell were amplified and converted to voltage. The voltage signals were transmitted to a pen recorder where they were displayed as "peaks".

Mobile phase for the HPLC-EC process (10.35 g NaH₂PO₄·H₂O, 340.5 mg EDTA, 206.5 mg sodium octylsulfate in 1 L H₂O) was adjusted to pH 3.1 with H₃PO₄, filtered and de-gassed under vacuum prior to use. During measurement procedures, flow rate through the system was maintained at 1 ml/min. Four standards for NE (0, 1875, 3750, 7500 pg/ml) and EPI (0, 625, 1250, 2500 pg/ml) were prepared in 2% acetic acid immediately prior to assaying samples. Twenty-microliters of standard and sample were applied to the column; both standards and samples were run in duplicate. Standard peak heights (Fig. 3) were plotted against concentration to generate a standard curve. Sample concentrations (Fig. 4) were determined by plotting sample peak heights against the standard curve. Between assay CV for NE and EPI was 11% and 10%, respectively. Within assay CV for NE and EPI was 9% and 4%, respectively. Recovery of NE from extracted goat plasma averaged 56%; EPI recovery averaged 60%. The range of standards was chosen based on preliminary work with measuring plasma concentrations of catecholamines in conscious goats. A linear relationship was found between concentration of standard and peak height for all doses employed. The sensitivity of the amperometric detector was adjustable in a range from 0.1 to 500 nA. A range
Figure 3. Representative HPLC-EC recording of peaks obtained from three dilutions of norepinephrine (NE) and epinephrine (EPI) standards. Detector sensitivity was 1 nA. Time of injection referred to as "i" on diagram. Chart paper ran from left to right.
Figure 4. Representative HPLC-EC recording of two extracted plasma samples. Both norepinephrine (NE) and epinephrine (EPI) peaks are discernible. Detector sensitivity was 1 nA. Time of injection labelled "i" on diagram. Chart paper ran from left to right.
of 0.5 to 2 nA was routinely used in the present study. The changes in
detector sensitivity corresponded to linear changes in recorded peak heights.
Thus, a two-fold change of detector sensitivity lead to a two-fold change in
recorded hormone peak height. In this way, detector sensitivity could be
increased for anticipated low hormone concentrations (control periods), or
decreased for anticipated high concentrations (hypotension periods). With a
detector sensitivity of 1 nA, the limits of detectability for NE and EPI were 18.8
and 31.3 pg/ml, respectively.

**Plasma Renin Activity and Atrial Natriuretic Factor Assays.** PRA and ANF
were measured utilizing commercially available kits (New England Nuclear and
Peninsula Laboratories, respectively), and the methods described above.
Within- and between-assay CVs for PRA were 9 and 11%, respectively. All
ANF samples were measured on two assay days. Within-assay CV for ANF
was 5%, with a calculated sample recovery of 64%.

**Plasma Osmolality.** Plasma osmolality was measured from 250 ul samples
by a freezing point depression osmometer (Advanced Digimatic model 3D2).

**Hematocrit.** Hematocrit (Hct) was measured in duplicate by microcapillary
centrifugation.
Statistical Analyses

A one way analysis of variance (ANOVA) for repeated measures was performed on all data. A Duncan's Multiple Range Test was performed to determine significance between means (Winer, 1971). Significance was set at $P<0.05$. Due to heterogeneity of variance, plasma AVP and epinephrine values were converted to $\log_{10}$ before analyses were made. Unless otherwise indicated, values presented are means $\pm$ standard error of the means ($\pm$ SEM).

60 Minute Hypoxic Exposure with Serial Sampling

Experiments

Analysis of the cardiovascular and catecholamine responses from the hemorrhage and SNP studies prompted this additional study. Basically, it was found that conscious female goats did not display an increase in MABP after 60 min of hypoxic exposure ($\text{FiO}_2 = 10\%$). Additionally, no changes in plasma concentrations of norepinephrine or epinephrine were measurable during hypoxic exposure. Some conscious animal models exhibit an increase in MABP and plasma catecholamines during hypoxic exposure. Since the first blood samples taken during hypoxia in the previous studies were not made until after
60 min of exposure, it was possible that a transient increase in MABP or catecholamine concentrations might be overlooked. Thus, the intent of this small study was to follow the MABP and catecholamine changes during 60 min of hypoxic exposure.

As in the earlier studies, animals were placed in a stanchion the night prior to experimentation and allowed water ad libitum. The following morning, catheters were placed in the carotid artery and saphenous vein. The carotid artery catheter was connected to a pressure transducer by a saline filled line. This catheter allowed for pulse rate and blood pressure measurements, as well as removal of arterial blood for samples. Following catheterization, the animals were allowed a 60 min equilibration period. During this period, the animals were fitted with the same mask used in the previous studies. Immediately prior to all blood sampling, HR and MABP were noted. Then, 4 ml of arterial blood were drawn from the carotid line and discarded. One-milliliter of arterial blood was then collected in a heparinized syringe for blood gas measurements. Five-milliliters of arterial blood were then withdrawn and placed in an iced heparinized tube. Simultaneous to this blood sampling was an infusion of 5 ml isotonic lactated Ringer's Solution. This blood sampling procedure was done while the animals breathed room air, and at 10 min intervals during 60 min of hypoxia (FiO₂ = 0.10; see Fig. 5).
Figure 5. Time line for 60 minute hypoxic exposure experiments. Time is in minutes. CATHS = catheterization period. RAC = period of room air control. b1, b2, b3,... = blood sample, blood pressure and heart rate, and blood gas measurement period. <--- HYPOXIA ---> = period of hypoxic exposure.
Blood Measurements

Plasma Catecholamine Assays. Plasma norepinephrine and epinephrine were assayed from extracted plasma by the HPLC-EC methods described in detail above. Within assay CV for NE and EPI were 9% and 4%, respectively. Between assay CV for NE and EPI were 11% and 10%, respectively. Hormone recovery was calculated from externally spiked plasma samples extracted simultaneous to the experiment samples. Recovery for NE and EPI was 56% and 60%, respectively.

Plasma Osmolality. Osmolality was measured from 250 ul of plasma by a freezing point depression osmometer (Advanced Digimatic model 3D2).

Hematocrit. Hematocrit was measured in duplicate by microcapillary centrifugation.

Statistical Analyses

A one way analysis of variance (ANOVA) for repeated measures was performed on all data from this series. A Duncan's Multiple Range Test was performed to determine significance between means (Winer, 1971). Significance was set at P<0.05. Unless otherwise indicated, data are
presented as means ± standard error of the means (± SEM).
CHAPTER III

RESULTS

Hemorrhage Series

Results from this series of experiments have been published in the American Journal of Physiology (Eichinger and Claybaugh, 1992). The normoxic control animals (NC; n = 5) showed no changes in any cardiovascular or blood gas parameters over the two hour experimental period (Table 1). Thus, any effects of time alone can be ruled out of the experimental design.

As expected, conscious goats breathing 10% oxygen (hypoxic control, HC; n = 5) developed a significant and sustained tachycardia (Table 1). Heart rate was more than doubled at the 60 min point of hypoxia, and stayed more than 35 bpm above the control value for the two hour period. However, mean arterial blood pressure was not different from control at any time during hypoxia, indicating a response similar to that observed in human subjects. Arterial oxygen partial pressure (PaO₂) was significantly reduced from 97.4 ± 2.3 mm Hg to an average of 35.2 ± 2.0 mm Hg over the two hour hypoxic period. In addition, arterial carbon dioxide partial pressure (PaCO₂) was significantly reduced from 36.0 ± 0.9 mm Hg to an average of 32.0 ± 1.1 mm Hg, while arterial pH was increased from 7.41 ± 0.02 to 7.44 ± 0.02. Thus, the method of gas delivery employed in this study was effective in producing
Table 1. Cardiovascular and blood gas responses in hemorrhage series normoxic controls (NC) and hypoxic controls (HC). HR= heart rate, beats/min; MABP = mean arterial blood pressure, mm Hg; PaO₂ = arterial oxygen partial pressure, mm Hg; PaCO₂ = arterial carbon dioxide partial pressure. * = p<0.05 from time 0. + = p<0.05 from corresponding NC value.

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hypocapnic hypoxemia in the spontaneously breathing animals. Hematocrit was significantly increased with hypoxic exposure from $30.4 \pm 1.2$ to $33.9 \pm 0.9$ % (Table 2), most likely indicating contraction of the spleen. However, an isosmotic fluid shift cannot be ruled out as plasma osmolality remained unchanged by hypoxic exposure at $290.8 \pm 2.0$ mosmol/kgH$_2$O. Hypoxia reduced plasma sodium concentration after 60 and 120 min slightly, but significantly, in the HC group (from $144.4 \pm 0.9$ mEq/l to $143.6 \pm 1.0$ and $143.6 \pm 0.9$ mEq/l, respectively). However, plasma potassium was unaffected by hypoxia ($4.2 \pm 0.1$ mEq/l).

As presented in Table 2, no changes in plasma AVP, PRA or ANF were detectable in the NC group over the two hour time period. Likewise, hypoxic exposure elicited no significant changes in any of the plasma hormones measured. Due to low circulating levels, plasma AVP could not be detected in two NC and HC animals. Therefore, the lowest detectable limit for the assay (0.06 uU/ml) was substituted for these two animals to allow for analyses.

MABP was maintained at 10 and 20 minutes of a controlled hemorrhage of 0.5 ml/kg/min under normoxic conditions (NH; n = 5), but was reduced upon cessation of blood removal (time 120, Figure 6). At this point, MABP was reduced to $75 \pm 5$ mm Hg. Clearly, the tendency toward MABP reduction was evident at the 30 min period of hemorrhage (time 90), although this value was not significantly lower than control period. Heart rate (HR; Fig. 7) was
Table 2. Hormonal, electrolyte and hematocrit responses in hemorrhage series normoxic controls (NC) and hypoxic controls (HC). AVP = arginine vasopressin, uU/ml; PRA = plasma renin activity, ng/mI/hr; ANF = atrial natriuretic factor, pg/ml; OSM = plasma osmolality, mosm/kgH$_2$O; $P_{Na}$ = plasma sodium, mEq/l; $P_{K}$ = plasma potassium, mEq/l; Hct = hematocrit, percent packed cell volume. * = p<0.05 from time 0. + = p<0.05 from corresponding normoxic value.

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Figure 6. Mean arterial blood pressure (MABP) response to hemorrhage during normoxia (open circles; n = 5) and hypoxia (closed circles; n = 4). Time is in minutes. Period of hemorrhage was from time 60 to time 90. Points represent means ± SEM. * = p<0.05 from time 0. + = p<0.05 from corresponding normoxic value.
Figure 7. Heart rate (HR) response to hemorrhage during normoxia (open circles; n=5) and hypoxia (closed circles; n=4). Time is in minutes. Period of hemorrhage was from time 60 to time 90. Points represent means ± SEM. * = p<0.05 from time 0. + = p<0.05 from corresponding normoxic value.
significantly increased in the NH group only at the 30 min period of blood loss (time 90). This increase in heart rate was of a magnitude similar to that observed with hypoxia alone in the HC group. However, the development of hypotension was associated with a relative bradycardia in the NH group as HR was no longer different from control period during the post-hemorrhage measurement.

In contrast to the NH observations, MABP was significantly reduced in with HH at only 20 min of blood loss (time 80). Thereafter, MABP remained decreased during the last 10 minutes of blood removal and after 30 min following cessation of the hemorrhage (time 120). Final MABPs during the post-hemorrhage periods were not different between groups; in fact, they were remarkably similar (75 ±5 mm Hg vs. 75 ±4 mm Hg; NH vs. HH, respectively). Hemorrhage did not increase heart rate above the level associated with hypoxic exposure alone in the HH group. Similar to the response observed in the NH group, heart rate was depressed following the development of hypotension in the HH group. By the completion of hemorrhage (time 90), heart rate was no longer significantly different from control period in the HH group. However, there was a resumption of tachycardia in the HH group during the post-hemorrhage period (time 120) whereby heart rate was again greater than both control and the corresponding NH value.

The AVP responses to hemorrhage are presented in figure 8. Note the
Figure 8. Plasma arginine vasopressin (AVP) response to hemorrhage during normoxia (open circles; n = 5) and hypoxia (closed circles; n = 4). Time is in minutes. Period of hemorrhage was from time 60 to time 90. Points represent means ± SEM. * = p < 0.05 from time 0. + = p < 0.05 from corresponding normoxic value. Log_{10} conversions of the data were made before statistical analyses. Note the log scale for Plasma AVP.
log scale for AVP concentration in the figure. Similar to the blood pressure responses to hemorrhage, significant changes in plasma AVP concentration were observed first in the HH group. AVP was significantly increased above control and corresponding NH values at 10 and 20 min of blood loss (time 70 and time 80) in the HH group (2.60±2.08 and 160.40±49.74 uU/ml, respectively). In contrast, plasma AVP levels were not significantly elevated until 30 min of hemorrhage (time 90) in the NH group (to 87.33±67.18 uU/ml). AVP levels were significantly increased in both groups at 30 min of blood loss (time 90). Post-hemorrhage (time 120) AVP levels also were not different between groups, as final MABPs were not different. A large amount of variability in the AVP responses to hemorrhage was observed in both groups, and as mentioned in the methods section, log₁₀ conversion was done on the values before statistical comparisons were made.

In contrast to the AVP responses, the initial PRA responses (Fig. 9) to hemorrhage were similar between groups. PRA was significantly elevated earlier above control in the HH group (time 70), although it is apparent from figure 9 that the PRA responses of the NH group paralleled those of the HH group. In fact, there were no differences in PRA between groups until the post-hemorrhage period (time 120) where the HH value was found to be significantly lower than the NH value. The post-hemorrhage HH PRA value was no longer different from control. Although the post-hemorrhage NH PRA value remained significantly greater than its control, there was a trend toward
Figure 9. Plasma renin activity response (PRA) to hemorrhage during normoxia (open circles; n = 5) or hypoxia (closed circles; n = 4). Time is in minutes. Period of hemorrhage was from time 60 to time 90. Points represent means ± SEM. * = p < 0.05 from time 0. + = p < 0.05 from corresponding normoxic value.
a reduction in PRA following the development of hypotension in both groups. Thus, similar, near parallel responses in PRA were observed between groups during and following blood loss, with an approximate 10 min "shift" between groups observed with regard to statistically significant findings.

Decreases in plasma ANF were observed in both groups in response to hemorrhage (Fig. 10). Unlike the AVP and PRA responses, a significant change in plasma ANF was first noted in the NH group. While ANF was decreased and stayed reduced after 10 min of blood loss (time 70) in the NH group, a significant reduction was not noted until 30 min of hemorrhage (time 90) in the HH group. However, there were no differences between groups, undoubtedly due to a large variability in hormone concentration (especially apparent in the HH group).

**Sodium Nitroprusside Infusion Series**

Both normoxic and hypoxic gas mixtures were delivered to the animals in this series via a fitted mask and two-way non-rebreathing valve instead of the large, fitted box utilized in the previous series. Also, as mentioned in the methods section, 70 min of both hypoxic and normoxic gas exposure were accomplished on one experimental day. The order of gas exposure was randomly determined, with a 60 min room air control (RAC) "recovery" period.
Figure 10. Plasma atrial natriuretic factor (ANF) response to hemorrhage during normoxia (open circles; n = 5) or hypoxia (closed circles; n = 4). Time is in minutes. Period of hemorrhage was from time 60 to time 90. Points represent means ± SEM. * = p<0.05 from time 0.
(with mask still on) placed between gas exposures. 70 min of normoxic gas exposure was well tolerated in the goats as no changes in HR or MABP were observed in the normoxia control (NC) period (Table 3). Delivery of normoxic air also did not affect Hct, PaO\textsubscript{2}, PCO\textsubscript{2}, or pH. Likewise, plasma sodium and plasma potassium concentration were unchanged (144.5±1.2 mEq/l, 4.1±0.1 mEq/l, respectively) with breathing normoxic air through the fitted mask (Table 4). Plasma osmolality was found to be lower after 65 and 70 min of normoxia, although these time points were not different from RAC or corresponding hypoxic values. Thus, delivery of compressed normoxic air through the fitted mask was not associated with any alterations in measured plasma constituents.

Delivery of 10% oxygen via the fitted mask resulted in a significant reduction in PaO\textsubscript{2} during the hypoxia control (HC) from 102.8±1.9 mm Hg to an average of 40.6±2.7 mm Hg. Likewise, PaCO\textsubscript{2} was significantly decreased with hypoxia (from 38.3±1.4 mm Hg to 33.6±0.8 mm Hg by 70 min), although no significant increase in pH was observed. Thus, like the previous study, this method of gas delivery also produced a hypocapnic hypoxemia in the spontaneously breathing goats. HR was significantly increased in the hypoxia control period and remained approximately 30-40 bpm above RAC and corresponding NC values, while MABP remained unchanged at an average of 101±2 mm Hg. Hct was significantly increased with hypoxia (from 33.0±1.7 to 35.5±1.6), and was greater than the RAC and corresponding NC values at
Table 3. Cardiovascular and blood gas responses in SNP infusion normoxic controls (NC) and hypoxic controls (HC). RAC = room air control. HR = heart rate, beats/min; MABP = mean arterial blood pressure, mm Hg; PaO₂ = arterial oxygen partial pressure, mm Hg; PaCO₂ = arterial carbon dioxide partial pressure, MM Hg. * = p<0.05 from RAC. + = p<0.05 from corresponding NC value.

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</table>

| PaO₂ NC    | 102.8±1.9| 103.4±2.0| 101.2±3.3| 104.6±1.4|     |
| HC         | ----| 40.5±1.8*+| 41.8±3.3**+| 39.5±3.0*+|     |
| PaCO₂ NC   | 38.3±1.4| 36.7±1.0| 36.8±1.9| 36.3±1.2|     |
| HC         | ----| 35.1±0.9*| 33.3±0.4**+| 33.6±0.8*|     |
| pH NC      | 7.42±.01| 7.43±.02| 7.44±.02| 7.43±.01|     |
| HC         | ----| 7.44±.02| 7.44±.01| 7.44±.01|     |
Table 4. Plasma osmolality, electrolyte and hematocrit responses in SNP infusion normoxic controls (NC) and hypoxic controls (HC). RAC = room air control. OSM = plasma osmolality, mosm/kgH2O; PNa = plasma sodium, mEq/l; PK = plasma potassium, mEq/l; Hct = hematocrit, percent packed cell volume. * = p<0.05 from RAC. + = p<0.05 from corresponding NC value.

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all time points. Plasma osmolality did not differ from RAC (289.5±1.0 mOsm/kgH₂O) with hypoxic exposure, and there were no differences between any hypoxic time periods. However, the 60 min normoxic value was found to be higher than the corresponding hypoxic value (290.9±1.6 vs. 288.4±1.2 mOsm/kg, respectively). Plasma sodium and potassium remained unchanged from RAC values (144.3±1.3 and 4.0±0.1 mEq/l, respectively) in both hypoxia and normoxia exposure periods over the course of the time control series.

The hormonal responses to 70 min of normoxic or hypoxic exposure are presented in Table 5. Difficulty was again encountered in measuring plasma AVP concentrations from the conscious goats. Plasma AVP was undetectable in three of the four animals during both the normoxic and hypoxic periods of the control experiments. The lowest detectable limit (0.06 uU/ml) was substituted for these animals for purposes of data presentation. While AVP concentration was measurable in one animal, it appeared to remain steady during the course of the experiment at an average of 0.18 uU/ml over the course of the experiment. While there was a tendency for the PRA values to be higher during the HC half of the experiments (0.57±0.06 ng/ml/hr) compared to the normoxic values (0.43±0.05 ng/ml/hr), statistical differences could not be demonstrated between any time points. Similarly, the plasma NE values were consistently higher during hypoxia than normoxia, yet no differences were present. A fairly large degree of variability was noted in the
Table 5. Hormonal responses in SNP infusion normoxic controls (NC) and hypoxic controls (HC). RAC = room air control. AVP = arginine vasopressin, uU/ml; PRA = plasma renin activity, ng/ml/hr; NE = norepinephrine, pg/ml; EPI = epinephrine, pg/ml; ANF = atrial natriuretic factor, pg/ml. No significant differences were found.

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<tr>
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<td>234.7±50.6</td>
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<tr>
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<td></td>
<td></td>
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</tr>
<tr>
<td>NC</td>
<td>149.4±29.2</td>
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<tr>
<td>HC</td>
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<td>160.9±50.9</td>
<td>147.8±23.7</td>
<td>123.6±34.5</td>
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<tr>
<td>ANF</td>
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<tr>
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<td>14±3</td>
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RAC values for NE (222.0±75.9 pg/ml) and a similar level of variability was present with all measurements. Plasma EPI concentrations were not affected with either normoxic or hypoxic gas exposure, and no significant changes were observed. In fact, the average plasma EPI concentration over the entire normoxic period (150.0±39.6 pg/ml) and hypoxic period (144.1±36.4 pg/ml) was very similar to the RAC value (149.0±29.2 pg/ml). Thus no indication of a hypoxic stimulation of NE or EPI could be determined. Plasma ANF concentrations were unchanged from RAC with 70 min of normoxia or hypoxia, and no differences were detected between the two exposures at any time point. As expected, then, no changes in any of the measured hormone concentrations were detectable in the NC group. Similarly, an FiO₂ of 0.10 also did not alter any of the plasma hormones measured, leaving HR and Hct as the only measured parameters altered by hypoxic exposure.

Sodium nitroprusside infusions were effective in reducing MABP in both normoxic and hypoxic goats. Figure 11 depicts the percent reduction during both exposures at 5 and 10 min of SNP infusion. The aim of the study was to reduce MABP 20% in both exposure periods; as is apparent in figure 11, the percent reduction in MABP tended to be greater during hypoxia at both 5 and 10 min of infusion, although no significant differences were observed between groups (24±2 vs. 19±2 mm Hg and 22±2 vs. 16±1 mm Hg; hypoxia vs. normoxia, 5 and 10 min of SNP, respectively). While a controlled reduction in MABP was achieved under both settings, pressure was more labile
Figure 11. Percent reduction in mean arterial blood pressure (MABP) after 5 and 10 min of sodium nitroprusside infusion. Open bars represent normoxic group values. Filled bars represent hypoxic values. Values are means ± SEM. No significant differences (N.S.) were observed between groups at either time point.
PERCENT REDUCTION IN MABP

TIME

5 min  10 min

N.S.  N.S.
during hypoxia than normoxia, and several experiments were terminated when MABP was rapidly reduced more than 20% (often more than 35%). The average volume of SNP (200 ug/ml) infused during normoxia tended to be greater than that required to reduce and maintain a reduced pressure during hypoxia (7.05±2.21 vs. 4.78±1.22 ml, N.S.), although continuous adjustments in flow rate were made during both normoxic and hypoxic experiments. Heart rate was significantly increased from 67±5 bpm to 103±9 and 98±14 bpm after 5 and 10 min of SNP infusion during normoxia, indicating stimulation of the baroreceptor reflex in the conscious goat (Fig. 12). In contrast, no change in HR accompanied the reduction in MABP with hypoxia. As indicated in figure 12, hypoxic exposure increased HR from 59±4 bpm to 116±17 bpm prior to SNP infusion; however, HR was not further increased after 5 or 10 min of SNP infusion (116±16 and 116±20 bpm, respectively).

The SNP infusions, and resultant hypotension, produced increases in plasma AVP concentrations (Fig. 13). Sample variability increased as AVP concentrations increased, and a Box's test indicated heterogeneity of sample variances. Thus, log₁₀ conversions of the data were made prior to statistical analyses (Winer, 1971). Statistically, plasma AVP concentrations were not increased until 10 min of SNP induced hypotension during normoxia (from 0.25±0.10 to 8.82±5.40 uU/ml). However, plasma AVP was increased after 5 and 10 min of SNP infusion during hypoxia (from 0.11±0.4 to
Figure 12. Heart rate (HR) response to SNP infusion during normoxia (open circles; n = 4) and hypoxia (closed circles; n = 4). Time is in minutes. RAC = room air control. Points represent means ± SEM. * = p < 0.05 from time 0. + = p < 0.05 from corresponding normoxic value.
Figure 13. Plasma arginine vasopressin (AVP) response to SNP infusion during normoxia (open circles; n = 4) and hypoxia (closed circles; n = 4). Time is in minutes. RAC = room air control. Points represent means ± SEM. * = p < 0.05 from time 0. + = p < 0.05 from corresponding normoxic value. Log₁₀ conversion of the data was made prior to statistical analyses. Note log scale for AVP.
55.23±24.33 and 35.57±11.82 uU/ml, respectively). Further, plasma AVP concentrations during hypoxia were significantly greater than corresponding normoxia values after 5 and 10 min of hypotension, suggesting a hypoxic enhancement of the AVP response to hypotension.

Unlike the AVP responses, PRA was not different between the two oxygen exposures after 5 or 10 min of SNP induced hypotension (Fig. 14). PRA increased from 0.39 ng/ml/hr to 1.09±0.05 and 0.93±0.14 ng/ml/hr after 5 and 10 min of hypotension, respectively. Thus, PRA was approximately tripled in response to SNP infusion during the normoxia exposure. SNP infusion also produced increases in PRA during the hypoxia exposure. PRA doubled after 5 min of infusion (from 0.63±0.11 to 1.21±0.09 ng/ml/hr), and remained increased after 10 min of infusion (0.95±0.10 ng/ml/hr). While the magnitude of increase in PRA appeared to be higher during normoxia, no significant differences were observed in the pre-infusion values (time 0) between the two exposures. Also, no differences were observed at either time of SNP infusion between the two exposures, indicating a similar PRA response to hypotension in normoxic and hypoxic goats.

Not all of the hormones measured were affected by the SNP induced hypotension. Figure 15 illustrates the ANF response to SNP infusion. Plasma ANF concentration was unchanged from baseline during SNP infusion in the normoxic animals (from 12±2 to 11±2 and 12±2 pg/ml; 5 and 10 min of infusion, respectively). Similarly, plasma ANF was unchanged from a baseline
Figure 14. Plasma renin activity (PRA) response to SNP infusion during normoxia (open circles; n = 4) and hypoxia (closed circles; n = 4). Time is in minutes. RAC = room air control. Points represent means ± SEM. * = p < 0.05 from time 0. No significant differences were observed between points.
Figure 15. Plasma atrial natriuretic factor (ANF) response to SNP infusion during normoxia (open circles; n = 4) and hypoxia (closed circles; n = 4). Time is in minutes. RAC = room air control. Points represent means ± SEM. No significant differences were observed.
hypoxic value of 15.±3 pg/ml after 5 and 10 min of infusion in the hypoxic group (13.±4 and 13.±3 pg/ml, respectively). Also, no differences were observed at any time point between the two exposures, further demonstrating a lack of effect of SNP induced hypotension on plasma ANF concentration.

Plasma NE concentrations also were not significantly altered by the SNP induced hypotension (Fig. 16). A test for homogeneity of variance, identical to that employed with the AVP data from this series, indicated similar variances between samples. Thus, no log_{10} conversions of the data were made. As a consequence, no significant changes were observed when comparisons were made with the raw data, despite the trend for plasma NE to increase during both hypoxia and normoxia. Pre-infusion NE concentrations were not different between normoxia and hypoxia (195.6.±66.0 vs. 220.8.±57.8 pg/ml, respectively), further supporting the results from the time control experiments.

Plasma EPI concentrations, unlike those of plasma NE, were found to increase with the hypotension (Fig. 17). Due to heterogeneity of variance in the EPI responses to SNP infusion during both normoxia and hypoxia, data were converted to log_{10} before comparisons were made. EPI appeared to be increased after 5 min of SNP infusion in both normoxia and hypoxia (from 170.1.±26.0 to 404.6.±109.9 pg/ml, normoxia; from 132.2.±26.7 to 431.3.±247.3 pg/ml, hypoxia), although no statistical differences could be demonstrated. After 10 min of SNP infusion, however, plasma EPI
Figure 16. Plasma norepinephrine (NE) response to SNP infusion during normoxia (open circles; n = 4) and hypoxia (closed circles; n = 4). Time is in minutes. RAC = room air control. Points represent means ± SEM. No significant differences were observed.
Figure 17. Plasma epinephrine (EPI) response to SNP infusion during normoxia (open circles; n = 4) and hypoxia (closed circles; n = 4). Time is in minutes. RAC = room air control. Points represent means ± SEM. * = p < 0.05 from time 0. No significant differences were observed between corresponding time points. Log₁₀ conversion of the data was made prior to statistical analyses.
concentration was significantly increased in both exposures (532.1±130.0 and 364.4±59.5 pg/ml; normoxia and hypoxia, respectively). Plasma concentration of the hormone was not different between exposures before or during the induced hypotension.

60 Minute Hypoxic Exposure with Serial Sampling

Hypoxic gas mixture (FiO₂ = 0.10) was delivered to conscious goats in the same manner as the previous SNP infusion experiments. Following a room air control (RAC) measurement, hypoxia was begun, and the FiO₂ was reduced uniformly over a 10 min period. HR and MABP were monitored continuously, and blood samples were taken at 10 min intervals for a period of 60 min. The first blood sample was taken 10 min after the RAC sample, and thus coincided with the first full min of breathing 10% oxygen.

The induction of hypoxia significantly reduced PaO₂ from 104.0±2.8 to 40.8±3.1 mm Hg at the first 10 min sample period (Table 6). Thereafter, PaO₂ was further reduced and averaged 33.9±2.6 mm Hg over the next 50 min of hypoxic exposure. Unexpectedly, PaCO₂ was unchanged with hypoxic exposure during this series, perhaps partly due to the relatively low starting RAC value (33.8±0.5 mm Hg). Arterial blood pH was found to be higher than RAC (7.41±0.02) after 30, 50 and 60 min of hypoxia (all at 7.45±0.01). Hct
Table 6. Blood gas, osmolality, plasma electrolyte, and hematocrit responses to 60 min hypoxia. RAC = room air control. \( \text{PaO}_2 \) = arterial oxygen partial pressure, mmHg; \( \text{PaCO}_2 \) = arterial carbon dioxide partial pressure, mm Hg; OSM = plasma osmolality, mosm/kgH_2O; \( P_{Na} \) = plasma sodium, mEq/l; \( P_K \) = plasma potassium, mEq/l; Hct = hematocrit, percent packed cell volume. * = \( p<0.05 \) from RAC.

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was significantly increased by 20 min of hypoxic exposure (from 27.1 ± 0.6 to 30.6 ± 0.6) and was increased further after 40 min (34.0 ± 1.8). Plasma sodium and potassium concentrations (143.8 ± 0.9 and 3.9 ± 0.1 mEq/l, respectively) were unaffected by hypoxic exposure and did not change over the 60 min period. Plasma osmolality was decreased with the induction of hypoxia (from 290.6 ± 1.4 to 288.6 ± 1.5 mosm/kgH₂O). Thereafter, plasma OSM remained unchanged for the duration of hypoxic exposure.

Figure 18 details the HR response to 60 min of hypoxic exposure. An immediate, significant increase in HR was demonstrated (from 66.0 ± 3.8 to 90.3 ± 11.9 bpm). HR continued to increase, and by 40 min of exposure had reached a plateau of approximately 121 ± 11 bpm. In contrast to the HR response, figure 19 demonstrates no significant change in MABP over the 60 min hypoxic period. As is obvious from the graph, MABP tended to increase during the early period of exposure. The analysis of variance report for the data did not indicate a significant F-ratio. However, a Duncan's post-hoc test (Steel and Torrie, 1960) reveals significant increases in MABP after 10 and 20 min of hypoxia (from a RAC value of 106 ± 1 mm Hg to 112 ± 2 and 111 ± 2 mm Hg; 5 and 10 min, respectively).

Plasma NE concentrations (Fig. 20) were unchanged with hypoxic exposure and averaged 231.5 ± 49.9 pg/ml over the 60 min period. A large, but consistent variability is indicated in the figure and demonstrates a large and maintained range in the NE response between animals. Figure 21 shows
Figure 18. Heart rate (HR) response to 60 minutes of hypoxia (n = 4). Time is in minutes. RAC = room air control. Points represent means ± SEM. * = p < 0.05 from time 0 (RAC value).
Figure 19. Mean arterial blood pressure (MABP) response to 60 minutes of hypoxia (n = 4). Time is in minutes. RAC = room air control. Points represent means ± SEM. No significant differences were observed.
Figure 20. Plasma norepinephrine (NE) response to 60 minutes of hypoxia (n = 4). Time is in minutes. RAC = room air control. Points represent means ± SEM. No significant differences were observed.
Figure 21. Plasma epinephrine (EPI) responses to 60 minutes of hypoxia (n = 4). Time is in minutes. RAC = room air control. Points represent means ± SEM. No significant differences were observed.
that plasma EPI, like plasma NE, is unaffected by hypoxic exposure in conscious goats. Plasma EPI concentrations appear to decrease after 30 min of exposure, although the reduction in concentration was not significant (from 196.9 ± 31.1 to 125.2 ± 23.3 pg/ml; RAC to 30 min, respectively).
CHAPTER IV

DISCUSSION

The cardiovascular and endocrine responses to both hypoxic exposure and hemorrhagic hypotension have each been separately described in detail by many investigators. For example, it is now recognized that most conscious animal models display a two phase cardiovascular and endocrine response to a progressive blood loss (Schadt and Ludbrook, 1991). Additionally, it has been demonstrated that hypoxic exposure requires several cardiovascular, neurohumoral and endocrine adjustments to maintain normal blood pressure, flow and delivery. Even so, both intact and sino-aortic denervated animals are capable of regulating blood pressure during hypoxia (Miki et al, 1987). Yet, this study conclusively supports the notion that a hemorrhage during hypoxia represents a greater challenge to the cardiovascular system than a hemorrhage under normoxic conditions. Thus, unique endocrine responses are elicited during blood loss with hypoxia. Based on the findings of Heistad and Wheeler (1970), I hypothesized that a hemorrhage during hypoxia, compared with normoxia, would result in an earlier and greater development of hypotension, and thus hormones regulated by pressure changes would also respond differently. The research detailed in this report has demonstrated that a blood loss during hypoxia elicits an earlier development of hypotension. However,
the magnitude of the hypotension associated with hemorrhage during hypoxia was not found to be greater than that associated with blood loss during normoxia. Clearly, compensatory mechanisms allow the intact conscious goat to maintain blood pressure following a hemorrhage during hypoxia. The cardiovascular and endocrine responses to both hemorrhage and chemically-induced hypotension during hypoxia suggest unique compensatory adjustments allowing for the maintenance of arterial blood pressure.

**Cardiovascular Responses to Hypoxia in the Conscious Goat**

Not surprisingly, the conscious adult female goat, exposed to hypoxia ($\text{FiO}_2 = 0.10$), responds with a profound and sustained tachycardia. Similar observations have been made in the most all conscious animal models, including the rat, dog, and human (Raff et al., 1991; Walker, 1983; Claybaugh et al., 1978, respectively). As mentioned briefly in the introduction, the increase in heart rate associated with hypoxic exposure is most certainly due to both an increase in sympathetic nerve activity and a decrease in vagal control of the heart. However, the degree of involvement of the two neural influences appears to vary among species, and to my knowledge, no study has investigated the nature of the hypoxia induced tachycardia in the conscious goat. However, the catecholamine response to hypoxia was addressed twice in this study, with one experiment specifically designed to closely follow the
norepinephrine and epinephrine responses to hypoxia over a 60 minute time period. While the measurement of plasma norepinephrine cannot be directly equated with sympathetic nerve activity, studies have indicated a good correlation between the two (Wallin et al., 1980). In this study, no significant increase in plasma norepinephrine (see Fig. 20) could be documented during hypoxic exposure, suggesting that the associated tachycardia in the conscious goat was due primarily to vagal withdrawal. At least, if the increased heart rate with hypoxia was due to increased sympathetic nerve activity, any increase in norepinephrine spill-over into the plasma was not great enough to be detected. This, of course, is a very real possibility, although other data and further discussion presented below favors a withdrawal of vagal control of the heart with hypoxia in the conscious goat.

The observation that the conscious adult female goat does not increase mean arterial blood pressure with hypoxic exposure is also not surprising. Human subjects exposed to a similar level of hypoxia also do not demonstrate an increase in blood pressure (Ashack et al., 1985; Claybaugh et al., 1978). However, an increase in blood pressure during hypoxia is a consistent finding in the conscious dog (Raff et al., 1981; Rose et al., 1984; Walker, 1983). Thus, important species differences exist in the cardiovascular responses to hypoxia and must be considered when making comparisons of research findings.

An increase in cardiac output with hypoxic exposure has also been
observed in rats (Walker, 1986) and dogs (Bagshaw et al., 1986; Walker, 1983). While cardiac output was not directly measured in this study, an increase in HR with no change in MABP strongly suggests that cardiac output was increased. Further, then, a decrease in total peripheral resistance in the conscious goats with hypoxia would also be suggested. Local vascular hypoxemia elicits vasodilation, with certain vascular beds being more responsive than others (Heistad and Abboud, 1980), while chemoreceptor reflexes act to partially offset the vasodilation and maintain blood pressure and flow. Undoubtedly, similar cardiovascular adjustments are made in the conscious goat that allow for the maintenance of arterial blood pressure during moderately severe hypoxia.

Figures 18 and 19 of the results section document the HR and MABP responses to a one hour hypoxic exposure period. Heart rate was increased at the first measurement point (after 10 min) and continued to further increase until about 30 to 40 minutes of exposure. The last 30 minutes of hypoxic exposure were characterized by a relative plateau in HR, with the tachycardia representing an approximate doubling in HR over the room air level. Mean arterial blood pressure tended to rise early on with hypoxic exposure in the conscious goat (Fig. 19), although a one-way ANOVA failed to demonstrate a significant F-ratio. However, the ANOVA incorporates a pooled variance term, and it is for this reason that statisticians allow for post-hoc tests without a significance of F (Steel and Torrie, 1960). Thus, a Duncan’s Multiple-Range
test was applied to the MABP pressure data from the 60 min hypoxic exposure experiment. MABP was then found to be significantly increased after 10 and 20 minutes of hypoxic exposure; MABP was returned to room air levels for the remainder of hypoxia. Therefore, the apparent immediate cardiovascular responses to hypoxia in the conscious goat include a significant tachycardia with a transient rise in MABP. The sustained tachycardia and return of MABP to control levels suggest vascular adjustments to hypoxemia, including a reduction in total peripheral resistance. The importance of these apparent vascular adjustments becomes evident in the interpretation of cardiovascular responses to hemorrhage with hypoxia.

**Endocrine Responses to Hypoxia in the Conscious Goat**

Hormonal responses to hypoxia (FiO₂ = 0.10) were assessed several times in the studies outlined herein. Time control experiments that employed only normoxic exposure or hypoxic exposure were run to specifically address the influence of hypoxemia on the status of plasma hormones. Additionally, I have investigated the influence of acute hypoxic exposure on plasma hormones through serial blood sampling over a 60 min time period. The administration of hypoxic gas was successful in reducing PaO₂ during all experiments, although plasma concentrations of AVP, PRA, ANF, and the catecholamines NE and EPI were not altered.
The AVP response to normoxia and hypoxia alone in the hemorrhage series time control experiments is presented in table 2. As mentioned in the results section, plasma AVP was undetectable in two of the normoxic control and two of the hypoxic control animals. Thus, despite an assay sensitivity (0.06 uU/ml) greater than that most often reported in the literature (see Arnauld et al., 1977; Larsson et al., 1978; Wang et al., 1983), I was unable to detect basal concentrations of plasma AVP. In the three animals that had measurable concentrations of AVP, hypoxia was ineffective in altering plasma levels of the hormone. Further, plasma concentrations of AVP were undetectable in three of the four animals during the normoxic and hypoxic portions of the SNP infusion series time control experiments (Table 4). While the findings from the two experiments are in agreement, they further support the fact that hypoxia does not increase plasma AVP concentrations in the conscious goat.

Raff and colleagues (1983b) have shown a hypoxic stimulation of AVP in anesthetized, artificially ventilated dogs. The investigators determined the hypoxemic threshold for increased plasma AVP concentrations to be about $P_{aO_2} = 35$ mm Hg in normocapnic animals, a level of hypoxemia close to that observed with the present experiments. Other studies, utilizing conscious dogs have also demonstrated an increase in plasma AVP concentrations at a similar level of hypoxemia (Walker, 1983; Rose et al., 1984). Interestingly, hypoxia also increases MABP in anesthetized and conscious dogs, indicating that the chemoreceptor stimulation of AVP is strong enough to overcome baroreceptor...
inhibition of AVP release. In fact, if the increase in MABP with hypoxia is prevented in anesthetized dogs, a greater AVP response is observed (Raff et al., 1983a). Like the conscious dog, the conscious, spontaneously breathing goats in the present studies developed an associated hypocapnia with hypoxic exposure and no change in plasma osmolality. Unlike the dog, no change in MABP was observed after 60 min of hypoxia in the goat. Thus, the conscious goat would seem an ideal model for the chemoreceptor control of AVP stimulation. However, it is clear that strong species differences in the AVP response to hypoxia exist, with the observations in the conscious goat being very unlike those from dogs, and more similar to those from human subjects (Claybaugh et al., 1989). The threshold PaO\textsubscript{2} for AVP stimulation in the conscious goat remains unknown.

Hypoxia has been associated with reduced renin release in human subjects. Hogan et al. (1973), and Maher and colleagues (1975a) studied human subjects at high altitude and noted a decrease in PRA and content, respectively. However, Lawrence and associates (1990), were unable to show an effect of normobaric hypoxia (O\textsubscript{2} = 12\%) on PRA in humans. Likewise, normobaric and hypobaric hypoxia were ineffective in stimulating renin release in human subjects studied by Heyes et al. (1982). An FiO\textsubscript{2} = 0.10 was not associated with a change in PRA in conscious goats in the present studies (Tables 2 and 4). As mentioned earlier in the introduction, several other circulating hormones have been shown to have a direct effect on renin release.
Increases in plasma AVP within the physiological range have been shown to decrease renin secretion (Malayan et al., 1980; Reid, 1983), as have increases in plasma ANF concentrations (Henrich et al., 1988). Further, increases in circulating catecholamines, especially epinephrine, are effective in increasing PRA (Johnson et al., 1979a, 1979b). A role for AVP regulation of renin during hypoxic exposure in the goat would not be anticipated as no change in plasma AVP was associated with the reduction in PaO₂. Likewise, as will be discussed below, no change in the plasma catecholamines or ANF were detected in response to hypoxia in the goat. Thus, there would be little reason to suspect a hormonal alteration in PRA in hypoxic goats. An unaltered PRA with hypoxia in the conscious goat, then, is not unique, and is also not contradicted by humoral changes.

Interest in a hypoxic stimulation of ANF has been generated from studies such as that by Baertschi et al. (1988), that demonstrated an increase in the hormone in hypoxic anesthetized rabbits. Similarly, Baertschi and Teague (1989) have shown an increase in plasma ANF in conscious lambs breathing 10% oxygen. Decreased metabolism of circulating ANF could not account for the increase in plasma concentrations of the hormone from their study. Conflicting results from human studies, however, cast some doubt on the role of hypoxia in stimulating ANF release. DuSouich and co-workers (1987) exposed human subjects to 120 min of 10% oxygen and noted an increase in plasma ANF concentrations. However, exposure to hypoxic gas was also
accompanied by a slight hypercapnia and increase in diastolic pressure, making interpretations of the hormonal responses difficult. A study in the following year by Bartch and colleagues (1988) examined the effects of high altitude exposure (4,559 m) on plasma ANF concentrations in human subjects. After 24 hr of high altitude exposure, subjects were divided into two groups based on symptoms associated with acute mountain sickness (AMS). Those subjects presenting signs of AMS had significantly elevated levels of plasma ANF, while the hormone concentration was unchanged in the asymptomatic group. In addition, the AMS group displayed significantly elevated increases in plasms NE and EPI concentrations. Findings from this study indicate that hypoxic exposure is not accompanied by an increase in plasma ANF in conscious, spontaneously breathing goats. Table 2 displays the ANF response to 120 min of hypoxic gas exposure in the hemorrhage series time control experiments. No changes in ANF are seen. Similarly, plasma concentrations of ANF were not altered with hypoxic exposure in the time control experiments from the SNP infusion experiments (Table 4). Thus, I am confident that hypoxic exposure is not associated with a change (increase or decrease) in plasma ANF concentrations in the goat.

A possible explanation for the difference in ANF responses to hypoxia may be due to hormonal interactions during hypoxia. In investigating mechanisms for increased ANF secretion with hypoxia in rat hearts, Lew and Baertschi (1988) demonstrated that prior catecholamine depletion in intact animals
partially prevented the rise in ANF release with hypoxia. Further, the authors concluded that both alpha- and beta-andrenergic stimulation of the atria were responsible for approximately half of the increase in ANF seen with hypoxia. This possible mechanism for increased ANF release with hypoxia is especially attractive as the symptomatic subjects in the above study (Bartsch, et al., 1988) demonstrated increased levels of both plasma NE and EPI. Further, no increase in plasma ANF was observed in hypoxic goats in the present study, as plasma NE and EPI concentrations also remained unchanged.

Hypoxic exposure failed to increase plasma concentrations of the catecholamines norepinephrine and epinephrine in the present study. Table 4 of the results summarizes the findings of NE and EPI after 70 min of normoxia and hypoxia in conscious goats. Plasma concentrations of both NE and EPI were unchanged from room air values after 70 min of normoxic or hypoxic exposure, and no differences were observed between groups. The observation of unchanged plasma concentrations were initially met with skepticism, as most animal models studied (except humans) have shown an increase in both hormones. This fact, along with the possibility that a transient rise in the hormones had occurred during the first 60 min of exposure (that is, prior to the first blood sample; plasma half-life of NE = 1.5 min; Goldstein, 1987), led to a closer investigation of the hormonal responses to acute hypoxia. However, findings from the 60 min hypoxia series of experiments also could not demonstrate an increase in plasma NE or EPI with hypoxia (Fig.s 20 and 21).
While the conscious dog has been shown to have increased plasma levels of both NE and EPI with hypoxia, increases in the hormones have not been observed in human subjects acutely made hypoxic (Ashack et al., 1985). Thus, the cardiovascular and hormonal responses to hypoxia in the conscious goat are closely matched by observations from human studies. This study has shown that the conscious goat is an excellent model for the study of physiological responses to hypoxia, and presumably the responses to hemorrhage as well.

While this study has focused on the responses of plasma AVP, PRA, ANF and the catecholamines, it should be recognized that several other vasoregulatory factors are known to be influenced by hypoxic exposure. Isolated guinea pig hearts show increased release rates of the coronary vasodilator adenosine upon perfusion with a 30% O₂ medium (Schrader et al., 1977). In addition, inosine and hypoxanthine release rates are also found to increase with hypoxic perfusion, and the increases in all three substances strongly correlate with reductions in coronary resistance (Schrader et al., 1977).

The circulating prostaglandins are also known to have strong vasoactive properties. Animal studies have shown direct, potent vasodilatory actions of the E and A series prostaglandins. The E and A series prostaglandins are also known to dilate the pulmonary vasculature, while PGF₂α has been shown to increase pulmonary arterial pressure in dogs, cats and calves (Malik and
McGiff, 1976). However, a definite role for prostaglandins in regulating arteriolar tone with hypoxia has yet to be shown. In fact, one study discounts any prostaglandin involvement in hamster cheek arteriolar responses to changes in oxygen tension (Jackson, 1986). Finally, PGE$_1$ is known to have antagnoizing effects on the pressor actions of angiotensin II and AVP in rabbits and rats (Malik and McGiff, 1976). Thus, a more encompassing study on the hormonal regulation of blood pressure during hemorrhage could include an assessment of interactions of prostaglandins with angiotensin II and AVP.

Finally, several other circulating substances, including leukotrienes, serotonin and endothelium-derived relaxant factor (EDRF) have potential vasoregulatory effects (Letts and Cirino, 1985; Vanhoutte, 1990; Pohl and Busse, 1989; Pohl, 1990). Recent evidence indicates an oxygen-sensing capability of vascular endothelial cells as demonstrated by a PO$_2$-dependent release of EDRF. Increases in plasma EDRF (nitric oxide) are associated with hypoxemia, suggesting involvement of the autacoid in vascular responses to hypoxia (Pohl and Busse, 1989). The present study was designed to investigate the responses of well characterized hormonal systems to hemorrhage and hypoxia. A brief review of the literature suggests that other circulating factors could be, in part, responsible for the early development of hypotension with blood loss during hypoxia. Further avenues of research, then, could examine the responses of other vasoactive agents, including the prostaglandins, leukotrienes, adenosine, and EDRF under conditions similar to
those outlined in this study.

Cardiovascular and Endocrine Responses to Hemorrhage and Hypotension

The removal of arterial blood at 0.5 ml/kg/min in the conscious goats produced a significant hypotension only after the completion of blood loss. Clearly, however, pressure was declining during the 30 min of blood removal. Regardless, a lengthy period of nonhypotensive blood loss existed under normoxic conditions (Fig. 6). An reflex increase in HR was observed after 30 min of hemorrhage (time 90 in Fig. 7) in the normoxic animals; the change in heart rate was reversed during the post-hemorrhage, hypotensive period (time 120). These observations are consistent with a two-phase cardiovascular response to progressive blood loss, and are supported by a number of other conscious animal studies (Schadt and Ludbrook, 1991).

The hemorrhage with hypoxia experiments provided much different results and indicated that blood loss concurrent with acute hypoxemia presents a greater challenge to the cardiovascular system than hemorrhage under normoxic conditions. MABP was significantly reduced after only 20 min of blood loss during hypoxia. This observation, together with the fact that MABP was unchanged at the same time in the NH group indicates that the regulation of blood pressure during hemorrhage is altered. In fact, based on the findings of Heistad and Wheeler (1970), such a hypothesis was advanced at the
beginning of this study. A reduction in peripheral resistance is commonly
observed with hypoxia, and cardiovascular contractile reflexes have been
shown to be diminished while breathing hypoxic gas. However, this study
documents the first time that blood pressure responses to hemorrhage with
hypoxia have been observed during the course of blood loss in a conscious
animal model. Two studies by Raff and colleagues (1986, 1991) have also
examined the combined effects of hypoxia and hemorrhage, although the first
observations of blood pressure changes were not made until 10 min following
completion of the blood loss. Also, the design of the studies dictated a rapid
withdrawal of blood. No significant differences in MABP were observed
between the two groups of conscious rats studied. The authors (Raff et al.,
1986, 1991) concluded that hypoxic exposure did not alter the blood pressure
response to hemorrhage. The same conclusion could be reached from the
present study if only the post-hemorrhage blood pressure measurements (time
120, Fig. 6) were compared. The final MABPs produced by hemorrhage were
nearly identical under normoxic and hypoxic conditions, although clearly the
development of hypotension was greatly accelerated by hypoxemia.

As mentioned above, an increase in HR was detected during hemorrhage
under normoxic conditions, and this tachycardia was presumably a result of
reflexes induced by unloading of the arterial baroreceptors. The SNP infusion
experiments more clearly demonstrated the reflex increase in HR with arterial
hypotension under normoxic conditions (Fig. 12). In the same way,
assessments of baroreceptor reflexes have been repeatedly made with SNP in a host of other animal models (Brizzee et al., 1991; Fritsch et al., 1989). While hypoxic exposure alone produced a significant tachycardia in the conscious goats, no further increase in HR was observed with blood loss. However, owing to the rapid reduction in MABP during HH, it was possible that a further increase in HR could not be observed as the animals advanced quickly into the "second phase" of hemorrhage (that is, the period of sympatho-inhibition and vaso-vagal activation with prolonged hypotension). However, findings from the SNP infusion experiments have also shown a lack in heart rate response to hypotension during hypoxia. A reduction in baroreceptor mediated reflexes has been documented with aging, hypertension, and other disease states (Smith and Kampine, 1984). The data from this report indicates that hypoxia abolishes the baroreceptor reflex tachycardia associated with hypotension. Undoubtedly, the inability to increase heart rate with hypotension lead to a rapid reduction in cardiac output in a system where peripheral resistance was already reduced.

The AVP responses to hemorrhage, like the MABP responses, differed with regard to time. In both hemorrhage conditions, AVP was increased prior to a significant reduction in MABP. Clearly, though, MABP was already declining in both groups at the time of increased AVP levels (time 70 and 90, HH and NH, respectively). An earlier report of hemorrhage in the goat (Larsson et al., 1978) supports this finding, suggesting "normal" arterial baroreceptor
regulation of AVP. In the goat, as in the dog, then, high-pressure arterial baroreceptors appear to be the primary regulators of AVP release during hemorrhage. Cowley and co-workers (1987) have shown that a blood loss in conscious dogs that reduced atrial pressure without altering arterial pressure produced only minimal increases in plasma AVP concentrations. Constrictor levels of the hormone were not observed until arterial hypotension was produced.

However, a role for the low-pressure cardiac (left atrial) baroreceptors in regulating AVP release during hemorrhage in the goat cannot be excluded. The significant increases in plasma AVP concentrations before significant reductions in MABPs would argue in favor of low-pressure baroreceptor control (Goetz and Wang, 1988). However, the possibility of atrial baroreceptor control of AVP release in the goat remains an unsolved issue since no measurements of cardiac or central pressures were made.

In the present study, no significant differences were observed in the magnitude of AVP concentrations associated with hemorrhage during hypoxia or normoxia. In addition, a comparison of the slopes of the AVP and MABP responses generated through regression analyses was not statistically significant (p<0.1). Yet, a number of previous studies suggest a possible hypoxic enhancement of baroreceptor regulation of physiological functions, including the control of AVP. A comprehensive review of conscious animal studies by Korner (1971) indicates that hypoxia is capable of resetting the
baroreceptor reflexes. Also, an enhancement of chemoreceptor control of ventilation during hypoxia and hypotension was demonstrated by Heistad and co-workers (1975). Finally, Anderson et al. (1978) have provided evidence that a baroreceptor stimulation of AVP is responsible for the antidiuresis and decrease in free water clearance observed during hypoxia.

Indeed, in direct contrast to the findings from the present hemorrhage series of experiments, Raff et al. (1991) have reported an increased AVP response to hemorrhage in rats maintained in a hypoxic environment ($\text{FiO}_2 = 0.10$). Hemorrhage was induced in that study by removing 15 ml of blood /kg body weight rapidly over a 2 minute period. Although no measurements of blood pressure during or immediately following the hemorrhage were provided in the report, it can be assumed that the rapid removal of such a large volume of blood rapidly induced hypotension in both groups. Post-hemorrhage MABPs, at 10 and 20 minutes following the initiation of blood loss, were not different between the normoxic and hypoxic rats. Plasma AVP was significantly higher in the hypoxic rats at both time periods following hemorrhage (Raff et al., 1991).

Ideally, comparisons of hormonal responses to changes in blood pressure under normoxic and hypoxic conditions should incorporate an equal degree of hypotension imposed over similar time periods. In my studies, intravenous infusions of SNP in conscious goats reduced blood pressure rapidly in both instances, and the percent reduction in blood pressure between groups was
not different at 5 or 10 min of hypotension. This study has demonstrated that a reduction in MABP of approximately 20% through SNP infusion produces significantly greater plasma AVP levels during hypoxia than normoxia (Fig. 13). Seemingly, then, this work is in agreement with that of Raff et al. (1991). Unlike the study by Raff et al. (1991), the present study in conscious goats has directly examined a mechanism for the control of AVP, and in doing so has shown a hypoxic enhancement of the AVP response to hypotension, per se.

Lastly, with regard to the AVP responses to SNP induced hypotension, this study and work by others (Raff et al., 1990) has shown a high degree of variability in the AVP responses to SNP induced hypotension. Raff et al. (1990) reports that a reduction in MABP of 30 mm Hg increased plasma AVP to only 27 pg/ml (about 10.8 uU/ml) in one conscious dog, while a reduction in pressure of only 20 mm Hg was associated with an increase in AVP to 1,195 pg/ml (about 475 pg/ml) in a second animal. The authors contend, however, that the differences in AVP responses to hypotension between dogs were consistent within each animal over the course of several weeks. In a similar fashion, I have observed that the AVP responses to SNP induced hypotension in the conscious goat are also highly variable. For instance, in the normoxic time period, a reduction in MABP of 17.5% resulted in an increase in plasma AVP to 27.1 uU/ml, while a reduction in MABP of 17% in another goat did not elicit any change in hormone concentration. The AVP response to hypotension within animals was fairly consistent between the normoxic and
hypoxic time periods. That is, the animal with the greatest AVP response to hypotension during normoxia also showed the greatest response during hypoxia. As mentioned above, no increase in plasma AVP was observed in one animal made hypotensive with normoxia. The experiment was repeated in this animal, and again no increase in hormone concentration was observed with SNP infusion under normoxic conditions. The same animal responded to SNP induced hypotension during hypoxia with a significant increase in plasma AVP (from undetectable levels to 15.4 and 72.4 uU/ml after 5 and 10 min of hypotension, respectively). This observation, combined with the fact that SNP infusion was associated with greater AVP concentrations in every animal during hypoxic exposure, further supports a hypoxic enhancement of the AVP response to hypotension.

The PRA responses to hemorrhage in conscious goats were affected by hypoxia. The PRA responses to the early stages of blood loss were essentially identical between NH and HH (Fig. 9), with PRA being increased with blood loss before significant reductions in MABP were noted. Increases in PRA prior to reductions in MABP are suggestive of low pressure baroreceptor regulation of renal sympathetic nerve activity (RSNA). An unloading of the atrial baroreceptors decreases tonic inhibitory vagal afferent activity and leads to a reflex increase in renal sympathetic nerve efferent activity (Skoog et al., 1985). However, it is clear that PRA "peaked" in both NH and HH and was reduced toward the latter stages of the experimental period. In fact, the post-
hemorrhage (time 120) PRA value for HH was reduced to levels no longer
different from control. Closer observation indicates that the peak PRA
responses were conincident with the development of hypotension, and that the
reductions in PRA that followed also occurred during a period of established
hypotension. This is especially clear in the HH responses to hemorrhagic
hypotension. As mentioned in the introduction, it is now understood that the
development of arterial hypotension in conscious animals is accompanied by
a reduction in sympathetic nerve activity, most often measured as RSNA (Davis
Further with an estimated renin plasma half-life of approximately 4 to 15 min,
and the fact that a constant stimulation is required to maintain increased
release rates of renin (Keeton and Campbell, 1981), it is highly possible that
the reduction in PRA observed with hemorrhage under both conditions is due
to a reduction in renin release, and metabolism of the enzyme. The apparent
difference in the reduction in PRA following blood loss between NH and HH
may only be due to the time course difference in the development of
hypotension. It is not hard to visualize "shifting" the line representing the
MABP and PRA response in figures 6 and 9 such that it overlays the HH line.

Results from the SNP induced hypotension experiments are helpful in the
interpretation of the PRA responses to blood loss. With equal reductions in
MABP occuring over similar time periods, any apparent hypoxic alteration of
PRA is eliminated (Fig. 14). The increases in PRA that do occur with SNP
induced arterial hypotension may be mediated by reductions in atrial pressure (observations from Brooks, 1989; Brooks and Hatton, 1991) or withdrawal of RSNA following the rapid reductions in arterial pressure. The differences in the PRA responses observed with hemorrhage in the normoxic and hypoxic animals could also have been partly explained by the AVP responses. Reductions in stimulated renin secretion have been documented with concentrations of AVP much lower than those associated with the hemorrhages from this study (Malayan et al., 1980; Shade et al., 1973). Since high AVP levels were detected first with HH, it could be theorized that an AVP attenuation of PRA was occurring during hemorrhage with hypoxia, and thereby preventing measurable differences between the normoxic and hypoxic settings. However, while hypoxia augmented the AVP response to SNP induced hypotension, no differences were observed in the PRA increases. Thus, an AVP inhibition of PRA during hemorrhage with hypoxia seems unlikely.

This report has shown plasma ANF to be decreased during hemorrhage in both the normoxic and hypoxic settings (Fig. 10), although the time required to reduce the concentrations was different. While some researchers have reported increases in ANF with hemorrhage (Carlson et al., 1989), the present findings are supported by reports that have also shown decreases in the hormone after blood loss (Edwards et al., 1988). The reason for the delay in the ANF reduction in the HH group remains uncertain. Lew and Baertschi
(1989) have proposed a hypoxic stimulation of the hormone, and it is possible that the concurrent hypoxic exposure prevented an earlier reduction in ANF with hemorrhage. However, as I could not demonstrate a hypoxic stimulation of ANF in the time control experiments, this seems a remote possibility.

Seemingly also unexplainable is the lack of ANF response to hypotension induced by SNP infusion (fig. 15). Brooks (1989) has shown both left and right atrial pressures to be reduced in conscious dogs with SNP doses similar to those employed in the present study. Thus, there is little reason to believe that atrial pressures were not affected in the conscious goats.

Many studies have investigated increased atrial stretch and pressure on ANF release, while few studies have addressed the effect of atrial hypotension on the hormone. Prior to the experiments detailed in this report, seemingly no information was available on the ANF response to SNP induced hypotension. It is becoming increasing clear, however, that factors other than atrial pressure or stretch mediate the release of ANF from the heart. For example, Phillips et al. (1989) have shown a tonic inhibition of ANF release by the vagus nerves in rats. In their experiments, vagotomy resulted in a large increase in ANF despite reductions in atrial pressures produced by the procedure. Additionally, Lang and colleagues (1987) have provided evidence that circulating epinephrine acts to increase ANF release through both beta- and alpha-adrenoceptor pathways.

Unfortunately, plasma catecholamines were not measured in the
hemorrhage series experiments, as the assay was not yet established in the laboratory. However, plasma NE and Epi were measured in the 60 min hypoxia exposure experiments (above), as well as the SNP induced hypotension experiments. Hypoxic exposure was ineffective in increasing either plasma NE or EPI. Similarly, SNP induced hypotension was not associated with an increase in plasma NE concentrations. Arterial hypotension, as produced through progressive hemorrhage, has been associated with a reduction in sympathetic nerve activity (Hesser and Schadt, 1992; Skoog et al., 1985), while SNP induced hypotension is without effect on NE (Brooks and Hatton, 1991). Also, at least one other study (Brooks, 1991) has measured plasma NE in a conscious animal model following SNP induced hypotension and also found no change in hormone concentration. In that study, SNP infusion into conscious dogs pretreated with an AVP antagonist significantly reduced MABP without altering plasma NE concentrations. However, plasma EPI concentrations were found to significantly increase. The author concluded that the SNP infusions had activated the Bezold-Jarisch reflex, since a general sympathetic nerve activity decrease with selective adrenal nerve increase had seemingly occurred. Activation of the same reflex (Smith and Kampine, 1984) is indicated in the data from the present experiments.

Plasma EPI concentrations were significantly increased after 10 min of SNP induced hypotension during both normoxia and hypoxia (Fig. 17), indicating an increase in adrenal nerve activity. However, no difference in the EPI
response to SNP induced hypotension was present between normoxic or hypoxic exposure. Thus, hypoxia neither enhances nor attenuates the EPI response to arterial hypotension in the conscious goat.

The increased EPI concentrations following SNP induced hypotension indicates a role of the arterial baroreflexes in increasing adrenal release of the hormone. However, the same conclusion was not reached by Fater et al. (1983) with conscious dogs. In their study, occlusion of the common carotid arteries for a period of 5 minutes did not significantly increase plasma EPI concentrations in a group of aortic arch denervated dogs. The differences in the results obtained by Fater et al. (1983) and those presented in this study may be due to both the species examined and the methods employed.

Finally, the lack of a hypoxic influence on the EPI response to SNP induced hypotension in the conscious goat is compatible with other hormonal responses. That is, both PRA and ANF have been shown to be affected by increased circulating levels of EPI. No differences in ANF concentrations or PRA were observed between normoxic and hypoxic exposure periods as no differences in EPI were detectable.

Summary of Findings

In summary, the experiments detailed in this report have shown the conscious adult female goat to be a suitable model for the study of hypoxic
exposure, hemorrhage, and chemically induced hypotension. The cardiovascular and endocrine responses of the goat to the individual stimuli appear to be very similar to those observed in humans. Thus, the conscious goat also appears to be a most suitable model for the study of physiological responses to combined hemorrhage/hypotension and hypoxia.

This study has demonstrated that a hemorrhage under hypoxic conditions results in an earlier development of hypotension than an identical blood loss performed with normoxia. However, certain mechanisms are present in the intact conscious animal that prevent a greater absolute reduction in MABP. Also, a hypoxic attenuation of the baroreceptor reflex increase in HR has been shown with both hemorrhagic hypotension and SNP induced hypotension in the conscious goat.

Similar to the observed responses in MABP, the hormonal responses to hemorrhage have been shown to be altered with hypoxic exposure. An apparent hypoxic attenuation of the PRA response to hemorrhage was observed in this study. However, further investigation employing a reduction of blood pressure during normoxia and hypoxia over equal time periods failed to produce significant differences between exposures. Thus, the similarity in PRA observed with hemorrhage during normoxia and hypoxia may represent a continued stimulation of renin release on one hand (normoxia, through atrial baroreceptors) and attenuated release of the enzyme on the other hand (hypoxia, reflex suppression of RSNA with arterial hypotension).
Hemorrhage with hypoxia produces an earlier increase in plasma AVP concentrations, just as MABP is reduced sooner with that exposure. The release of AVP during hemorrhage with hypoxia, then, probably results from an unloading of arterial baroreceptors. The increase in plasma AVP during hemorrhage with normoxia appears to be mediated first through atrial baroreceptors (as increases in hormone concentrations are observed before reductions in MABP are present), and later through arterial baroreceptor involvement. However, hypoxia has been shown to augment the AVP response to hypotension when blood pressure is reduced over an equal time frame during normoxia and hypoxia. The exact mechanism for this apparent hypoxic enhancement of AVP control is unknown at this time but may involve an interaction of carotid chemoreceptors and baroreceptors.

Finally, a rapid reduction in MABP with SNP fails to significantly increase plasma NE concentrations during normoxia or hypoxia in the conscious goat. However, plasma EPI concentrations are significantly elevated with the same maneuver, although no difference in the response is seen between the normoxic or hypoxic exposures. Thus, the selective sympathetic activation resulting from SNP induced hypotension appears not to be affected by hypoxia.
APPENDIX

Assay Procedures

Arginine vasopressin radioimmunoassay. The assay methods for the detection of plasma arginine vasopressin have been developed in the laboratory of Dr. John R. Claybaugh at the Department of Clinical Investigation, Tripler Army Medical Center.

A). Preparation of 0.2 M Phosphate Assay Buffer.

1. Dissolve 14.197 g 0.2M Na$_2$HPO$_4$ (dibasic) into 500 ml distilled H$_2$O.
2. Dissolve 6.895 g 0.2M NaH$_2$PO$_4$ (monobasic) into 250 ml distilled H$_2$O.
3. Combine 450 ml of first solution with approximately 120 ml of second solution until pH = 7.2.
4. Add 3.3 g NaCl and 1.0g BSA to 500 ml of combined solution. Bring up to volume with 500 ml distilled H$_2$O.


1. Wash 4 g Norit carbon 6 times, 15 minutes each, with 500 ml wash solution (NaCl, 3.3 g/L; 500 ml assay buffer; 500 ml filtered H$_2$O).
2. Add 800 mg BSA to 500 ml wash solution. Mix with washed carbon and store refrigerated until use.


1. Collect whole blood into iced heparinized tubes.
2. Centrifuge blood under refrigeration. Separate plasma (4 ml), acidify with 0.1 ml/ml plasma of 1 N HCl, and store in -70 freezer until assay date.


1. Slowly thaw plasma samples in ice bath. Also thaw three acidified goat plasma stock samples. Vortex and centrifuge all samples. Add 10 ul AVP (1mU/ml) to 2 acidified goat plasma stock samples.
2. For every sample to be extracted, obtain a Sep-Pak cartridge (Waters, Millipore Corp). Wash each cartridge with 5 ml methanol, 5 ml 8 M urea, and 10 ml double-distilled H_2O.

3. Apply samples to cartridges. Wash with 10 ml dd-H_2O, 10 ml 4% acetic acid. Elute with 5 ml 40% ethanol/4% acetic acid. Save eluates.

4. On the following day, dry samples under slight heat and vacuum.

5. Resuspend residues with 0.5 ml assay buffer.

6. Prepare AVP standards in doses of 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 uU/tube.

7. Prepare dilutions of standards and controls in duplicate (200 uL/tube).

8. Prepare each sample in two doses (150 and 200 uL/tube). Add 50 uL of a 1:30,000 dilution of antisera to standards, controls and samples. Cover and refrigerate all tubes.

9. After 72 hr, add 50 uL of ^{125}I-AVP (New England Nuclear; 2,000-3000 cpm) to standards, controls and samples. Cover and refrigerate for an additional 48 hr.

10. Add 0.3 ml assay carbon to all tubes. Centrifuge for 20 min at 2000xG. Decant supernatant. Count both supernatant (antisera bound AVP) and carbon (free AVP) on gamma counter. The logit of the percent bound ^{125}I-AVP is plotted against the log of concentrations of standards used. Sample concentrations are calculated from this standard curve. Recovery of extracted sample is determined from the "spiked" and "unspiked" acidified goat plasma stock samples.

E). Cross reactivities. Antisera-96 has been determined to have the following cross-reactivities: AVP 100%, vasotocin <0.05%, oxytocin <0.05%, desglycinamide AVP <0.05%, pressinoic acid 0% (Uyehara and Claybaugh, 1988).

Plasma renin activity radioimmunoassay. The radioimmunoassay for the generation and measurement of angiotensin I from plasma was accomplished with a commercially available kit from New England Nuclear (Rianen Assay System Angiotensin I Radioimmunoassay Kit). All reagents were supplied with the kit.
A). Sample preparation. Whole blood is collected into iced Na-EDTA tubes. Plasma is separated from blood with centrifugation and is kept frozen until assay date.


1. Plasma samples and controls (New England Nuclear) are slowly thawed in ice bath.

2. 10 ul dimercaprol, 10 ul 8-hydroxyquinoline, and 2 ml maleate buffer are added to 1 ml of plasma sample and controls.

3. 1 ml aliquots of the above mixture are placed in a 37 degree centigrade shaker water bath for 60 min. The remainder of the mixture is kept on ice.

4. After 60 min, the incubated samples are placed on ice and angiotensin I standards are prepared in doses of 0.01, 0.025, 0.05, 0.1, 0.25 and 0.5 ng/ml.

5. 500 ul of antisera and 100 ul of $^{125}$I-angiotensin I is added to standards, plasma samples and controls. All tubes are incubated at 4 degrees centigrade for 18 hr.

6. 1.0 ml charcoal suspension is added to the assay tubes. Tubes are then centrifuged at 1200xG for 20 min. Supernatant is decanted from all tubes and counted on a gamma counter. A standard curve is constructed from the counts obtained from the known angiotensin I standards. Sample concentrations are calculated from this curve. Ungenerated angiotensin I (from sample portion kept on ice) is subtracted from generated angiotensin I (from 1 hr incubation) to accurately reflect plasma renin activity.

Atrial natriuretic factor radioimmunoassay. The measurement of goat atrial natriuretic factor(s) was achieved through use of a commercially available radioimmunoassay kit (Peninsula Laboratories radioimmunoassay for alpha human atrial natriuretic ploypeptide). Assay buffer, alpha-hANP, rabbit anti-alpha-hANP serum, goat anti-rabbit IgG serum, normal rabbit serum and $^{125}$I-hANP were provided with the kit.

A). Sample preparation. Whole blood is collected and placed in iced Na-EDTA tubes. Plasma is separated from blood with centrifugation and is treated with 50 ul aprotinin (10,000 kIU/ml) per 2 ml plasma volume. Plasma samples are kept frozen (-70 degrees centigrade) until assay date.

1. Plasma samples and control plasma ("unspiked" and "spiked" with 20 ul 6,400 pg/ml) are slowly thawed in an ice bath.

2. All samples are acidified with a volume of 1 N HCl equivalent to 10% of sample volume and vortexed.

3. Sep-Pack cartridges (Waters, Millipore Corp.) are prepared with washes of 5 ml 100% methanol followed by 10 ml distilled H₂O. Next, acidified plasma samples are added to the cartridges, followed by an additional 10 ml distilled H₂O wash.

4. Samples are eluted from cartridges with 2 separate 2.0 ml washes of 80% methanol; 0.1% trifluoroacetic acid; 19.9% distilled H₂O. The two eluates are combined and saved.

5. Eluates are evaporated under slight heat and vacuum until dry.

6. On the day following extraction, dilutions of alpha-hANP standard are prepared to equal 10, 20, 40, 80, 160, 320, 640, and 1280 pg/ml.

7. Dried eluate residues are resuspended in a volume of assay buffer equal to 25% of original plasma volume. 100 ul rabbit anti-alpha-hANP serum is added to 100 ul aliquots of samples and standards. All tubes are left for 24 hr at 4 degrees centigrade.

8. On the third day, 100 ul ¹²⁵I-alpha-hANP is added to standards and samples. All tubes are kept at 4 degrees centigrade for an additional 24 hr.

9. On the fourth day, 100 ul goat anti-rabbit IgG serum and 100 ul normal rabbit serum is added to samples and standards. Tubes are left at room temperature for 150 min.

10. 0.5 ml assay buffer is added to all tubes, and tubes are centrifuged at 1700xG for 20 min. Supernatant (free ¹²⁵I-hormone) is decanted from pellet (bound ¹²⁵I-hormone). Both supernatant and pellet are counted on a gamma counter. Construction of standard curve and calculation of sample concentration is achieved as described above in AVP assay.
C). Cross reactivities. Manufacturer's supplied cross reactivities with rabbit anti-alpha-hANP serum are as follows: alpha-hANP 100%, beta-hANP 94%, rat atriopeptin III 100%, ANP(18-28) 57%, oxytocin 0%, arginine vasopressin 0%, urodilatin 100%.

Plasma catecholamine HPLC-EC assay. The measurement of plasma norepinephrine and epinephrine was achieved with high pressure liquid chromatography with electrochemical detection (HPLC-EC). The extraction and detection methods presented herein have been modified from other reports (Goldstein, 1986; Goldstein et al., 1981; Jiang and Machacek, 1987). Unless otherwise noted, all reagents, supplies and apparatus were obtained from BAS, Inc.


1. 10.35 g NaH₂PO₄·H₂O, 340.5 mg EDTA and 206.5 mg sodium octylsulfate is dissolved in 800-900 ml distilled H₂O.

2. Above solution is adjusted to pH 3.1 with H₃PO₄, and brought up to a volume of 1000 ml with distilled H₂O. Next, 10 ml of solution are removed and replaced with 10 ml CH₃CN to make a 1% acetonitrile mobile phase solution.

3. The mobile phase is filtered and de-gassed under vacuum before use. Flow rate through the HPLC-EC apparatus (BAS, Inc.) is kept at 0.5 ml/min during standby, and 1.0 ml/min during sample measurement periods.


1. Whole blood is collected in iced heparinized tubes.

2. Blood is centrifuged under refrigeration. Plasma (1 ml) is immediately separated from cells and frozen at -70 degrees cetigrade until extraction date (within two weeks).

C). Sample extraction.

1. Slowly thaw plasma samples and two goat plama stock aliquots in ice bath. To one aliquot of goat plasma stock, add 10 ul of a NE/EPI solution (75 and 25 ng/ml, respectively). Transfer thawed
plasma to glass extraction vials containing 50 mg acid washed alumina.

2. Next, 10 ul of an EDTA-Na₂S₂O₅ solution (50 mg EDTA, 50 mg Na₂S₂O₅ in 1 ml H₂O) and 500 ul of a 0.5 M Tris-HCl/Trizma base (Sigma Chemical Co.) solution (pH=8.4) is added.

3. Immediately vortex and shake samples for 5 min. Allow the alumina slurry to settle and aspirate and discard supernatant. Wash alumina 3 times with 0.5 ml ice cold distilled H₂O. Add an additional 1 ml H₂O, and transfer slurry to a microfilter apparatus.

4. Centrifuge microfilter units at 1000xG for 1 min. Place new receive tube on microfilter units and add 100 ul of 2% acetic acid to each sample. Vortex units briefly and allow to stand for 5 min. Centrifuge microfilter units at 1000xG for 3 min, and collect 100 ul extract. Store stable extract at -70 degrees centigrade until HPLC-EC measurement.

D). HPLC-EC measurement of plasma extracts.

1. Stabilize HPLC-EC apparatus with the following parameters: flow rate = 1 ml/min; applied potential = +720 millivolts; pen recorder zeroed to baseline.

2. Prepare four dilutions of NE (0, 1875, 3750, 7500 pg/ml) and EPI (0, 625, 1250, 2500) in 2% acetic acid. Slowly thaw plasma extracts.

3. Apply 20 ul of standards, samples and known extracted controls to column with glass syringe (Hamilton, Inc.). Allow sufficient time between sample injections for clearance of all detectable substances.

4. Plot standard peak heights against concentration and extrapolate sample concentrations from the resultant curve. Recovery of extracted catecholamines is determined from extracted "spiked" and "unspiked" goat plasma stock aliquots.
REFERENCES


