THE UNIVERSITY OF CALGARY

Age-dependent Effects of Furosemide on Cardiovascular Control in Conscious Lambs

by

Julie J. Thomson

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF CARDIOVASCULAR

AND RESPIRATORY SCIENCES

CALGARY, ALBERTA

JUNE, 1999

ABSTRACT

Previously, we observed that furosemide elicits a transient increase in blood pressure (BP) whilst heart rate (HR) remains unchanged. From this observation, we hypothesized that furosemide might alter the arterial baroreflex control of HR. Previous studies also suggest that some physiological responses to furosemide may be age-dependent. In conscious, chronically instrumented lambs aged, one and six weeks, we measured the baroreflex control of HR and hemodynamic responses, before and at 30-minute intervals after I.V. furosemide or vehicle. After furosemide, but not after vehicle, the baroreflex was altered at 30 minutes compared to control. This effect occurred in both age-groups, however, it occurred only transiently in older lambs. HR, BP and renal blood flow responses to furosemide were age-dependent. These data provide new evidence that furosemide alters the baroreflex and hemodynamic responses in an age-dependent manner. Developmental regulation of neurohumoral factors and/or their receptors may be involved in these responses.

ACKNOWLEDGEMENTS

I would like to thank Dr. Francine Smith for providing me with the opportunity to earn my Masters degree in her laboratory. I would also like to thank the members of my supervisory committee, Dr. James Fewell and Dr. Doug McMillan, for their insight and critical analysis of my Masters project. I would like to thank Dr. Fritz Lorschieder for acting as my external examiner. A special note of thanks for the encouragement and personal guidance that was kindly provided by: Dr. John Tyberg, Dr. Doug McMillan, Dr. David Severson, and Dr. Wayne Giles. Thanks also to Ms. Marney Cudmore and Ms. Judy Gayford for their assistance in my graduate study.

James Dobson, Heather Thomson and Teresa Killam were not only a great editing team but also an excellent support system and source of encouragement. To them, I will be forever grateful. Thanks to Alp Sener, who on a daily basis was always there to assist me in the lab, to share some laughs and the occasional two for one. Thanks to Andrea Cobbold, my grad school roommate, who always had the time to provide a listening ear and cook dinner after a very long day of experiments.

This acknowledgement would not be complete without thanking my family, Audri, Barry, Heather and Scott for their unrelenting support both financial and moral, throughout the course of my studies and my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

Introduction

1.1 CARDIOVASCULAR REGULATION

In 1926, Cannon in *The Wisdom of the Body* eloquently stated,

> *In an open system, such as our bodies represent, compounded of unstable material and subjected continually to disturbing conditions, constancy is in itself evidence that agencies are acting, or ready to act, to maintain this constancy* (1).

In this regard, the constancy of blood pressure in the body thus alludes to regulatory mechanisms such as a feedback control loop that maintains blood pressure within narrow limits around a "set-point", despite perturbations. The body achieves this homeostasis via a sophisticated interplay between numerous physiological mechanisms that act in concert. Of these mechanisms, the arterial baroreflex is paramount in its ability to respond to beat-to-beat changes in blood

pressure (2). It does so by modulating sympathetic and parasympathetic nerve activities (3).

1.11 Arterial Baroreflex

The arterial baroreflex operates around a "set-point" corresponding to the mean blood pressure; deviations from this "set-point" activate restoring mechanisms. Consequently, central regulation of the "set-point" may serve as a major determinant of neural outflow and hemodynamic stability (3).

Precise regulation of the cardiovascular system is vital to an animal's survival. Consequently, it is not surprising that the mechanisms devised for its control are diverse, rapid, redundant and adaptive. Hence, it must be emphasised that the arterial baroreceptors are one of many important baroreceptors involved in cardiovascular regulation. The cardiopulmonary baroreceptors, located in the heart and lungs, respond to changes in blood pressure but, unlike the arterial baroreceptors, respond preferentially to long-term changes in blood pressure (2). Evidence has revealed that, in most situations, the two sets of baroreceptors act in concert to maintain blood pressure and thus, lend to the redundancy of cardiovascular homeostasis (2,4). Also of importance are the renal baroreceptors. The renal baroreceptors are modified smooth muscle cells of the juxtaglomerular apparatus that respond to decreases in afferent arteriole

pressure by releasing the enzyme, renin (5). Renin is the rate-limiting enzyme in the formation of angiotensin II. In this thesis, discussion of neural mechanisms involved in cardiovascular regulation will be limited to the arterial baroreflex.

The arterial baroreflex is a negative feedback mechanism by which the central nervous system (CNS) controls arterial blood pressure (2). The baroreflex detects blood pressure and through the same reflex arc, affects blood pressure. The baroreflex thus uses its proprioceptive, or self-monitoring, reflex abilities to maintain arterial blood pressure within a narrow physiological range (6).

The components of the baroreceptor reflex arc include sensory afferents (stretch receptors) originating in the carotid sinus and aortic arch, an efferent component consisting of autonomic innervation of the heart and vasculature and a central neural component that links afferent input to efferent output (7).

Sensory afferents (stretch receptors) originate in the carotid sinus and aortic arch. Impulses arising from the carotid sinus are sent via the sinus nerve to the glossopharyngeal nerve and finally, to the nucleus tractus solitarius (NTS) in the medulla oblongata. Impulses arising from the aortic arch reach the NTS via afferent fibres of the vagus nerve (8). Changes in arterial pressure that activate or deactivate stretch receptors alter the firing frequency of baroreceptor afferent

nerves (6,7). For example, an increase in arterial pressure enhances the activity of baroreceptor afferents, which relay information centrally to the NTS (6,7).

For decades the CNS component of the arterial baroreflex was treated as the proverbial "black box" in which the mediators and the mechanism(s) by which afferent input was processed into the appropriate level of efferent output was largely unknown. More recently, CNS components of the arterial baroreflex have been identified along with neural pathways and neurotransmitters (Figure 1). Regulation of the baroreflex mediated sympathetic activity involves an excitatory neural pathway from the NTS to the caudal ventrolateral medulla (CVLM). The CVLM sends inhibitory projections to the "vasomotor centre" of the rostral ventral lateral medulla (RVLM) (7,9). Sympathoexcitatory neurons of the RVLM directly innervate and excite sympathetic pre-ganglion neurons involved in regulation of vasomotor and cardiac tone. Central control of vagal parasympathetic nerves must diverge from sympathetic control; the site of diversion is thought to be the nucleus ambiguus (7,10).

Baroreceptor afferents, responding to increases in arterial pressure, signal the NTS to make autonomic adjustments to restore blood pressure to homeostatic levels. The end result is an increase in vagal inhibitory activity and a decrease in sympathetic outflow, which act to reduce heart rate and vascular resistance allowing arterial pressure to be restored (2). Conversely, a decrease in arterial

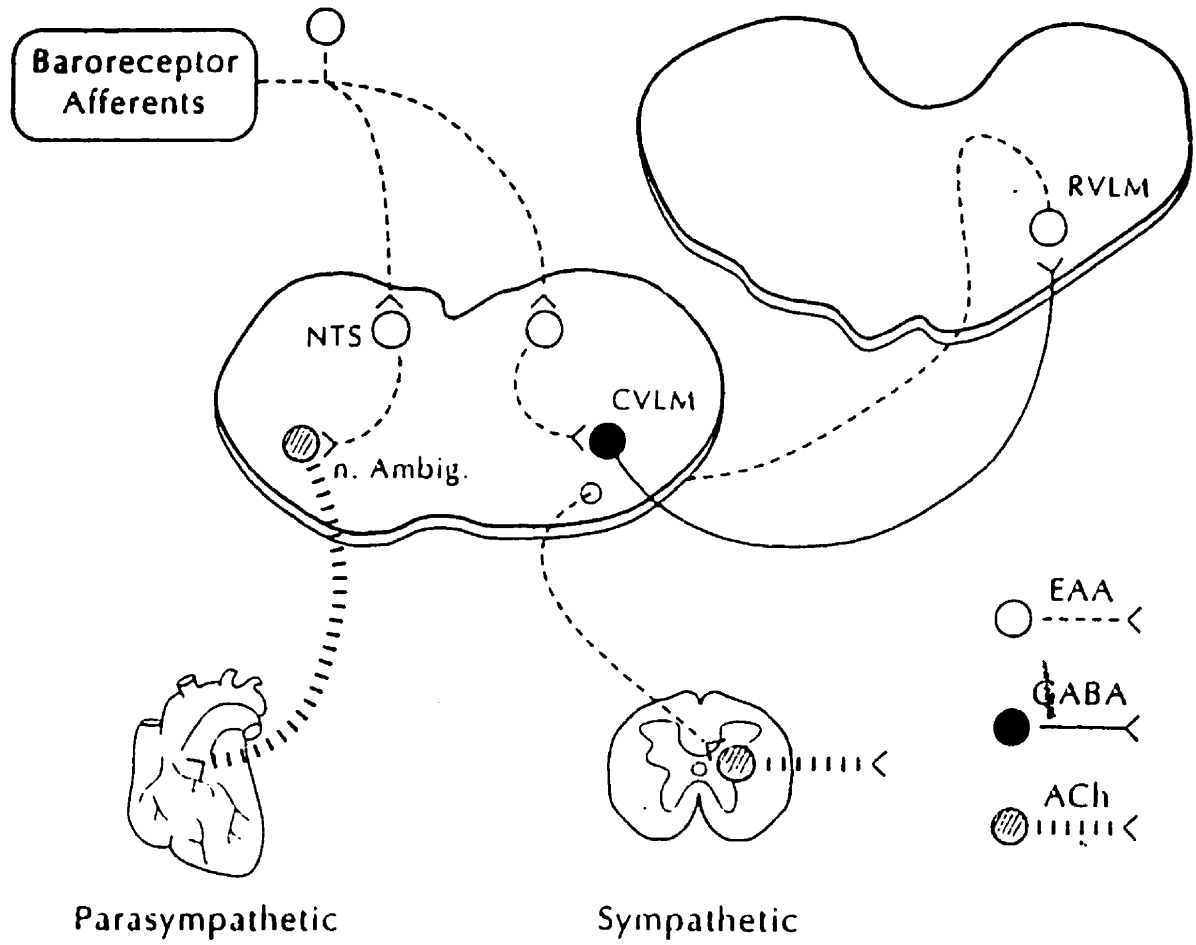


Figure 1. Diagram of the central connections of arterial baroreflex. ACh, acetylcholine; CVLM, caudal ventrolateral medulla; EAA, excitatory amino acid; GABA, γ-aminobutyric acid; n. ambig., nucleus ambiguus; NTS, nucleus tractus solitarius; RVLM, rostral ventrolateral medulla. Adapted from Sved and Gordon, 1994.

pressure diminishes baroreceptor afferent firing and subsequent alterations in autonomic activity, namely vasoconstriction and tachycardia, act to increase arterial pressure (2).

Alterations in heart rate are but one component of the arterial baroreflex response. Arterial and venous dilation and modifications in the force of cardiac contraction also contribute to the baroreflex response to an increase in blood pressure. It was not the objective of this study to evaluate the various components to the baroreflex but to investigate one component, the arterial baroreflex control of heart rate. Thus, future discussions will be confined to the arterial baroreflex control of heart rate.

1.12 Relationship of Blood Pressure to Heart Rate

Baroreflex curves were developed to quantitatively describe the physiological parameters of the relationship between heart rate (HR) and blood pressure (BP). The relationship between heart rate and blood pressure was illustrated to be inversely related and sigmoidal in nature (11). The mathematical model described by Kent and colleagues (1972), made it possible to calculate the parameters of the baroreflex, namely: gain (slope), BP at the midrange and maximum and minimum heart rates (11).

1.13 Angiotensin II's Modulation of the Arterial Baroreflex

Like most homeostatic mechanisms, regulation of the arterial baroreflex is finely tuned by other physiological interactions (i.e. hormones). For example, angiotensin II (ANG II) and arginine vasopressin (AVP) are two important peptides that modulate the "set-point" of the arterial baroreflex, as detailed below (12-14).

ANG II exerts important actions on the kidney, adrenal cortex and sympathetic nervous system. ANG II can stimulate the release of epinephrine and norepinephrine from the adrenal medulla and facilitates sympathetic transmission at adrenergic nerve terminals (15). In addition, ANG II acts directly on the adrenal cortex to stimulate the release of aldosterone biosynthesis and release. The aforementioned events assist in restoring blood pressure via renal mechanisms by increasing sodium reabsorption and potassium secretion (aldosterone) and by decreasing glomerular filtration rate and renal blood flow (vasoconstriction of the efferent arteriole)(15).

Of significant interest to this thesis, is the fact that ANG II interacts with the baroreflexes, particularly with the baroreflex control of heart rate. Studies in

adult humans, dogs, rabbits and sheep have observed that the pressor response of ANG II is accompanied by little or no change in heart rate in comparison to that seen with other vasoconstrictors such as phenylephrine (12,15,16). This suggests a "resetting" of the baroreflex towards higher pressures. A "resetting" of the baroreflex is defined as a shift toward the prevailing pressure (3). This "resetting" by ANG II would alter the HR:BP relationship such that the same heart rate corresponded to a higher blood pressure. Indeed, studies in conscious rabbits have confirmed that the primary action of ANG II is to reset the baroreflex control of heart rate towards higher pressures (17).

The mechanism by which ANG II resets the baroreflex could be explained by an increase in sympathetic tone, a decrease vagal tone or both, as it is known that ANG II has no direct effect on the arterial baroreceptors themselves (12). Recent evidence obtained from conscious rabbits has shown that administration of propanolol, a β-adrenoreceptor-blocking agent, is unable to prevent the shifting of the HR: BP relationship by ANG II(17). Propanolol has a direct action on the heart and is known to prevent reflex tachycardia, suggesting that increasing sympathetic tone is not the mechanism by which ANG II resets the baroreflex curve (17,18). Direct evidence supporting a decrease in vagal tone has not yet been found, however it is hypothesized to be the mechanism of baroreflex resetting via ANG II (17).

The fundamental site of action of ANG II, in the resetting of the baroreflex, is the area postrema, a circumventricular organ in the medulla oblongata (13,15,19,20). The area postrema is a logical candidate for many reasons: First, devoid of a blood brain barrier, the area postrema is in contact with blood-borne ANG II (21). Second, high concentrations of angiotensin type I (AT_1) receptors have been discovered in the area postrema (22). Lastly, the area postrema also has connections to the vagus and aortic nerves and has anatomical and functional connections to medullary cardiovascular control centres, mainly the NTS (20).

In fact, studies in conscious rabbits, in which the area postrema has been surgically ablated, have shown that ANG II failed to reset the baroreflex control of heart rate, suggesting that the area postrema is critical to angiotensin II's resetting of the arterial baroreflex (13,23). In these same studies, the area postrema lesion did not alter the bradycardia response to phenylephrine (13,23). This is the first evidence that ANG II resets the baroreflex control of heart rate via an influence through the area postrema. Recent extracellular recordings in anesthetized rabbits have reported that electrical stimulation of area postrema activates NTS neurons (20). Moreover, area postrema activation enhanced the NTS response to aortic depressor nerve activity, suggesting a possible interaction between the area postrema and peripheral afferents in the medulla oblongata

(20). Perhaps more convincing are the results obtained by Dampney et al (1996) which have shown that certain NTS neurons respond to alterations in circulating ANG II levels. Taken together, this evidence strongly suggests an ANG II sensitive pathway between area postrema and the NTS.

Binding studies have revealed that the majority of ANG II receptors in the area postrema are angiotensin type I (AT_1) receptors (22). Based on the above findings, studies were conducted in conscious rabbits to investigate if ANG II's "resetting" of the baroreflex occurs through activation of the AT_1 receptors. In these studies an AT_1 receptor antagonist, losartan, was systemically administered prior to ANG II infusion. It was found that losartan completely blocked the resetting of baroreflex (24). Thus, it was concluded that AT_1 receptors in the area postrema were responsible for the central modulation of the arterial baroreflex control of heart rate (24).

1.14 Arginine Vasopressin Modulation of the Arterial Baroreflex

AVP is a peptide hormone produced by hypothalamic neurons with cell bodies in the supraoptic and paraventricular nuclei. Axons of the hypothalamic neurons terminate in the posterior pituitary from which AVP is released (14). AVP release is stimulated by: 1) increased plasma osmolality 2) decreased blood volume and 3) increased circulating levels of ANG II (14). AVP-containing neurons have

inputs arising from both osmoreceptors and baroreceptors, which monitor plasma osmolality and blood volume, respectively (14). An increase in plasma osmolality by ~1 % is sufficient to increase AVP secretion (25). A decrease in blood volume by ~5-10 % activates the cardio-pulmonary baroreceptors located in the heart and lungs, and to a lesser extent, arterial baroreceptors located in the carotid sinus and aortic arch (25). Signals from these baroreceptors are relayed to AVP secretory neurons of the supraoptic and paraventricular nuclei via afferent fibers of the vagus and glossopharnygeal nerves and stimulate AVP release (25). In addition, these baroreceptors activate the renin-angiotensin system and, via increases in ANG II acting on AT_1 receptors in the supraoptic nucleus, stimulate AVP release (25). Increased angiotensin II levels can thus mediate AVP release in the absence of blood volume depletion.

AVP, also known as antidiuretic hormone, is perhaps most notable for its role in increasing the water permeability of the collecting tubules in the kidney, via V_2 receptors (25). In addition to AVP's renal actions, AVP is a very potent vasoconstrictor that acts on AVP type 1a (V_{1a}) receptors on vascular smooth muscle (14). Surprisingly, intravenous (I.V.) infusions of AVP cause no increase in systemic blood pressure. The absence of a pressor response

to AVP occurs because AVP resets the baroreflex control of heart rate to lower pressures (14,25,26). This resetting alters the HR: BP relationship such that the same heart rate now correlates to a lower pressure. AVP's modulation of the arterial baroreflex control of heart rate has been evidenced in many species to date, including adult dogs (26), rabbits (27) and sheep (28).

Studies by Bishop and colleagues (1985) have shown in conscious rabbits that surgical ablation of the area postrema prevents AVP's modulation of the baroreflex (29). Moreover, studies in which the AVP type I (V_1) receptor antagonist was systemically administered to intact conscious rabbits have shown AVP's modulation of the baroreflex control of heart rate was blocked (27). Collectively, these studies may suggest that AVP's resetting of the baroreflex occurs via V_1 receptors in the area postrema.

Studies have attempted to describe the neural connections between AVP neurons and cardiovascular centres, namely the NTS. Anterograde-labelling studies have identified bundles of nerve fibres connecting the paraventricular nucleus to the nucleus ambiguus, NTS, area postrema and dorsal motor nucleus of the vagus (6). These studies define anatomical connections through which AVP could elicit its action to centrally enhance the arterial baroreflex control of heart rate. It is possible that increased levels of circulating AVP could serve to buffer the modulating effects of ANG II on the baroreflex.

1.15 Ontogeny of the Baroreflex

Although the arterial baroreflex has been known to exist for over 100 years (2), many of its intricacies are only recently being understood. This is especially true in the newborn animal.

The assessment of mammalian newborns can be divided into two classifications: precocial newborns and altricial newborns (30). These classifications are general descriptive groups of neonatal developmental degrees (30). Precocial species are well developed at birth meaning they can move around within minutes/hours after birth, they are pelted and have open eyes. Altricial literally means, "nest dependent". Mammals of this classification are poorly developed, naked, blind and helpless (30). At birth, the degree of baroreflex function differs between animal species in accordance with maturity. In precocial species, like the newborn lamb, the baroreflex is well developed at birth (31,32). In more altricial species, such as the newborn rat, the baroreflex control of heart rate is less well developed (33). For example, functional sympathetic ganglionic transmission is absent in the newborn rat, whereas postganglionic activity can be recorded in the fetal sheep early in the third trimester (33).

Studies in sheep have shown that the central and efferent mechanisms of the baroreceptor reflex are present at birth (32,34). In sheep, the baroreceptors are

functional from 0.6 gestation (31). As gestation advances, the baroreflex control of heart rate matures, as evidenced by an increase in the proportion of positive arterial baroreflex responses to brief elevations in blood pressure. Concomitantly, there is an increasing sensitivity of baroreflex, such that the ratio of heart rate slowing to blood pressure elevation is greater (31).

The arterial baroreflex operates over a lower range of pressures in the newborn than in the adult. Resetting of the baroreflex is thought to occur during postnatal life (32), as it is known that the increase in pressure during postnatal life is associated with an increase rather than a decrease in heart rate (32).

1.16 Arterial Baroreflex Modulation in the Newborn

As previously stated, the arterial baroreflex is finely tuned by neurohumoral factors. Angiotensin II (ANG II) and vasopressin (AVP) are two peptide hormones known to alter the arterial baroreflex control of heart rate (28,35). ANG II shifts the HR: BP relationship towards higher pressures whilst AVP shifts the HR: BP relationship toward lower pressures. ANG II and AVP elicit their effects on the baroreflex by binding to AT_1 and V_1 receptors, respectively, located in the area postrema of medulla oblongata (36). The location and density of both AT_1 and V_1 receptors in the brainstem varies during postnatal

maturation and consequently, it is possible that differential responses of ANG II and AVP on the arterial baroreflex may be seen during development.

1.17 Angiotensin II Receptor Distribution in Development

It has been suggested that there are important developmental changes in the expression of the two ANG II receptor subtypes, AT_1 and AT_2, and that these differences are tissue specific. It is conceivable that receptor differences between newborn and older animals could account for unique responses to ANG II in terms of the baroreflex modulation.

Evidence of the ANG II receptor is first detected on day 11 in the rat fetus in the liver, kidney , adrenal gland, heart and brain (37). However, there is a predominance of AT_2 receptors in fetal and newborn life as opposed to a predominance of AT_1 receptors in the adult rat (37). It is thought that the AT_2 receptor plays an important role in organogenesis and development, which accounts for its high expression early in life.

Maximal binding of ANG II is found near term indicating there is an increase in ANG II receptors as gestation progresses (38). Renal AT_1 mRNA expression remains unchanged in the last trimester in fetal sheep (39). Significant

decreases in AT_1 mRNA are seen on postnatal day 10 in the kidneys of newborn lambs (39).

The question of whether ANG II receptor expression and distribution in the newborn can play a role in modulation of the baroreflex by ANG II has not been extensively studied. It is known from studies in the developing rat brain that AT_1 receptors are localized to brain structures of the circumventricular organs and the paraventricular nucleus, whereas AT_2 receptors were localized to the thalamic nuclei, cerebellum and cingulate cortex (40). Nonetheless, some brain tissues where found to contain both AT_1 and AT_2 receptor subtypes (40).

At this time it is unknown whether the upregulation of AT_1 receptors prior to 10 days of life occurs with a decreased sensitivity of the receptor or if this increased population of receptors is able to elicit a more potent response to ANG II.

1.18 Angiotensin II Modulation of the Arterial Baroreflex in the Newborn

To date, there is a paucity of information regarding hormonal modulation of the baroreflex control of heart rate in the conscious newborn animal. Studies by Segar et al (1997) examined the effect of removing ANG II by systemic administration of the AT_1 receptor antagonist, losartan, to one-week old

paralyzed, artificially ventilated lambs. They reported a shift of the baroreflex towards lower pressures with an increase in the gain (sensitivity) (35). These studies suggest that ANG II has reset the baroreflex towards higher pressures, via AT_1 receptors, in keeping with ANG II's effect on the baroreflex in the adult. This is evidence that early in life, endogenous ANG II exerts a tonic effect on the baroreflex. Further studies are needed to examine if this same response occurs in conscious newborn animals.

1.19 Arginine Vasopressin Modulation of the Arterial Baroreflex in the Newborn

Recently, it has been reported that AVP plays no role in modulating the arterial baroreflex control of heart rate early in life (28). In these experiments, the baroreflex control of heart rate was measured following I.V. infusion of AVP to sedated, paralyzed and artificially ventilated lambs and these responses were compared to adult sheep treated in the same manner (28). The baroreflex curves that were generated were shifted towards lower pressures during AVP infusion to adult sheep, but not when infused to 1 week-old lambs (28). Therefore, these data strongly suggest that AVP does not modulate the arterial baroreflex control of heart rate in the first week of life, at least in animals that are sedated and paralysed. It is conceivable that ANG II's effects on the baroreflex in the adult, but not in the newborn, may be minimised by increased

levels of AVP; this would assist in restoring the baroreflex toward a lower pressure. In addition, it is likely that age-dependent processes are responsible for the absence of AVP's effect on the baroreflex in the newborn animal. Possible mechanisms could include age-dependent processes responsible for the maturation of the baroreflex and/or changes in the quantity and location of V_1 receptors in the brainstem. However, the exact mechanism responsible for this phenomenon remains obscure.

1.2 PHYSIOLOGICAL EFFECTS OF FUROSEMIDE IN THE ADULT

Diuretics are pharmacological agents that induce a net loss of water and electrolytes by inhibiting the reabsorption of Na^+/Cl^- from the kidneys (41). One such agent is the loop diuretic, furosemide (42), which acts by inhibiting the Na^+-K^+-$2Cl^-$ symporter in the thick ascending limb of the loop of Henle, resulting in a rapid natriuresis and diuresis (43) and ultimately a decrease in blood volume (Figure 2).

It is well established that furosemide has several physiological actions other than its effects on the kidney (44,45). These actions include an increase in renin release independent of systemic volume change (46), and increases in circulating levels of AVP (47) and prostaglandin E_2 levels (26). The increase in circulating levels of these hormones that occurs in response to furosemide administration could influence systemic and renal hemodynamics as well as the baroreflex control of heart rate. These effects are detailed in the following paragraphs, and form the basis of our proposed hypotheses.

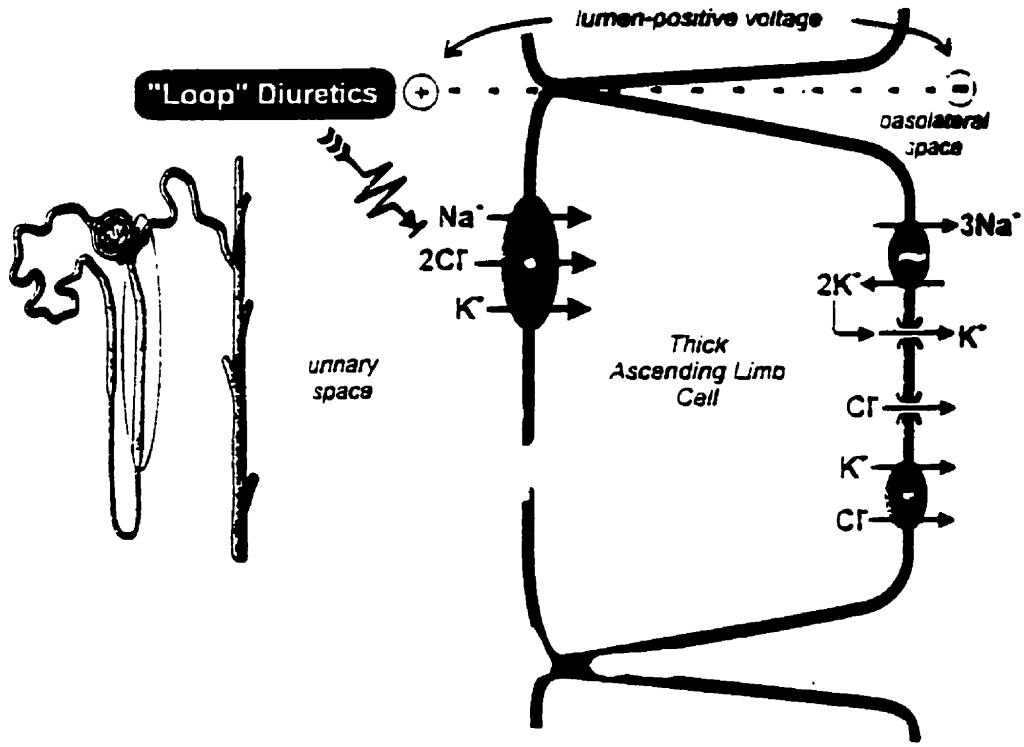


Figure 2. Illustration of the furosemide sensitive Na^+-K^+-$2Cl^-$ channel in the thick ascending limb (TAL) of the loop of Henle. Adapted from Friedman and Hebert, 1997.

1.21 Hemodynamic Responses to Furosemide

The first evidence of cardiovascular responses to furosemide were observed in adult congestive heart failure patients (48). Furosemide decreased cardiac output and increased venous capacitance soon after its administration and before any diuresis was apparent (48). These cardiovascular findings have been confirmed in mature experimental animals and in normal adult human subjects (49-51) and provide evidence that some of the earliest actions of furosemide reflect hemodynamic rather than renal actions. Furosemide's venodilator effects were not seen, however, in anephric patients and in patients pre-treated with indomethacin (prostaglandin synthesis inhibitor) (45). These studies suggest that the mechanism by which furosemide induces venodilation is via renal prostaglandin synthesis.

To date, there is little information on the effects of furosemide on blood pressure and heart rate in adult sheep. An acute pressor response to furosemide, however, has been documented in spontaneously hypertensive rats (49), dogs (52), normal and hypertensive patients (51) and in congestive heart failure patients (53,54). This pressor response occurs ~20-40 minutes after furosemide administration and is correlated to increases in circulating ANG II levels (51).

Furosemide's diuretic action results in a decrease in cardiac output and a decrease in blood volume and it is to these stimuli that the baroreflexes likely responds. The interaction of furosemide with the arterial baroreflex has been investigated by Janssen et al (1989) in conscious spontaneously hypertensive rats (49), who underwent sinoaortic denervation or sham denervation at the time of surgery. The experiments revealed that blood pressure and heart rate decreased in sinoaortic denervated rats but was preserved in sham operated animals. It was concluded that the arterial baroreflex normally prevents blood pressure from falling despite the decrease in cardiac output and blood volume evoked by furosemide (49). Similarly, a study carried out in anesthetized Sprague Dawley rats found that furosemide administration decreased cardiac filling pressure but not mean arterial pressure in intact animals (50). In sinoaortic denervated rats, however, mean arterial pressure fell in parallel with the reduced cardiac filling pressure induced by furosemide (50). This observation is compatible with the findings of Janssen *et al* (1989) and the notion that an intact arterial baroreflex is essential to maintaining arterial pressure during acute blood volume depletion elicited by furosemide (49). To date, the arterial baroreflex has not been directly assessed in adults and thus further investigation is warranted.

1.22 Effects of Furosemide on the Renin-Angiotensin System

Furosemide stimulates a cascade of neurohumoral factors to be released. Most prominent is its stimulation of the renin-angiotensin system (RAS)(46) via the release of renin. Furosemide administration stimulates renin release within minutes by directly binding to the Na^+-K^+-$2Cl^-$ symporter on the macula densa (55). Once the macula densa sensing mechanism is blocked by furosemide, renin will be released regardless of the Na^+/Cl^- concentrations in the distal tubule (55). Theoretically, over the long-term, furosemide administration will also indirectly stimulate renin release by inducing blood volume depletion (47). Blood volume depletion will activate baroreceptors, which will in turn, increase efferent renal sympathetic nerve activity (2).

1.23 Effects of Furosemide on Plasma Levels of Arginine Vasopressin

Plasma levels of AVP increase following furosemide administration to adult dogs, rabbits and humans (47,57,58). Since furosemide is a potent stimulator of the renin angiotensin system (46), it is likely that a large fraction of the AVP release occurs via ANG II stimulation. This theory is consistent with the findings of Siegel *et al* (60) who reported that in conscious newborn lambs, the increase in

circulating AVP levels, following furosemide administration, lags behind increases in circulating levels of ANG II.

It is important to note, that reabsorption of sodium in the TAL of the loop of Henle sets up the medullary intersitial gradient for water reabsorption (42). Thus, when reabsorption of sodium is inhibited by furosemide this medullary gradient is disrupted and AVP (antidiuretic hormone) has little effect in reabsorbing water from the collecting tubules (49, 60). Thus, it is concluded that ANG II is the main hormonal system counteracting the diuresis induced by furosemide, whereas antidiuretic hormone (AVP) seems to be of minor importance (47, 57).

1.3 PHYSIOLOGICAL EFFECTS OF FUROSEMIDE IN THE NEWBORN

Furosemide is one of the most commonly used diuretics in human neonates (61). Indications for its use include fluid overload associated with congestive heart failure (62), pulmonary edema (63), bronchopulmonary dysplasia (64), and acute renal failure (63). Despite its widespread application in the clinical setting, the physiological effects of furosemide in the newborn and during postnatal maturation are poorly understood. Several studies have focused on the pharmacokinetics and pharmacodynamics of furosemide in infants (63,65,66),

however, few studies have related age-dependent alterations in the responses to furosemide with physiological changes that occur with postnatal development.

1.31 Age-dependent Differences

There is now increasing evidence that postnatal age does play a role in the physiological response to furosemide. Previously, our laboratory has carried out experiments in conscious, chronically instrumented lambs in order to investigate some of the physiological responses to furosemide early in life and as maturation proceeds (56,59,67-69). We have discovered major differences in the response to furosemide in newborn versus older lambs in terms of cardiovascular, hormonal and renal variables.

In newborn animals (67,70) and infants (71), furosemide stimulates the release of renin. The increase in plasma renin activity (PRA) in response to furosemide is more prolonged and pronounced in newborns versus older animals (67). PRA remains elevated for at least two hours after administration of 2 mg/kg of furosemide to newborn lambs whereas in older lambs, PRA increases only transiently (67). This phenomenon occurs despite already elevated levels of renin, ANG II and aldosterone in the newborn period (72). Thus, it appears that the renin response to furosemide is developmentally regulated. Moreover, this observation provides evidence to suggest that there would be a prolonged

increase in ANG II levels following furosemide administration to the newborn. As a consequence, it is predicted that the increase in circulating ANG II levels following administration of furosemide might influence the physiological responses to furosemide.

1.32 Furosemide and its Relationship to the Arterial Baroreflex

Acute I.V. injection of 2 mg/kg of furosemide to conscious lambs resulted in a sustained increase in heart rate, whilst mean arterial pressure returned to control levels (59). Hence, it is conceivable that the arterial baroreflex control of heart rate is reset towards higher pressures in response to furosemide administration, accounting for the sustained increase in heart rate. Quantification and qualification of the parameters defining arterial baroreflex control of heart rate following furosemide administration to conscious lambs has not yet been investigated, but forms the basis of the proposed research.

1.4 HYPOTHESES

The objective of the current experiments was to investigate if the hemodynamic responses to furosemide were altered with postnatal maturation in conscious lambs and to test the following hypotheses:

1)...that the systemic and renal hemodynamic responses to furosemide are altered with maturation (Chapter 3).

2)...that the arterial baroreflex control of heart rate is altered in response to furosemide in an age-dependent manner (Chapter 4).

CHAPTER TWO

Materials and Methods

2.1 ANIMALS

Experiments were carried out in conscious chronically instrumented lambs aged one-week (9±2 days) and six-weeks (43±2 days). Lambs were obtained from a local source (Sheep Advisory Service, Alberta) and housed with their mother in individual pens in the vivarium of the Health Sciences Centre, except during surgery and experiments. All lambs were housed under controlled temperature of 22±1°C, 20-30% relative humidity and a 12:12 hour dark-light cycle, with lights on at 7 am. Lambs suckled from their mothers and had access to water ad libitum. Ewes were daily fed a diet of oats, barely and hay.

All surgical and experimental procedures described herein were carried out in accordance with the "Guide to the Care and Use of Experimental Animals" provided by the Canadian Council on Animal Care and approved by the Animal Care Committee of the University of Calgary.

2.2 SURGICAL PROCEDURES

Each lamb underwent one operation prior to experimentation. In preparation for surgery, the lamb's abdomen and right and left flanks were shaved. Surgery was performed on lambs using strict aseptic conditions. The previously shorn areas were cleaned with alcohol and antiseptic and a sterile drape was placed to cover all but the clean surgical areas. Briefly, anesthesia was induced with a mask and halothane (3-4%) in oxygen, the trachea was intubated and the lamb was ventilated using a positive pressure respirator (Harvard Apparatus, Model 613, South Natick, Massachusetts). Anesthesia was maintained throughout surgery with halothane (0.5-1.0%) in a mixture of nitrous oxide and oxygen (3:1).

Catheters (PE 160, Intramedic, Clay Adams, Parsippany, New Jersey) were inserted into the right and left femoral veins and arteries and advanced above the diaphragm to the inferior *vena cava* and descending aorta, respectively. Venous catheters were used for later intravenous (I.V.) injections and infusions as well as for the measurement of central venous pressure. Arterial catheters were used for mean arterial pressure measurements and for blood sampling. Catheters were tunneled subcutaneously to exit the lamb on the right and left flanks. The femoral incisions were then closed.

The bladder was exposed by a midline incision and catheterized directly through the bladder wall using a specially adapted catheter. The right kidney was exposed by a retroperitoneal approach and a precalibrated ultrasonic flow transducer (3S, 4S, Transonic Systems Inc., Ithaca, New York) was placed around the right renal artery for later measurement of renal blood flow. The flank incision was then closed. The renal flow transducer and all catheters were secured in pouches on the lamb's body jacket (Lomir Inc., Notre-Dame, Quebec) for safe storage between experiments (Figure 3).

Topical antiseptic ointment (Betadine, Purdue Fredrick Inc., Pickering, Ontario) was applied to suture lines to prevent infection. This procedure was repeated throughout the experiment as part of daily care. Antibiotics (0.5 mg/kg, enrofloxacin, Baytril, Bayer) were administered intramuscularly immediately before surgery and at 12 hour intervals thereafter, for 48 hours. At the completion of the surgical procedures, the lamb was allowed to recover from the effects of surgery and anesthesia in a critical care unit for small animals (Shoreline, Schroer Manufacturing Co., Kansas, Missouri) with adjustable oxygen supply. All lambs were able to stand within one hour of completion of surgery at which time they were returned to their mothers in the vivarium. At least two days of recovery from the effects of surgery and anesthesia were permitted before experiments were started. During the recovery period, lambs were

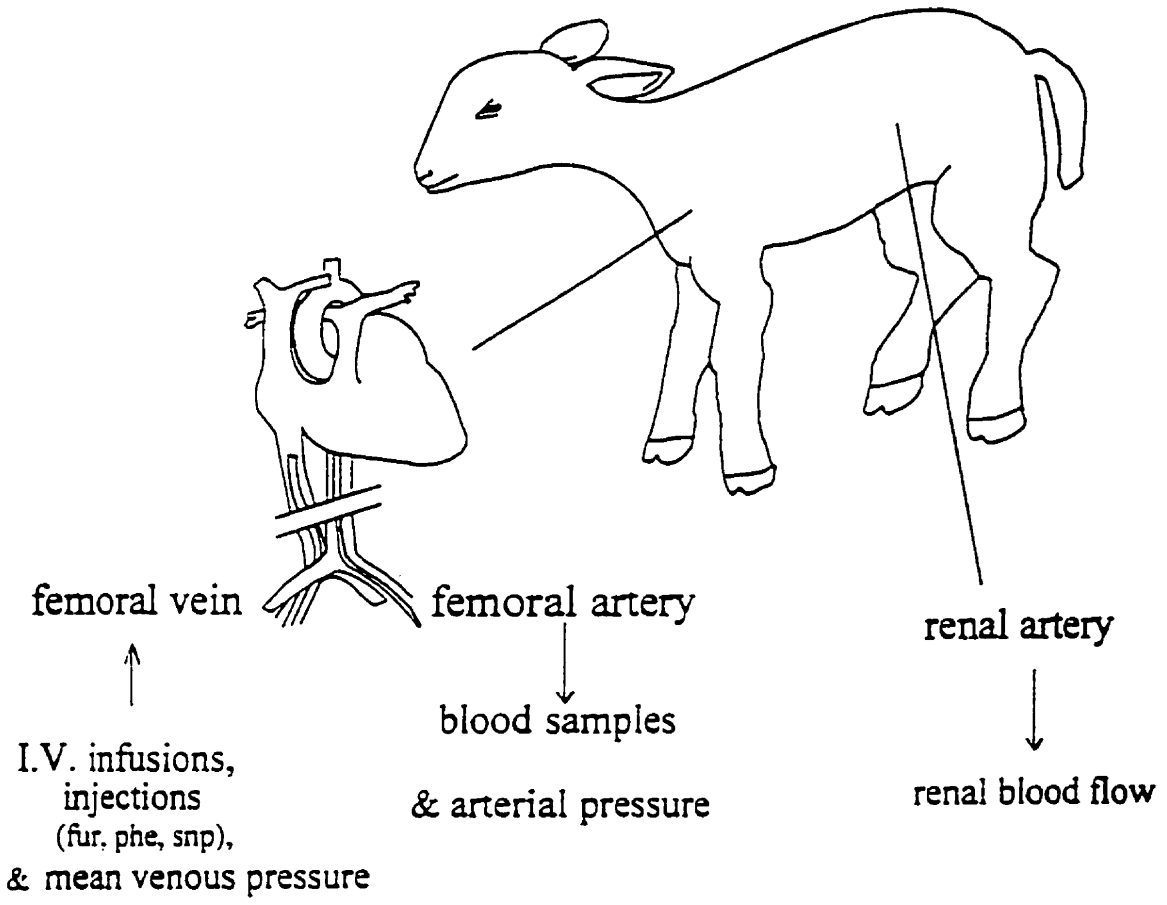


Figure 3. Diagrammatic representation of a conscious, chronically instrumented lamb.

trained to rest quietly in a sling in the laboratory environment. Daily at 0800 hours rectal body temperature and body weight was recorded.

All surgical procedures described herein were performed on all lambs studied.

2.3 EXPERIMENTAL DETAILS

On the day of an experiment, the lamb was removed from the vivarium and placed in a supportive sling in the laboratory environment for at least 60 minutes. During this time, the bladder was allowed to drain. In addition, an I.V. infusion of 5% dextrose and 0.9% sodium chloride (Baxter, Toronto, Ontario) was started (4.17 mg/kg/mL) to assist in maintaining fluid balance throughout the experiment.

Catheters for the continuous measurement of central venous pressure and mean arterial pressure were connected to Statham P23 XL pressure transducers (Statham Instruments Division, Gould) with the transducer placed parallel to the lamb's heart. Before each experiment, transducers were calibrated with a mercury manometer. The ultrasonic flow transducer was connected to the renal blood flow meter (T101, Transonics Systems Inc., Ithaca, New York) for the

measurement of renal blood flow. The accuracy of the flow probe in monitoring renal blood flow has been demonstrated in conscious sheep (73).

Mean arterial pressure and central venous pressures as well as renal blood flow were continuously recorded onto a polygraph (model 7, Grass Instruments, Quincy, Massachusetts). Simultaneously, they were digitised at 200 Hz to an IBM-PC over consecutive one-minute intervals, using the data acquisition and analysis software package CVSOFT (Odessa Systems Inc., Calgary, Alberta). Data were stored and analysed off-line using CVSOFT and an IBM-PC. Detailed experimental protocols for the two studies are described in Chapters 3 and 4.

At the completion of the study, lambs were euthanized with a lethal dose of sodium pentobarbitone (Euthanyl, MTC Pharmaceuticals, Cambridge, Ontario) and the zero offset of the flow transducer was determined. The placement of catheters and the renal flow transducer were verified by post mortem inspection; the right and left kidneys were immediately removed and weighed.

CHAPTER THREE

❖

Age-dependent Effects of Furosemide on Systemic and Renal Hemodynamics

3.1 INTRODUCTION

Although furosemide is one of the most commonly used diuretics to treat edematous states in the adult and newborn infant (63,66,74) little is known about the systemic and renal effects of furosemide in the newborn and during postnatal maturation.

In addition to its natriuretic and diuretic effects, furosemide is a potent stimulator of the renin-angiotensin system (46). Furosemide is also known to stimulate renal prostaglandin E_2 synthesis (75-77). Thus, furosemide's diverse renal actions, most notably the increase of angiotensin II and prostaglandin E_2 (PGE_2) production, may complicate the renal and hemodynamic responses to furosemide.

3.11 Age-Dependent Differences

Previously, our laboratory described studies that provide evidence that there are major differences in the physiological response to furosemide in newborns versus older lambs (56,59,67-69). For example, plasma renin activity (PRA) increased rapidly in response to furosemide. The response was, however, more dramatic and prolonged in newborn versus older lambs (67). PRA levels remained elevated at 120 minutes in the newborn animals while PRA returned to control levels in older animals(67). This age-dependent phenomenon occurs despite the already elevated ANG II levels in the newborn period (72).

In adult animals and humans, renal blood flow increases in response to furosemide administration due to a decrease in renal vascular resistance mediated in part by PGE_2 (26,78). Prostaglandins have been implicated in the increase in renal blood flow because administration of indomethacin, a cyclo-oxygenase inhibitor, prevents the increase in renal blood flow seen with furosemide (45). In contrast, renal blood flow decreases in response to furosemide in the three week-old lambs; interestingly this corresponds to no change in urinary excretion of PGE_2 (69).

Arterial pressure is well maintained in the face of volume depletion by furosemide, in both the adult and newborn animals and patients (49,51,59). Previous studies in our laboratory have shown that in the three week-old lambs there is an increase in heart rate that occurs at 40 minutes after I.V. administration of 2 mg/kg of furosemide (68). This effect occurs despite blood pressure remaining at control levels (68). This provides evidence that the arterial baroreflex control of heart rate may be altered with furosemide early in life, since blood pressure does not fall and heart rate increases.

3.12 Rationale

Age-dependent differences in the renal blood flow response to furosemide are coupled to differential stimulation of both renin and prostaglandin E_2 (67,69). It is possible that the age-dependent changes in the renal blood flow response, that occur following furosemide administration, could be correlated to these modulating factors.

Similarly, differences in the heart rate response to furosemide in newborn versus older lambs could be correlated to the activation of the renin-angiotensin system. Since data thus far has only been obtained in the 3-week old lamb and the adult sheep, further investigation needs to be conducted to assess if the renal blood

flow and heart rate responses to furosemide are altered in the newborn period and as maturation proceeds.

3.13 Objective

The objective of the present study was to measure the hemodynamic and renin responses to furosemide and to determine if the responses are age-dependent.

3.14 Hypothesis

The following experiments test the hypothesis...

1).... that the hemodynamic and endocrine responses to furosemide are altered with postnatal maturation in conscious lambs.

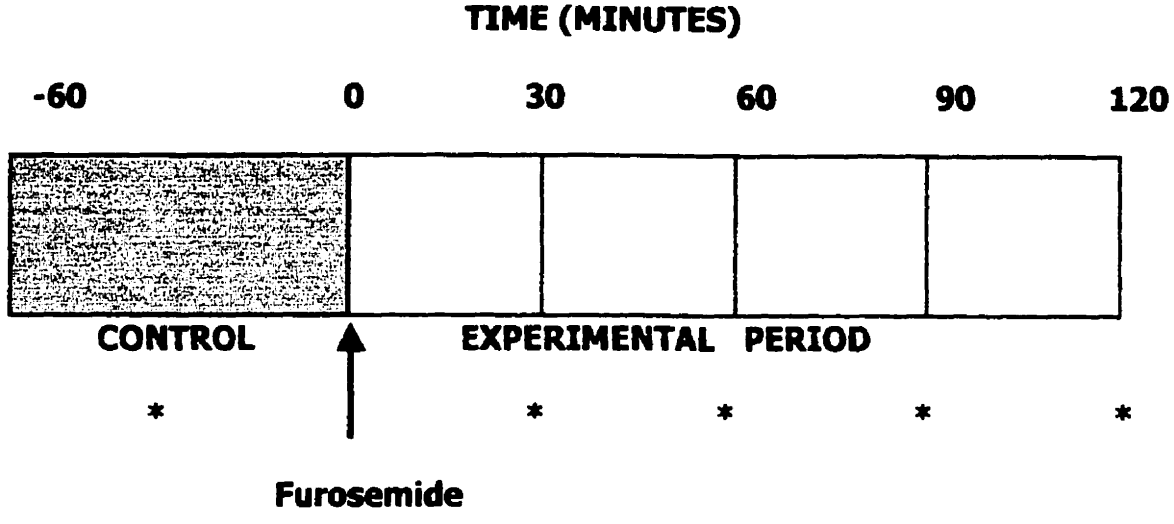
3.2 METHODS

3.21 Specific Protocol

Two age groups of lambs were studied: one-week (N=10, 9±2 days) and six-weeks (N=10, 43±2 days). After a one hour equilibration period, the experiment commenced consisting of hemodynamic and endocrine measurements one hour before (control period) and two hours after I.V. administration of furosemide at one of three doses (0.00 mg/kg, 0.25 mg/kg & 5.00 mg/kg). This protocol was repeated at intervals of 24-48 hours until all doses had been administered; the order of the experiments was randomized.

Previous studies in conscious lambs suggested that the renin response to a 0.25 mg/kg dose of furosemide is transient increased and returns to control by 90-120 minutes (59). In contrast, the renin response to a 5.00 mg/kg dose of furosemide was sustained for 120 minutes (59). Therefore 0.25 mg/kg and 5.00 mg/kg doses of furosemide were selected in this study to determine if the dose-dependent renin responses would influence hemodynamic variables.

A diagrammatic representation of the protocol timeline is shown below.



Timeline of protocol illustrating blood sampling (*) for the measurement of plasma protein concentration, hematocrit and PRA.

Blood sampling for the measurement of plasma protein concentration, hematocrit and plasma renin activity (PRA) was taken at 30 minutes in the control period and then at 30, 60, 90 and 120 minutes after furosemide administration. At the previously described intervals, blood was collected in chilled EDTA tubes and centrifuged at 4°C, the supernatant was then removed and stored at –70°C, for the later measurement of PRA. At the end of each experiment, the amount of blood removed was replaced with heparinized maternal blood to avoid any hemodynamic effects of sampling.

Urine was also continuously collected to determine the diuretic response to the various doses of furosemide. Two urine collections were performed: the first,

during the control period and the second, during the experimental period after furosemide administration. At the end of each collection period urine volume was recorded.

As mentioned previously (See Chapter 2, METHODS), catheters for the measurement of central venous pressure and mean arterial pressure were connected to Statham P23 XL pressure transducers (Statham Instruments Division, Gould). The ultrasonic flow transducer was connected to a renal blood flow meter (T101, Transonics Systems Inc., Ithaca, New York) for the measurement of renal blood flow.

Mean arterial pressures and central venous pressures as well as renal blood flow were continuously recorded onto a polygraph (model 7, Grass Instruments, Quincy, Massachusetts). Simultaneously, they were digitized to an IBM-PC, at 200 Hz over consecutive one minute intervals using the data acquisition and analysis software package CVSOFT (Odessa Systems Inc., Calgary, Alberta).

3.22 Computations

Cardiovascular variables were averaged over 20 minute intervals using CVSOFT (Odessa Systems Inc. Calgary, Alberta) and a spreadsheet program (Microsoft Excel 7.0). Renal vascular resistance (RVR) was calculated using the equation:

RVR = MAP-CVP/RBF, where MAP= mean arterial pressure, CVP= central venous pressure and RBF= renal blood flow (corrected for gram kidney weight). Beat-to-beat heart rates were determined from the systolic peak of the pressure waveform using CVSOFT (Odessa Systems Inc., Calgary, Alberta). Data collected during the control period were the same; averages were therefore calculated.

3.23 Analytical Procedures

Hematocrit values were determined using 1.0 ml of arterial blood. Two microcentrifuge tubes were filled with blood, sealed and microcentrifuged. Hematocrit readings were made using microcalipers and calculated as the ratio of packed cells to the total volume of the sample. The hematocrits were determined in duplicate and averaged. Plasma protein was determined using a refractometer (TS meter, American Optical Corp, Keene, New Hampshire). PRA was determined using standard radioimmunoassay procedures (79).

3.24 Statistical Analyses

Analysis was carried out using two-way ANOVA procedures for repeated measures. Significance was accepted at the 95% confidence interval ($p<0.05$). Where the F value was significant, Newman-Keul's multiple comparison post-hoc analysis tests were applied to determine where the significant difference(s) occurred. All data presented herein are expressed as means ± one standard deviation.

3.3 RESULTS

Baseline physiological measurements recorded during the (pre-furosemide) control period are shown in Table 1 for the two groups of lambs: newborn (one-week) and older lambs (six-weeks). Mean blood pressure, central venous pressure and renal blood flow were lower in newborn lambs than in older lambs (Table 1). Conversely, heart rate, renal vascular resistance, plasma protein concentrations and plasma renin activity were higher in newborn lambs than in older lambs (Table 1). Baseline levels for hematocrit were higher in older lambs than in the newborn lambs (Table 1).

3.31 Systemic Hemodynamic Responses to Furosemide

In older lambs, administration of furosemide was associated with a transient increase in mean blood pressure of ~ 7 mmHg ($p<0.05$) within the first 30 minutes (Figure 4). In newborn lambs, blood pressure remained unchanged after administration of 0.25 or 5.00 mg/kg doses of furosemide (Figure 4).

Table 1. Baseline physiological measurements

	NEWBORN	OLDER
Animals (N)	10	10
Age (days)	9±2*	43±2
Body weight (Kg)	7±1*	14±1
Kidney weight (g)	23 ±7	36±6
CVP (mmHg)	3 ± 4*	7 ± 6
MBP (mmHg)	82 ± 9*	91 ± 10
SBP (mmHg)	106±12	102±15
DBP (mmHg)	67±8	78±10
HR (beats/min)	195 ± 22*	110 ± 32
RBF (mL/min/gkw)	2.9 ± 1.1*	3.9 ± 1.2
RVR (mmHg/mL/min/gkw)	30 ±10.9*	22.7 ± 5.8
Plasma protein concentration(g/dl)	6.0 ± 0.2*	5.3 ± 0.3
Hematocrit (%)	26±3*	32±2
PRA (ng/ml/hr)	10±5	3±1

Note: CVP, central venous pressure; MBP, mean blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; RBF, renal blood flow; RVR, renal vascular resistance; PRA, plasma renin activity. Values are mean ± standard deviation. *$p<0.05$ compared to older lambs.

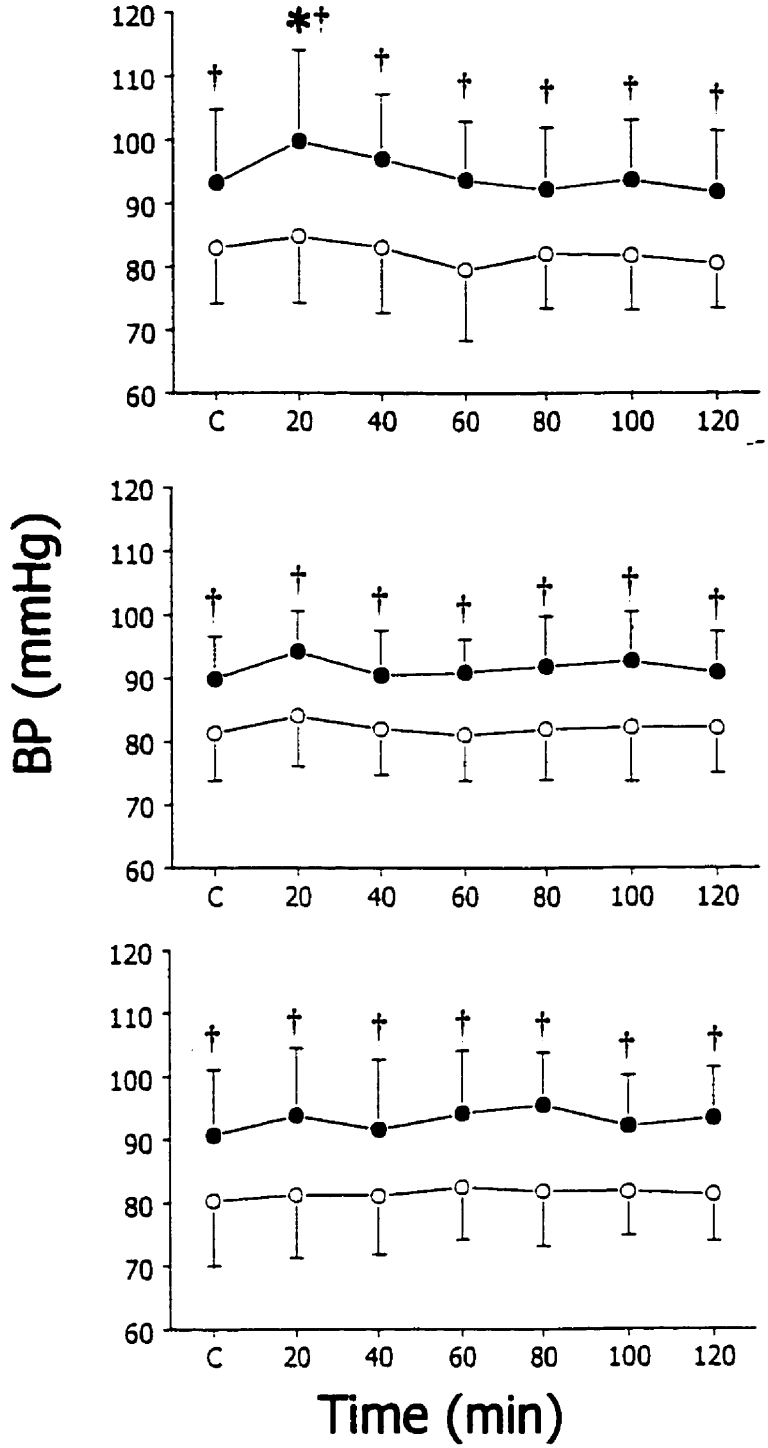


Figure 4. Effect of I.V. administration of furosemide on blood pressure (BP) in older lambs (solid circles) and newborn lambs (open circles). Three doses of furosemide are displayed: 5.00 mg/kg (top), 0.25 mg/kg (middle), 0.00 mg/kg (bottom). Values are mean ± one SD. *$p<0.05$ compared with control (C). †$p<0.05$ compared with newborn lambs.

In response to administration of I.V. furosemide, heart rate did not change from basal measurements; this response was different than measured in older lambs ($p=0.0001$; Figure 5). Heart rate increased 100 minutes after furosemide administration (5.00 mg/kg) in newborn lambs ($p<0.05$); this increase was sustained for the remainder of the experiment (Figure 5). Heart rate was unchanged following a 0.25 mg/kg dose of furosemide in both groups of lambs (Figure 5).

In both age-groups, there was a decrease in central venous pressure (CVP) ($p<0.05$) 40 to 60 minutes following the administration of 5.00 mg/kg of furosemide; this response was sustained for 120 minutes (Figure 6). The decrease in CVP was, however, greater in older lambs (-43%) compared with newborn lambs (-19%) ($p=0.029$; Figure 6).

3.32 Renal Hemodynamic Responses to Furosemide

The change in renal blood flow (RBF) following I.V. administration of furosemide was age-dependent ($p=0.0001$, Figure 7). The administration of a 0.25 mg/kg dose of furosemide was associated with a significant increase in renal blood flow in older lambs ($p<0.05$) at ~20 to 40 minutes whereas the same dose had no

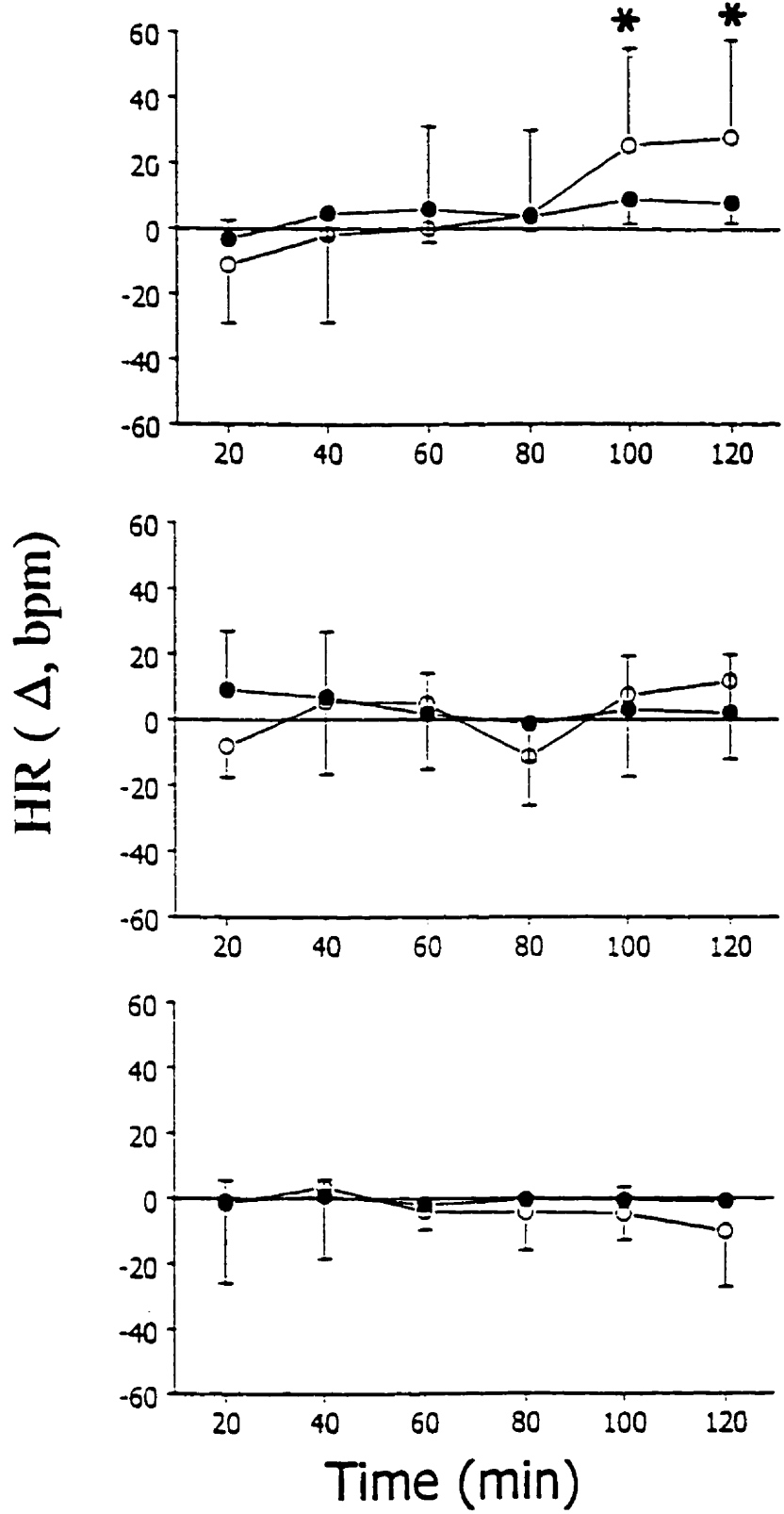


Figure 5. Effect of I.V. administration of furosemide on heart rate (HR), expressed as change from control, in older lambs (solid circles) and newborn lambs (open circles). Three doses of furosemide are displayed: 5.00 mg/kg (top), 0.25 mg/kg (middle) and 0.00 mg/kg (bottom). Values are mean ± 1 standard deviation. * indicates significant difference from control ($p<0.05$).

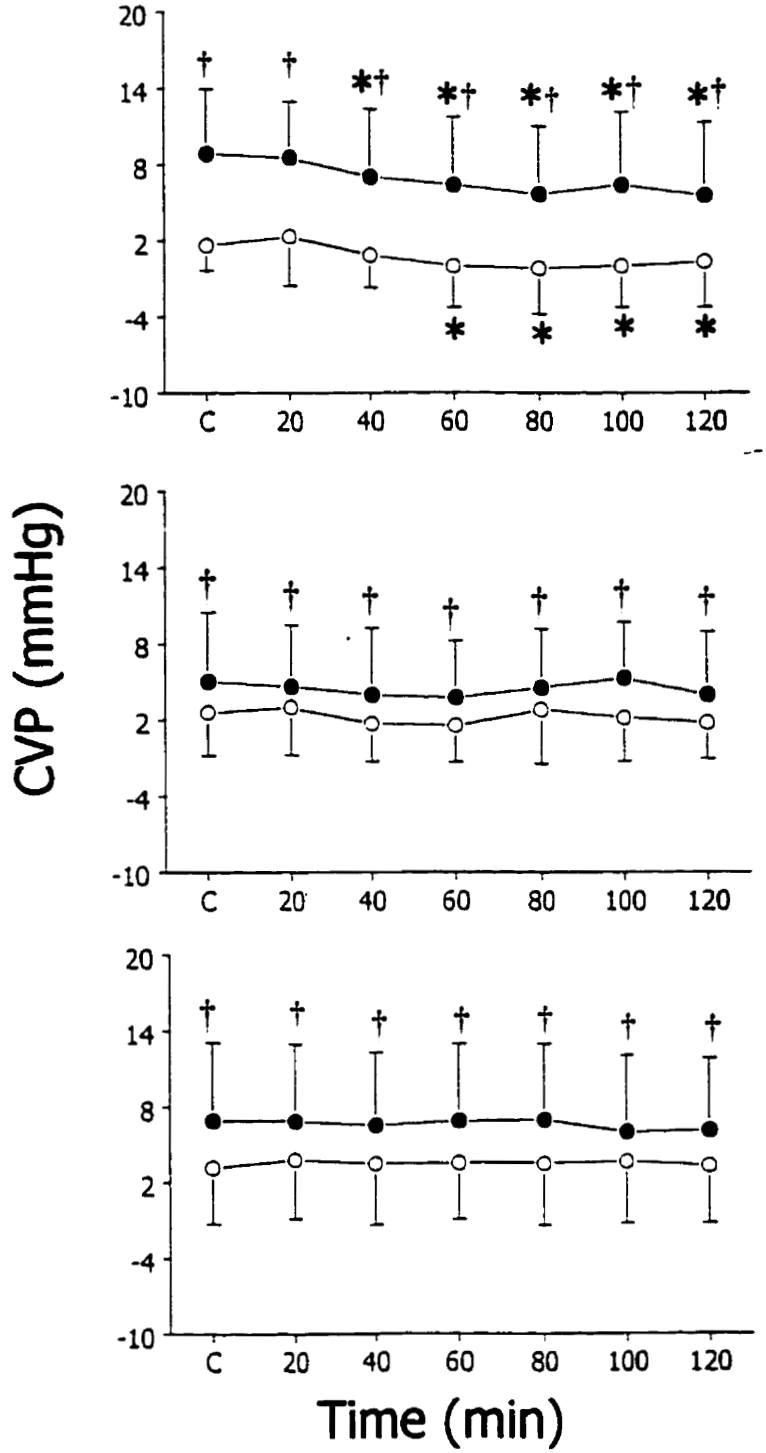


Figure 6. Effect of I.V. administration of furosemide on central venous pressure (CVP) in older lambs (solid circles) and newborn lambs (open circles). Three doses of furosemide are displayed: 5.00 mg/kg (top), 0.25 mg/kg (middle), 0.00 mg/kg (bottom). Values are mean ± one SD. *$p<0.05$ compared with control (C). †$p<0.05$ compared with newborn lambs.

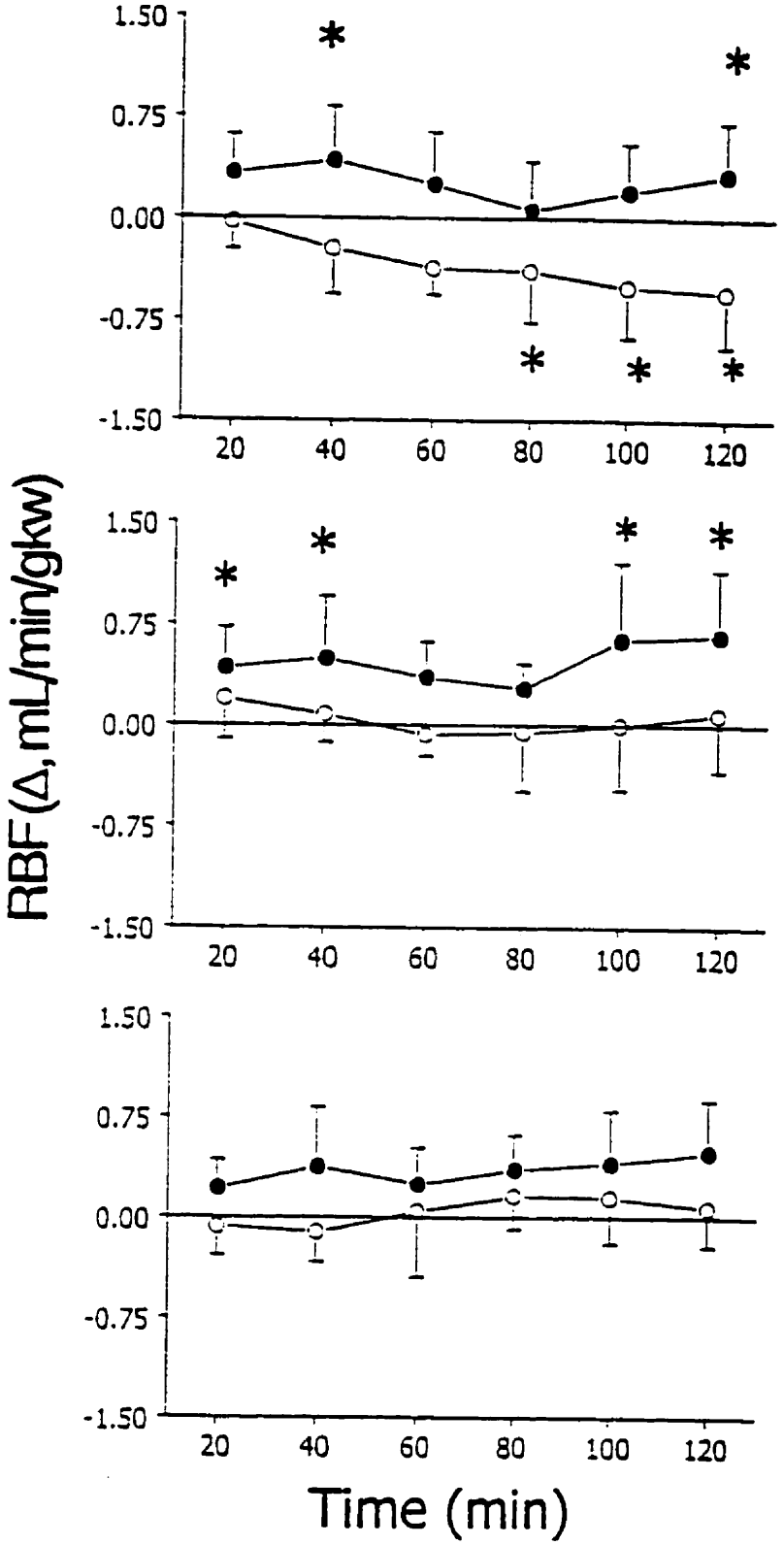


Figure 7. Effect of I.V. administration of furosemide on renal blood flow (RBF), expressed as change from control, in older lambs (solid circles) and newborn lambs (open circles). Three doses of furosemide are displayed: 5.00 mg/kg (top), 0.25 mg/kg (middle) and 0.00 mg/kg (bottom). Values are mean ± 1 standard deviation. * indicates significant difference from control ($p<0.05$).

effect in newborn animals (Figure 7). In newborn lambs, but not in older lambs, administration of a 5.00 mg/kg dose of furosemide was associated with a significant decrease in renal blood flow by 80 minutes; renal blood flow remained decreased for the duration of the experiment (Figure 7). There was no significant change in renal vascular resistance (RVR) in newborn lambs or older lambs (Figure 8).

3.33 Plasma Protein Concentration, Hematocrit, Urine Volume and PRA Responses to Furosemide

Plasma protein concentration increased in response to furosemide in both age groups. The response, however, was more dramatic in older lambs. Plasma protein concentration increased by 13.0 % after 5.00 mg/kg dose of furosemide in older lambs whereas in newborn lambs plasma protein concentration increased by 7.1%. Administration of 0.25 mg/kg of furosemide increased plasma protein concentration by 3.4% in older lambs and by 1.8% in newborn lambs. In both age groups, hematocrit increased in response to administration of a 5.00 mg/kg dose of furosemide (Figure 9). In older lambs, administration of a 0.25 mg/kg dose of furosemide was also associated with an increase hematocrit (Figure 9). The diuretic responses to furosemide, when corrected for kg of body weight, were similar in the two age-groups. Cumulative urine increased to 19±6 ml/kg after 5.00 mg/kg of furosemide in older lambs

In newborn lambs, cumulative urine increased to 26±8 mL/kg after a 5.00 mg/kg dose of furosemide. A 0.25 mg/kg dose of furosemide increased cumulative urine to 11±6 ml/kg in newborn lambs and 13±6 ml/kg in older lambs.

I.V. administration of furosemide increased plasma renin activity (PRA) in both newborn and older lambs (Figure 10). The renin response, was three fold greater in newborn lambs with the 5.00 mg/kg dose of furosemide. The 0.25 mg/kg dose of furosemide increased PRA transiently at 30 minutes in both age-groups (Figure 10).

Vehicle administration (0.00 mg/kg of furosemide) had no effect on any of the measured variables.

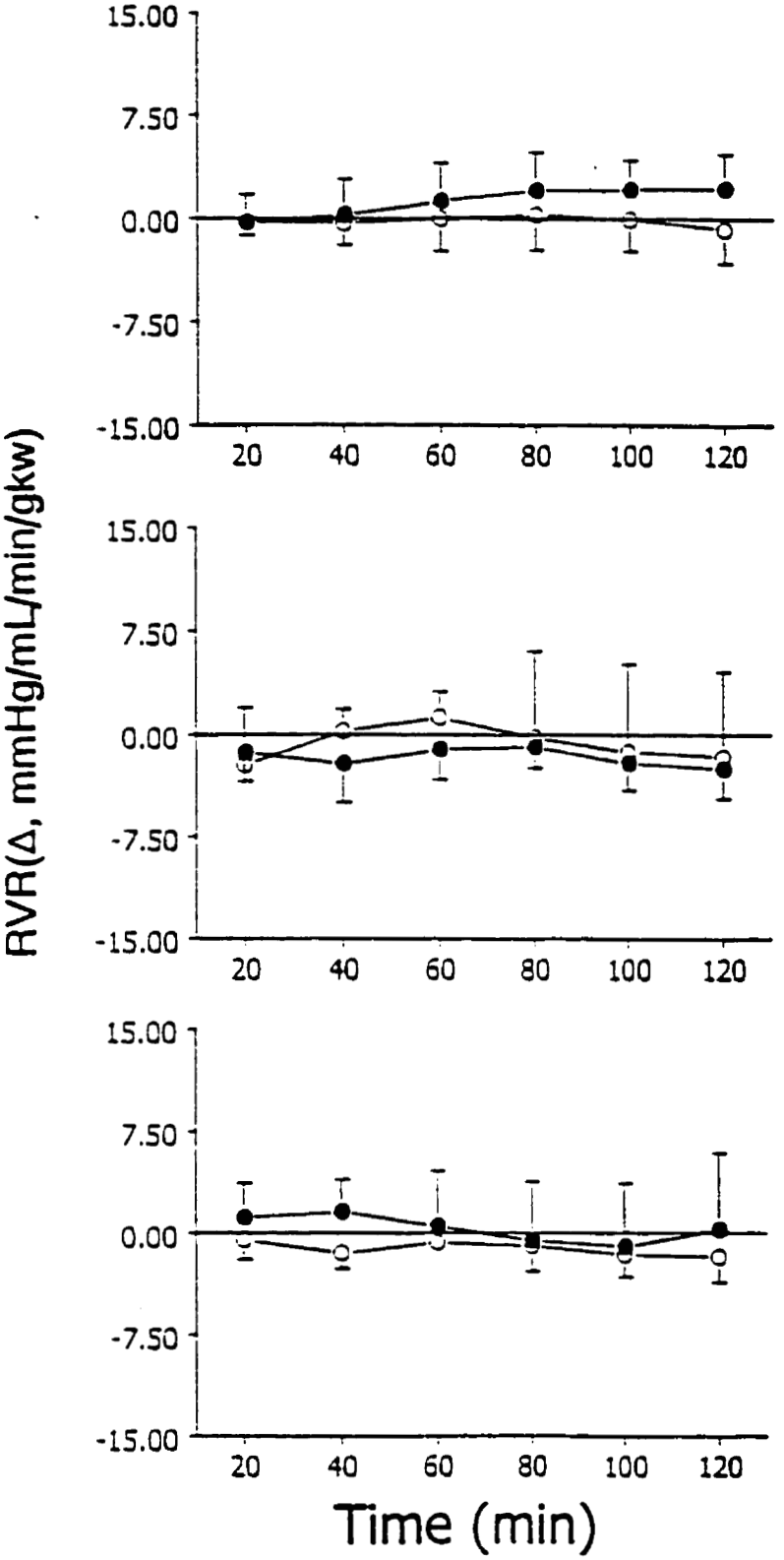


Figure 8. Effect of I.V. administration of furosemide on renal vascular resistance (RVR), expressed as change from control, in older lambs (solid circles) and newborn lambs (open circles). Three doses of furosemide are displayed: 5.00 mg/kg (top), 0.25 mg/kg (middle) and 0.00 mg/kg (bottom). Values are mean ± 1 standard deviation.

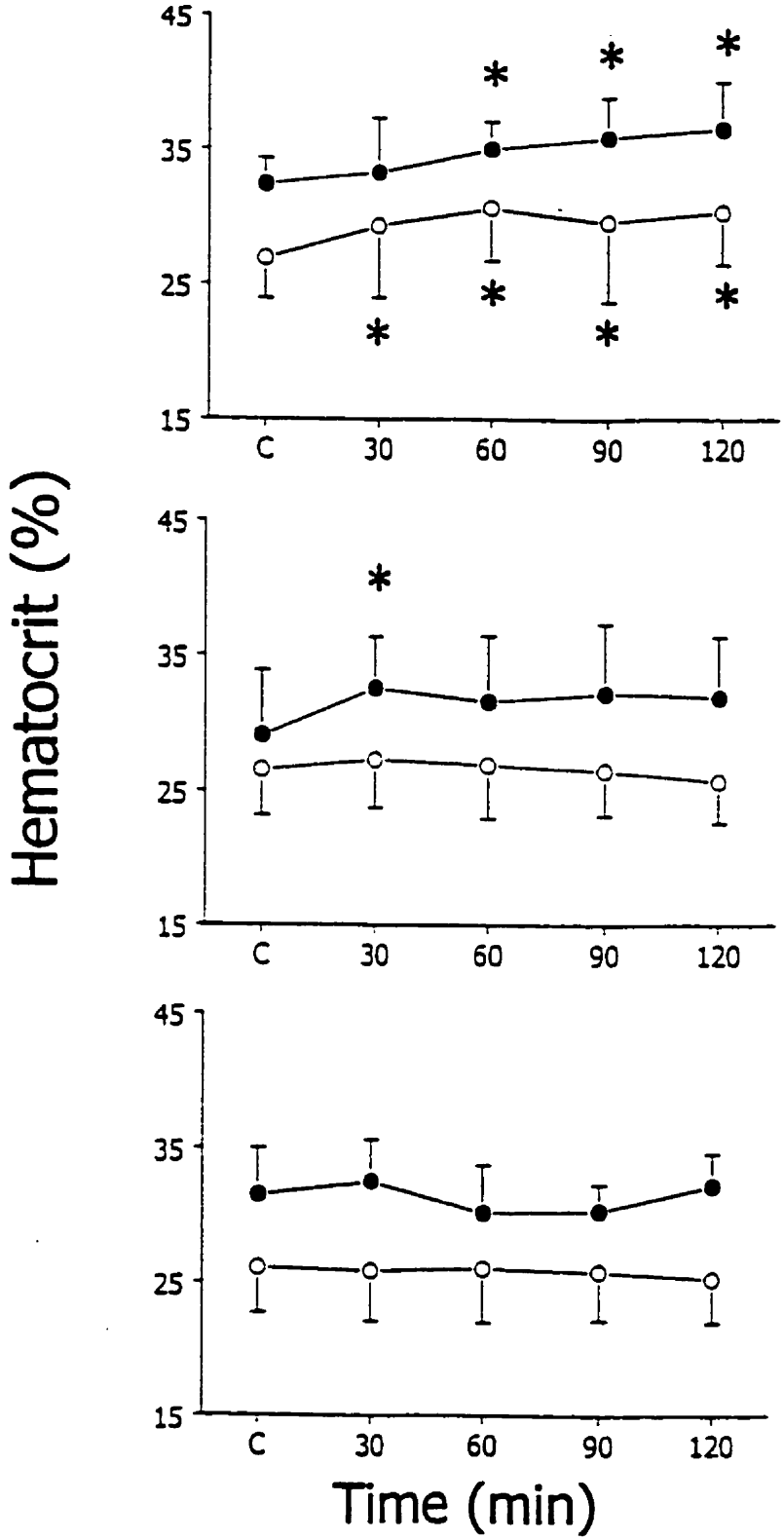


Figure 9. Effect of I.V. administration of furosemide on hematocrit, expressed as change from control, in older lambs (solid circles) and newborn lambs (open circles). Three doses of furosemide are displayed: 5.00 mg/kg (top), 0.25 mg/kg (middle) and 0.00 mg/kg (bottom). Values are mean ± 1 standard deviation. * indicates significant difference from control ($p<0.05$).

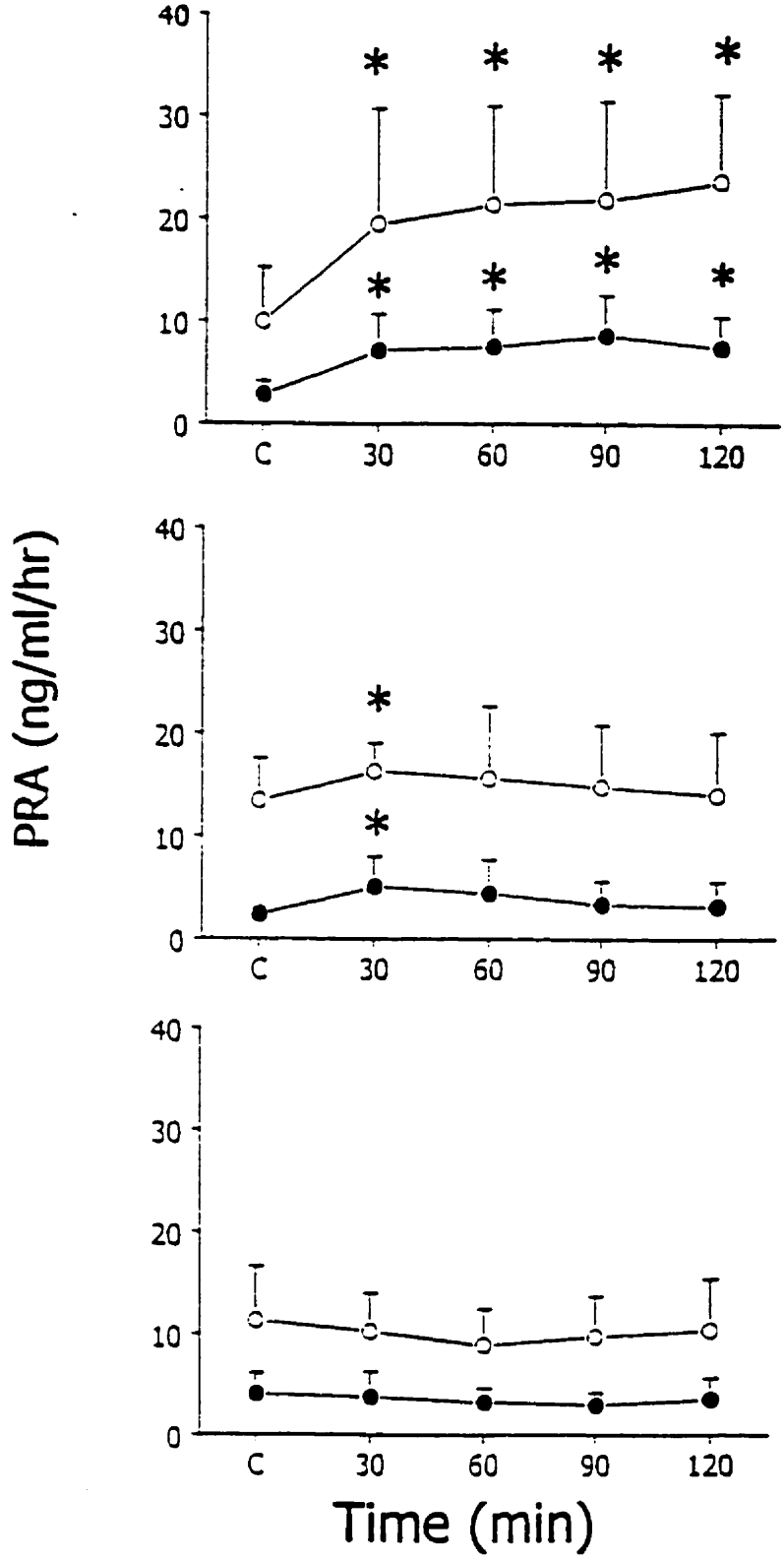


Figure 10. Effect of I.V. administration of furosemide on plasma renin activity (PRA), expressed as change from control, in older lambs (solid circles) and newborn lambs (open circles). Three doses of furosemide are displayed: 5.00 mg/kg (top), 0.25 mg/kg (middle) and 0.00 mg/kg (bottom). Values are mean ± 1 standard deviation. * indicates significant difference from control ($p<0.05$).

3.4 DISCUSSION

The purpose of the present experiments was to determine whether the hemodynamic and renin responses to furosemide administration were age-dependent. Novel findings include: 1) A transient hypertensive phase in response to a 5.00 mg/kg dose of furosemide in older, but not newborn lambs; 2) an increase in heart rate in newborn lambs, but not in older lambs, following administration of a 5.00 mg/kg dose of furosemide; 3) an age-dependent renal blood flow response to furosemide: in newborn lambs, renal blood flow decreased following furosemide, whereas in older lambs renal blood flow increased; 4) a decrease in central venous pressure in response to furosemide (5.00 mg/kg) in both age-groups, and 5) a more pronounced increase in renin secretion in newborn lambs when compared to older lambs following a 5.00 mg/kg dose of furosemide.

Previous studies by Johnston et al. (1984) reported that mean arterial pressure increased by ~5 to 10 mmHg in the 10 minutes immediately following administration of furosemide to healthy humans (51). This pressor response to furosemide was abolished upon pre-treatment with captopril, an angiotensin converting enzyme inhibitor, (30) or when furosemide-stimulated renin release was prevented by salt overload or indomethacin administration (45,51).

We have previously shown a transient increase in blood pressure occurs in young lambs and that this is dose-dependent (59). In the present study, we observed a transient pressor response only in older lambs which is consistent with our previous studies (59). This transient pressor response that occurs in older lambs is correlated to levels of plasma renin activity and again confirms previous observations in conscious lambs from our laboratory (59). The above stated observations suggest that stimulation of the renin-angiotensin system via furosemide is responsible for the transient hypertensive phase, since ANG II is a potent pressor agent.

We proposed in newborn lambs, where the renin response to furosemide is more prolonged and pronounced (67), that the pressor response to furosemide would be accentuated. The absence of a transient hypertensive phase in the newborn lambs following the administration of furosemide was surprising, therefore other factors in addition to ANG II, may be eliciting this pressor response. Maturational differences in the responsiveness of the newborn vasculature to ANG II cannot be ruled out. As it is known in the newborn lamb, as well as the human infant, that the renin-angiotensin system functions at a higher basal level than the adult (72), perhaps the vasculature is less sensitive to ANG II than in the adult due to decreased or downregulation of AT_1 receptors in the newborn vasculature (39).

Previously we measured similar natriuretic and diuretic responses to furosemide in newborns and older lambs (67). A 10-fold increase in urinary flow rate and sodium excretion was observed within the first 20 minutes of furosemide administration, representing a rapid decrease in blood volume (67). In the current study, we did not observe a decrease in mean arterial pressure in either age-group suggesting that arterial baroreceptor compensatory mechanisms prevented mean arterial pressure from falling. This theory is supported by the work of Petersen and DiBona who have shown in anesthetized rats that sinoaortic baroreceptors are essential for maintaining blood pressure after furosemide administration (50).

Furosemide caused a transient increase in blood pressure when administered to anesthetized dogs (52). Interestingly, the pressor response was blocked by pretreatment with the α_2-adrenergic partial agonist, clonidine, implying that the blood pressure response to furosemide was sympathetically mediated. Studies in congestive heart failure patients have documented an acute vasoconstrictor response to furosemide that corresponded to increased circulating levels of both renin and norepinephrine (53). Hence, there is evidence supporting a role for both the renin-angiotensin system and the sympathetic nervous system in contributing to this transient pressor response.

Matherne et al (1988) has studied the ontogeny of the α-adrenoreceptor responses in conscious newborn and adult sheep(81). They concluded that α_1-adrenergic responses to phenylephrine, an α_1-agonist, were similar in newborn lambs and adult sheep. In contrast, the α_2-adrenergic agonist, guanabenz, had a greater vasoconstrictor effect in adult sheep when compared to newborn lambs. These data suggest that the α_2-adrenergic responses to sympathetic activity are developmentally regulated in sheep, at least in the renal vasculature.

It is postulated that, in newborn lambs, the vasoconstrictor response to sympathetic activity is impaired at the level of the α_2-receptors, which may account for the lack of a transient pressor response to furosemide administration in newborn lambs. Further investigation in the systemic vascular bed in newborn lambs is warranted to clarify this postulate.

We have demonstrated an age-dependent heart rate response to furosemide. This increase in heart rate in response to furosemide confirms our previous observations in three week-old conscious lambs where heart rate increased by ~30 bpm (68,69).

In the adult rabbit (17), dog (12) and sheep (16) ANG II attenuates the arterial baroreflex inhibition of heart rate. For example, the pressor response to systemically administrated ANG II is accompanied by no change in heart rate or

a reduction in heart rate that, for a given increase in pressure, is much smaller than other vasoconstrictors, such as phenylephrine (12,13,16,17). This suggests the arterial baroreflex control of heart rate has been shifted towards higher pressures such that the same heart rate now corresponds to a higher blood pressure. It is thought that the mechanism by which ANG II diminishes the heart rate response to an increase in blood pressure is by inhibition of vagal tone to the heart (17). Prior administration of the β-blocker, propanolol, does not abolish the effects of ANG II effects on the heart, suggesting the resetting is not due to an increase in sympathetic tone (17). In the current study, 20 minutes after furosemide administration, when the transient hypertensive phase occurs, there is no change in heart rate in older animals suggesting that ANG II has reset the baroreflex towards higher pressures. In the newborn lambs however, blood pressure remains unchanged whereas heart rate increases 100 minutes after furosemide administration. This is evidence to support the postulate that ANG II has reset the baroreflex towards higher pressures since the same blood pressure now corresponds to a higher heart rate. This will be discussed further in Chapter 4 where the arterial baroreflex control of heart rate was directly assessed.

It is well established that furosemide causes an early increase in renal blood flow when administered to the adult as a result of decreased renal vascular resistance (48). This is thought to be mediated, at least in part, by the vasodilatory

prostaglandin, PGE_2 (48). The decrease in renal blood flow observed in the newborn lamb in the present study is in accordance with previous studies in our laboratory in 3-week old lambs (69). The mechanism(s) governing this response were not investigated in the current experiments however, previous studies have found that in the conscious 3-week old lamb urinary excretion of PGE_2 was not increased after furosemide, contrary with what is seen in the adult (69). It is postulated that the absence of an increase in vasodilatory prostaglandins may therefore explain the decrease in renal blood flow seen in 1-week old lambs. It is also possible that the vasoconstrictor effects of ANG II on the renal vasculature predominate in the absence of antagonist vasodilatory prostaglandins thus, decreasing renal blood flow. This warrants additional investigation.

It is well established that furosemide induces an early non-diuretic increase in venous capacitance (44). Accumulating evidence in adult humans suggests that the acute vascular effects of furosemide are mediated by renin release via the kidneys (45). Furosemide stimulated renin release results in ANG II formation (46), which in turn stimulates the release of vasodilators from the kidneys or venous vasculature. This theory is supported by the fact that ANG II is known to stimulate the production of vasodilatory substances (i.e. PGE_2 or PGI_2) from numerous vascular beds (30). The formation of vasodilatory substances from the kidney or the venous vasculature could result in an increase in venous

capacitance, as ANG II has little venoconstrictor effect (30). Indomethacin blocks the venodilator effect of furosemide in man (45), suggesting that prostaglandins are involved. Other studies have been unable to obtain evidence for an increase in circulating PGI_2 in response to furosemide (45). Collectively, these results demonstrate that the vasodilatory PGE_2 stimulated by ANG II in the vessel wall may be responsible for the acute venodilator effect observed with furosemide.

In the present study, CVP decreased in response to furosemide in both age-groups. The response was two-fold greater in the older lambs (43%) compared to newborn lambs (20%). Decreases in CVP could result from a decrease in blood volume, induced by the renal effects of furosemide (i.e. diuresis) and hence, result in a decrease in venous return to the heart. Alternatively, an increase in venous capacitance would also decrease venous return to the heart and decrease CVP. In newborn lambs, CVP decreased at 60 minutes following furosemide administration. The latency of this response is indicative of the indirect effects of furosemide to decrease blood volume. In the older lambs, CVP decreases 40 minutes after furosemide administration. The earlier onset of CVP decrease could imply that an increase in venous capacitance is occurring, lagging behind ANG II formation. The synergistic effects of venodilation and blood volume depletion could account for the greater decrease in CVP seen in older lambs, as the diuretic response and thus the degree of blood volume depletion was similar in both age-groups. The apparent age-dependent increase in venous

capacitance could be attributed to urinary excretion of PGE_2 remaining unchanged in response to furosemide in newborn lambs (69). This theory is speculative and was not directly investigated in these experiments.

Plasma protein increases in both newborn and older lambs in response to administration of 0.25 mg/kg and 5.00 mg/kg of furosemide. In newborn lambs, plasma protein concentration increased by 1.8% and 7.1% respectively. These findings are supported by previous studies by Bland et al (82) in conscious one week-old sheep. This differential increase in protein levels seen in the present study cannot be explained by differences in urine output since urine outputs, when corrected for body weight, were similar. In newborn lambs, furosemide reduces the transvascular gradient, hastening reabsorption of fluid from the interstitial space (82). Therefore, since the extravascular fluid volume is greater in the newborn, translocation of fluid into the plasma may offset the blood volume depletion induced by furosemide and thus minimise concentration of plasma proteins.

Baseline hematocrit values were age-dependent. Newborn lambs had a resting hematocrit of 26% whilst the hematocrit of older lambs was 32%. These values are in accordance with measurements made by Nuyt *et al* (28) and Bland *et al* (82) in conscious lambs. As we predicted, hematocrit increased in both age groups in response to furosemide, suggesting blood volume depletion.

The present study provides new information that there are age-dependent differences in the pressor response as well as renal blood flow responses to furosemide administration. In addition, age-dependent differences in the heart rate response to furosemide administration were observed, indicating possible modulation of the arterial baroreflex control of heart rate. The theory that furosemide administration alters the arterial baroreflex control of heart rate in an age-dependent manner will be further investigated in Chapter 4.

CHAPTER FOUR

❖

Age-dependent Effects of Furosemide on the Arterial Baroreflex Control of Heart Rate

4.1 INTRODUCTION

It is well known that furosemide administration increases circulating levels of ANG II and AVP in both newborn and adult animals (46,47,60). As mentioned previously in Chapter 1, both of these peptide hormones are known to alter the baroreflex control of heart rate (12-14). In fact, these two hormones have antagonizing effects on the arterial baroreflex, with ANG II shifting the HR:BP relationship toward higher pressures and AVP shifting the HR:BP relationship toward lower pressures. In both newborn and older animals, ANG II is able to modulate the baroreflex control of heart rate (12,35). Recently, however, it has been reported that AVP plays no role in modulating the arterial baroreflex control of heart rate early in life (28). Taken together, it is conceivable that the effects of increased levels of circulating ANG II on the baroreflex in older lambs, but not in newborn lambs, may be minimised by increased circulating levels of AVP; this would assist in restoring the baroreflex to a homeostatic level. In contrast, ANG

II may have a more pronounced effect on the baroreflex in newborn lambs where AVP modulation is immature.

Whether furosemide alters the arterial baroreflex control of heart rate in an age-dependent fashion will be investigated in the following experiments.

4.11 Rationale

Differences in the heart rate response to furosemide in newborn versus older lambs, as discussed in Chapter 3, can be correlated to the activation of the renin-angiotensin system. The increase in heart rate that occurs without a change in blood pressure suggests the arterial baroreflex has been altered (Chapter 3). To investigate this possibility requires that the relationship between HR: SBP be examined via baroreflex curves generated prior to (control) and following furosemide administration to two different age groups of lambs: newborn (one-week) and older (six-weeks).

4.12 Objective

Age-dependent findings described in Chapter 3, suggest the arterial baroreflex control of heart rate is altered with furosemide administration. The objective of the present study was to examine the effect of furosemide on the arterial baroreflex control of heart rate, focusing on age-dependent differences that might occur during postnatal maturation.

4.13 Hypothesis

1) .... that furosemide alters the arterial baroreflex control of heart rate in an age-dependent manner in conscious lambs.

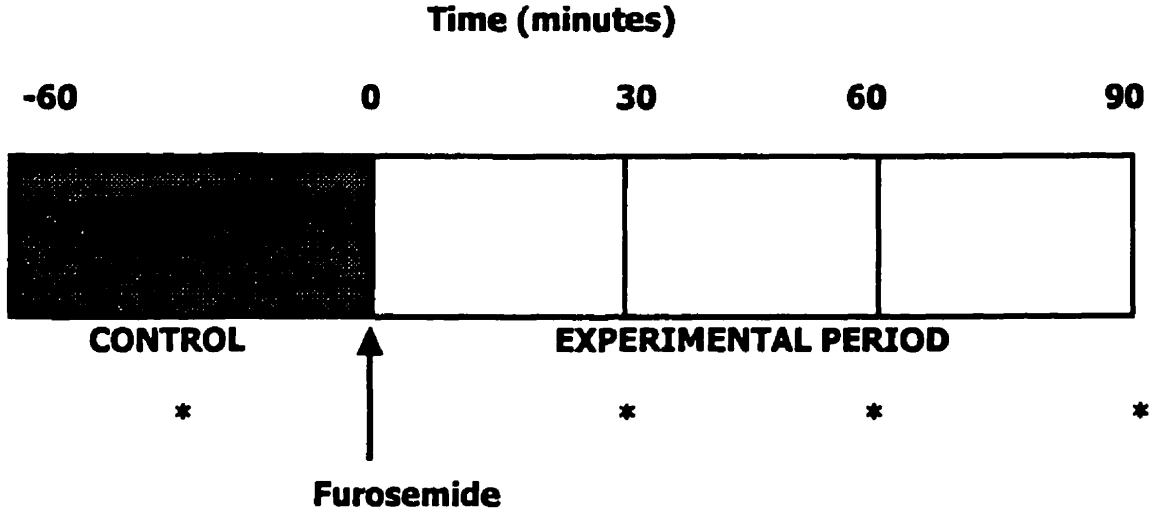
4.2 METHODS

Two experiments were carried out in each lamb at intervals of 24-48 hours. Two age-groups of lambs were studied: older (six week-old, N=6) and newborn (one week-old, N=6). Each experiment consisted of assessments of the arterial baroreflex control of heart rate before and after I.V. injection of one of two doses of furosemide (Lasix, Sabex, Boucherville, Quebec): 0.00 mg/kg and 5.00 mg/kg. The order of these experiments was randomised.

4.21 Specific Protocol

It is common practice to assess the baroreflex by increasing or decreasing the blood pressure using pressor or depressor agents, respectively (3,17,28,83). In order to assess the baroreflex before and after I.V. administration of furosemide, the pressor and depressor agents phenylephrine (10 μg/kg, Sabex, Boucherville, Quebec) and nitroprusside (10 μg/kg, Nipride, Roche, Mississauga, Ontario) were administered intravenously over 15 seconds, to increase and decrease the blood pressure by approximately 25 mmHg, respectively. The arterial baroreflex was evaluated by measuring changes in systolic blood pressure (SBP) and recording concomitant beat-to-beat heart rate responses (HR).

The arterial baroreflex was tested during baseline measurements (control period) and at 30 minute intervals after I.V. administration of furosemide for a maximum of 90 minutes. A diagrammatic representation of the protocol timeline is shown below.



Timeline of protocol illustrating baroreflex assessment (*).

4.22 Computations

Changes in SBP and HR occuring over ~2-3 minutes, following injection of phenylephrine or sodium nitroprusside, were used to assess the relationship between HR: SBP. The results of the phenylephrine blood pressure increase were combined with the results of the sodium nitroprusside blood pressure decrease. A stimulus response curve for the HR: SBP relationship was then constructed using SBP changes and corresponding heart rates. SBP for the

complete range of pressures were obtained by taking 10 control SBP: HR data points prior to the change in SBP and all subsequent points, until a plateau (max or min) occurred. One cardiac cycle (i.e. 1-beat) was allowed to pass before the corresponding HR was taken. All SBP:HR data points were then plotted (Sigmaplot ,Version 4.0 Jandel Scientific) and sigmoidal curves were fitted to the data according to a four parameter sigmoidal function using the following equation (32):

Heart rate (HR) = P4+[P1/(1+exp [P2(SBP-P3)])].

Where P1 is the range between the upper and lower plateaux, P2 is the coefficient to calculate the gain as a function of pressure, P3 is the SBP at midrange of the curve where the gain of the relationship is maximum and P4 is the lower plateau. The gain (slope) was calculated from the first derivative of the above equation. An illustration of these parameters is shown in Figure 11.

4.23 Statistical Analysis

Statistical analysis of differences in the arterial baroreflex curve parameters before (control) and after furosemide were performed using a paired students t-test. Statistical analysis of baseline differences in the arterial baroreflex curve parameters in newborn and older lambs were performed using a non-paired t-

test. Differences were considered significant when $p<0.05$. All results are presented mean ± one standard deviation.

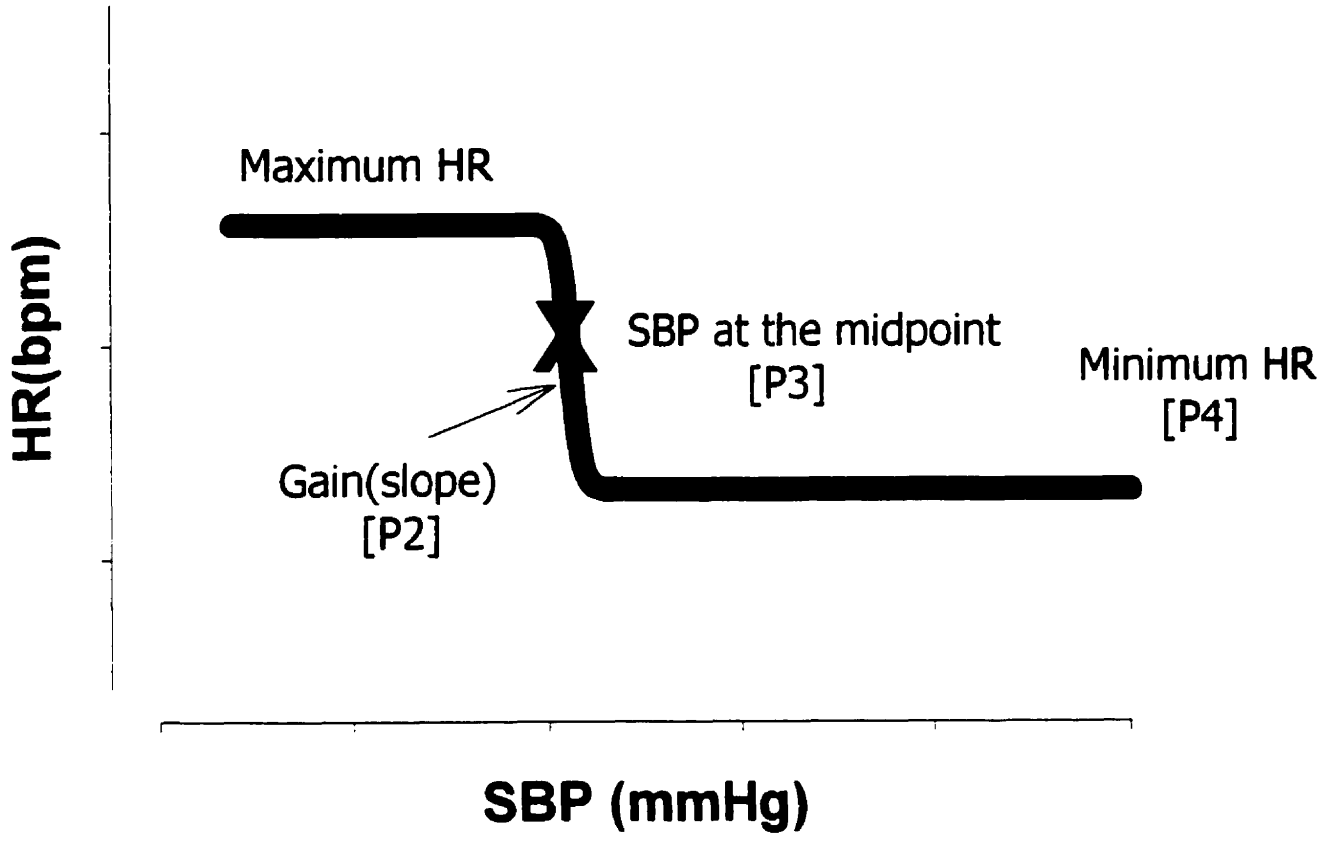


Figure 11. Diagrammatic illustration of the arterial baroreflex relationship between heart rate (HR) and blood pressure (BP). Physiological parameters of gain, SBP at the midpoint, maximum and minimum heart rates are shown.

4.3 RESULTS

4.31 Age-dependent Comparison of Normal Baroreflex Function

The arterial baroreflex, in older lambs, operates over higher BP and lower heart rate compared with newborn lambs (compare Figure 12 & 15). Interestingly, the SBP at the midrange is lower in older than newborns lambs (Table 2). The sensitivity of the arterial baroreflex control of heart rate, as measured by the gain, is comparable in newborn and older lambs (Table 2). As expected the maximum and minimum heart rate values were significantly different, however, the magnitude of the heart rate range over which the arterial baroreflex operated was similar (Table 2).

4.32 Effects of Furosemide on the Baroreflex Control of Heart Rate in Older Lambs

In older lambs, a 5.00 mg/kg dose of furosemide resulted in a significant change at 30 minutes, in the SBP at the midrange of the baroreflex curve or a shift of the baroreflex curve toward higher pressures with the gain remaining unchanged

TABLE 2 Comparison of baseline arterial baroreflex parameters in older and newborn lambs

	Older Lambs	Newborn Lambs
SBP midrange (mmHg)	92±7	120±10*
Gain (bpm/mmHg)	-4.9±6.1	-1.8±1.7*
HR range (bpm)	50 ± 17	62±2
Minimum HR (bpm)	74±7	160±10*
Maximum HR (bpm)	124±12	222±7*

*$p < 0.05$ compared to older lambs. SBP = systolic blood pressure; HR= heart rate

TABLE 3 Effects of 5 mg/kg IV furosemide on parameters describing the arterial baroreflex in older lambs

	CONTROL	30 min	60 min	90 min
SBP midrange (mmHg)	92±7	120±17*	98 ±7	101±7
Gain (bpm/mmHg)	-4.9±6.1	-4.4±17.8	-2.5±5.8*	-3.9±6.1*
HR range (bpm)	50±17	32±42*	35±12	41±17*
Minimum HR (bpm)	74±7	89±29*	73±12	69±12
Maximum HR (bpm)	124±12	121±37	108±12*	110±12*

* $p < 0.05$ compared to Control. SBP= systolic blood pressure; HR= heart rate.

(Figure 12). The HR: SBP relationship is returning to control by 90 minutes (Figure 13 & 14).

Table 3 displays the effects of furosemide on parameters describing the arterial baroreflex in older lambs. In older lambs, a 5.00 mg/kg dose of furosemide shifted the SBP at the midrange of the baroreflex curve 30 minutes after furosemide administration. This shift was transient as the SBP at the midrange of the baroreflex returned to control by 60 minutes (Table 3). Concurrent with the shift of SBP at the midrange was a decrease in the heart rate range and a increase in the minimum heart rate. The gain (sensitivity) of the baroreflex and the maximum heart rate were decreased at 60 and 90 minutes after furosemide administration.

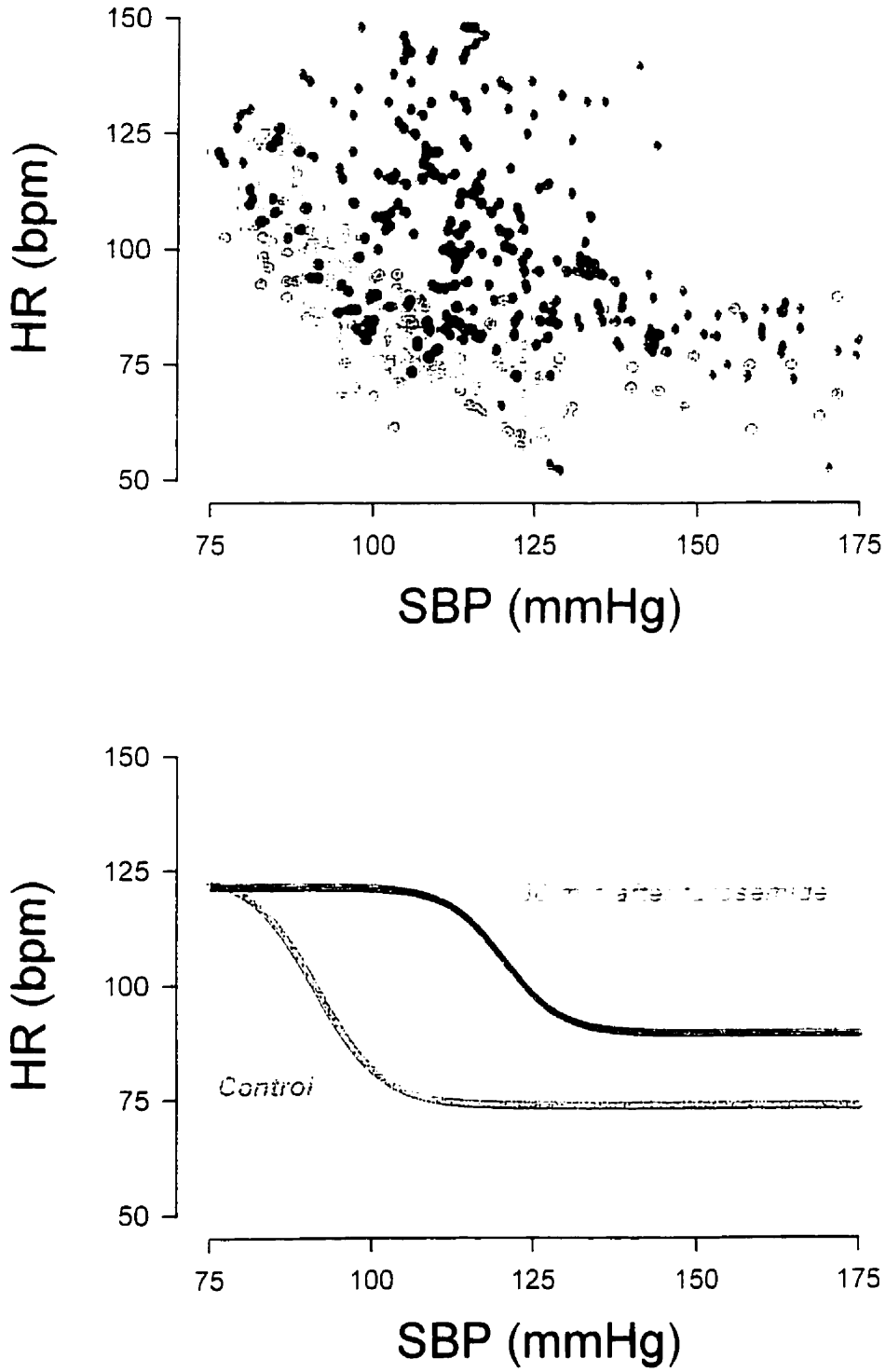


Figure 12. Relationship between heart rate (HR) and systolic blood pressure (SBP) in older lambs before (control) and 30 min after furosemide administration (5.00 mg/kg). Raw data from six conscious lambs (top). Averaged data using a four-parameter logistic sigmodial function (Sigmaplot) (bottom).

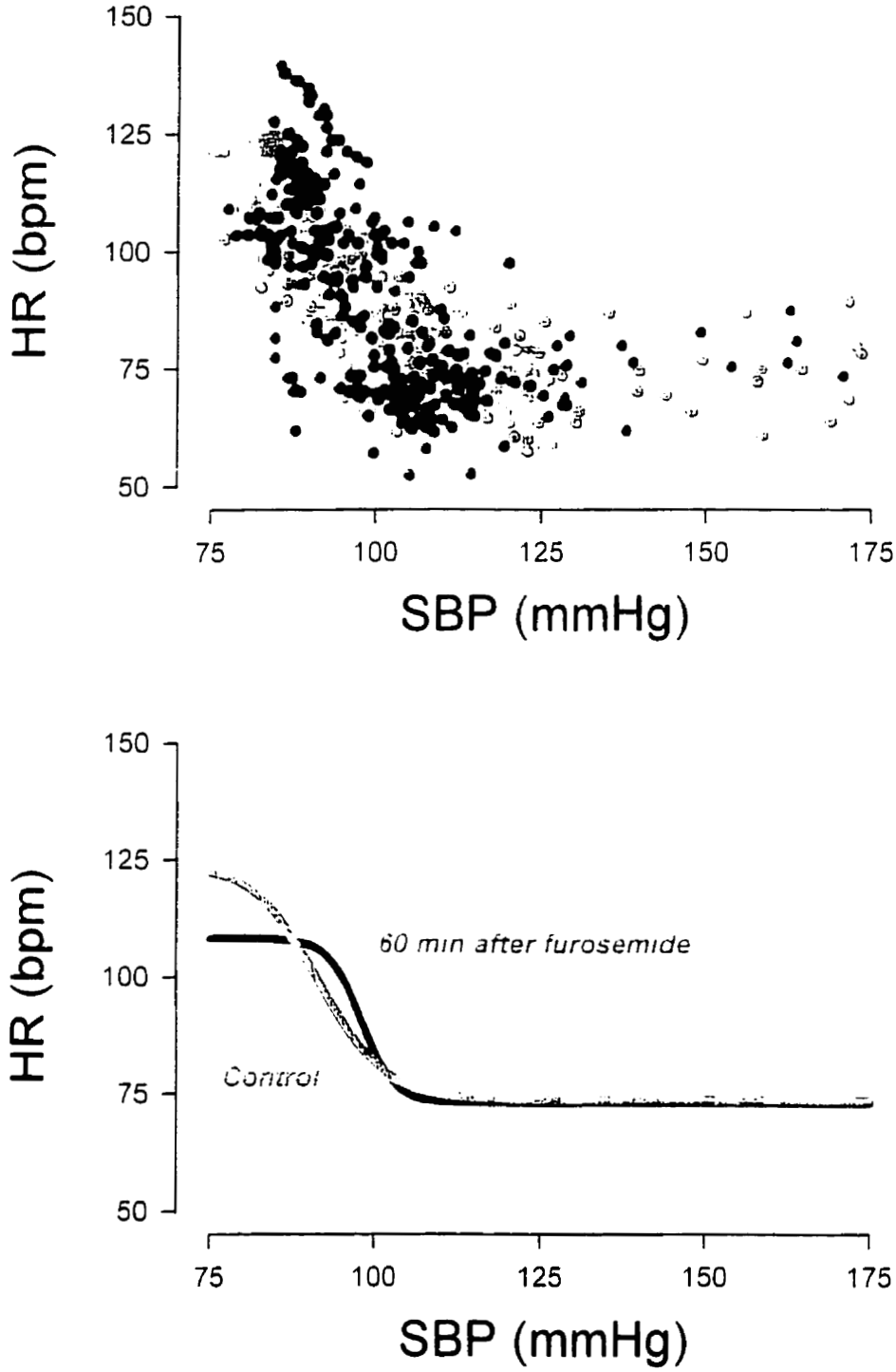


Figure 13. Relationship between heart rate (HR) and systolic blood pressure (SBP) in older lambs before (control) and 60 min after furosemide administration (5.00 mg/kg). Raw data from six conscious lambs (top). Averaged data using a four-parameter logistic sigmodial function (Sigmaplot) (bottom).

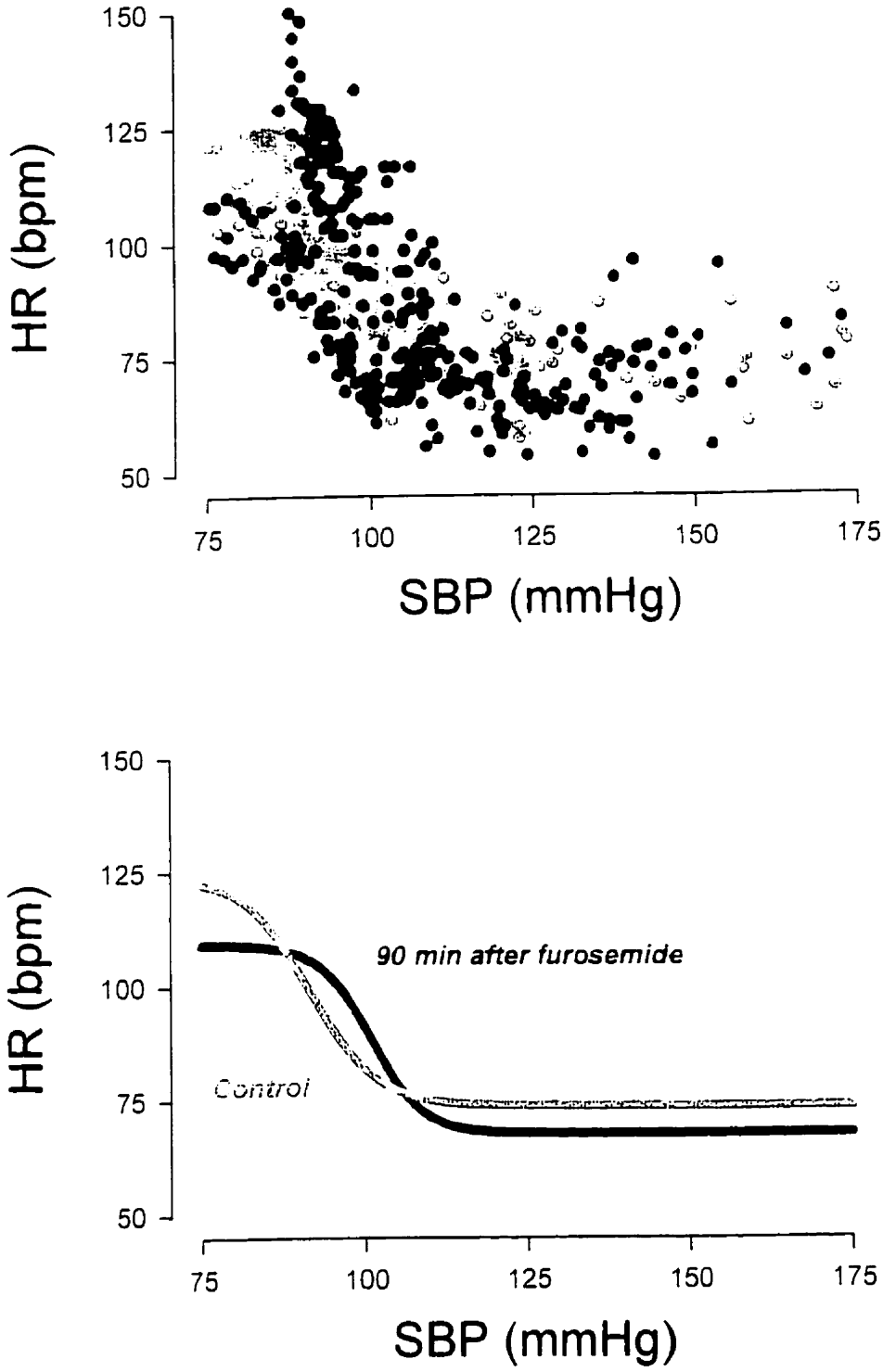


Figure 14. Relationship between heart rate (HR) and systolic blood pressure (SBP) in older lambs before (control) and 90 min after furosemide administration (5.00 mg/kg). Raw data from six conscious lambs (top). Averaged data using a four-parameter logistic sigmodial function (Sigmaplot) (bottom).

4.33 Effects of Furosemide on the Baroreflex Control of Heart Rate in Newborn Lambs

Figure 15 shows the effect of a 5.00 mg/kg dose of furosemide on the arterial baroreflex control of heart rate in newborn lambs at 30 minutes post administration. In newborn lambs, furosemide administration increased the gain (sensitivity) and shifted the SBP at the midrange of the arterial baroreflex curve (Table 4). These effects were sustained for the duration of the experiment. There also was a significant increase in the heart rate range at 30, 60, 90 minutes after furosemide administration ($p<0.05$)(Figure 16 & 17). The minimum heart rate was decreased 30, 60 and 90 minutes after furosemide while the maximum heart rate increased 60-90 minutes after furosemide ($p<0.05$) (Figure 16 & 17).

The baroreflex curves generated in both age-groups for the vehicle dose (0.00 mg/kg) were superimposible. Hence, there were no significant changes in the parameters describing the arterial baroreflex curves at any of the time points ($p<0.05$).

TABLE 4 Effects of 5 mg/kg of furosemide on parameters describing the arterial baroreflex in newborn lambs

	Control	30 min	60 min	90 min
SBP midrange (mmHg)	120±10	114±5*	133±15*	124±5*
Gain (bpm/mmHg)	-1.8±1.7	-7.7±3.9*	-12.3±8.7*	-7.8±3.4*
HR range (bpm)	62±2	78±20*	141±66*	134±29*
Minimum HR (bpm)	160±10	143±17*	133±15*	104±27*
Maximum HR (bpm)	222±7	221±17	274±37*	238±27*

*P< 0.05 compared with Control.

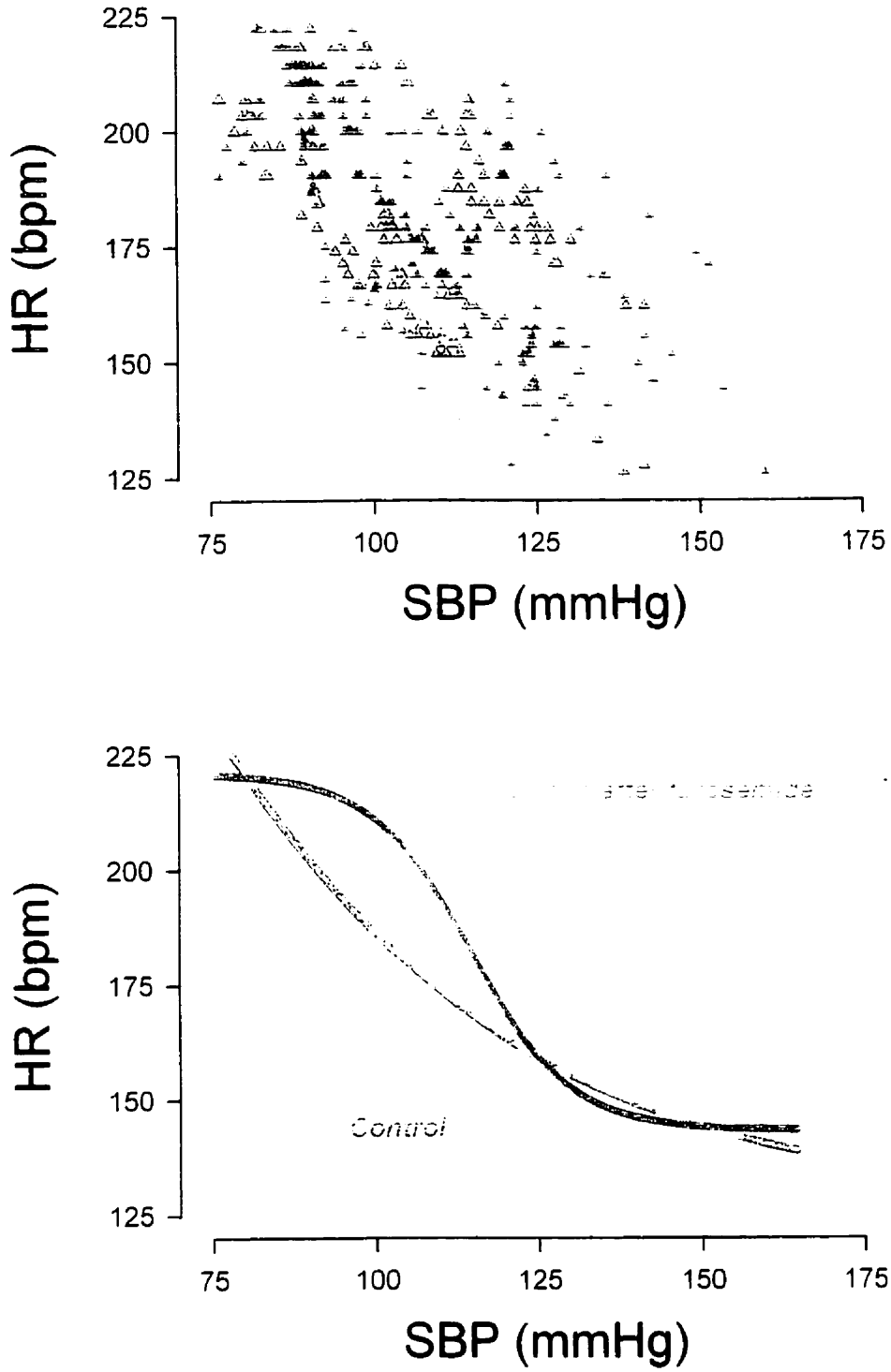


Figure 15. Relationship between heart rate (HR) and systolic blood pressure (SBP) in newborn lambs before (control) and 30 min after furosemide administration (5.00 mg/kg). Raw data from six conscious lambs (top). Averaged data using a four-parameter logistic sigmodial function (Sigmaplot) (bottom).

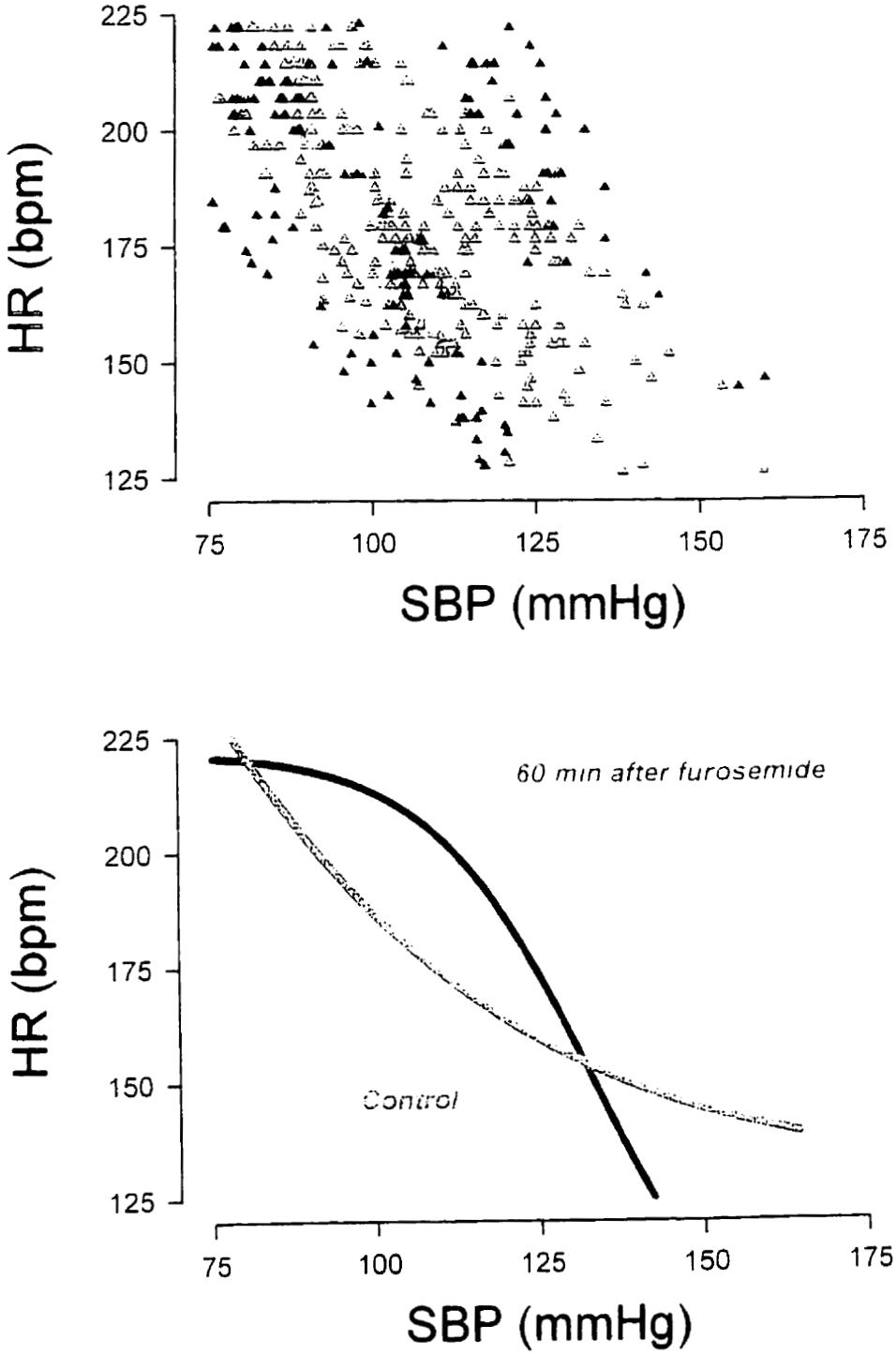


Figure 16. Relationship between heart rate (HR) and systolic blood pressure (SBP) in newborn lambs before (control) and 60 min after furosemide administration (5.00 mg/kg). Raw data from six conscious lambs (top). Averaged data using a four-parameter logistic sigmodial function (Sigmaplot) (bottom).

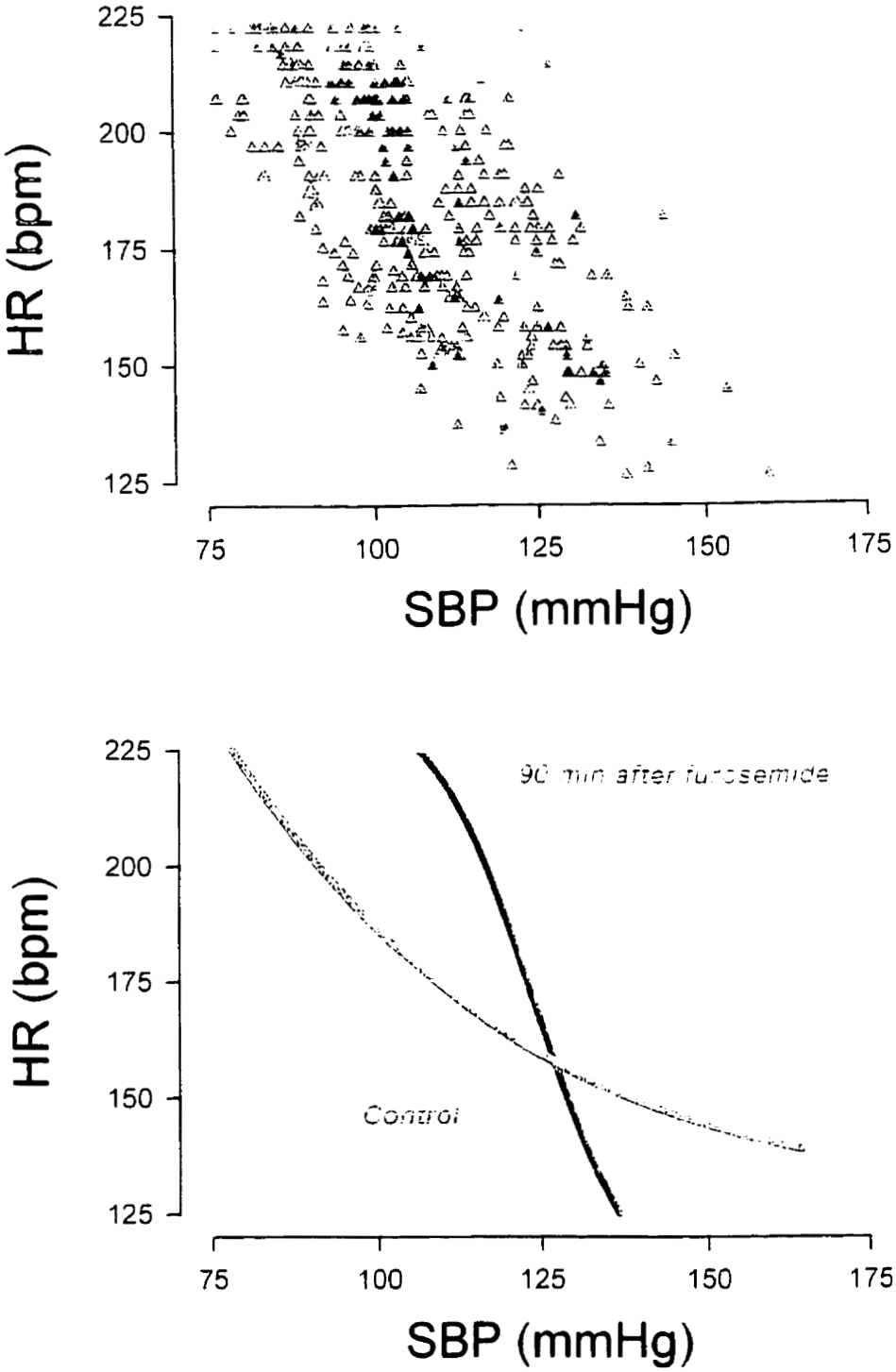


Figure 17. Relationship between heart rate (HR) and systolic blood pressure (SBP) in newborn lambs before (control) and 90 min after furosemide administration (5.00 mg/kg). Raw data from six conscious lambs (top). Averaged data using a four-parameter logistic sigmodial function (Sigmaplot) (bottom).

4.4 DISCUSSION

In the current study, we evaluated the arterial baroreflex control of heart rate at two stages of maturation (one and six weeks). In addition, we measured the effects of furosemide on the arterial baroreflex control of heart rate during postnatal maturation.

The major findings are: 1) A resetting of the baroreflex curve occurred with postnatal age which followed the normal decrease in heart rate concomitant with maturation 2) The sensitivity (gain) of the efferent limb of the baroreflex control of heart rate was comparable in both age-groups as was the range over which the arterial baroreflex operated. Maximum and minimum heart rates were however, altered with age. 3) The arterial baroreflex control of heart rate was altered in response to furosemide administration and 4) An age-dependent effect of furosemide on the arterial baroreflex control of heart rate was observed

Based on the above findings we accept our hypothesis that furosemide alters the arterial baroreflex control of heart rate in an age-dependent manner.

Age-dependent comparison of older and newborn lambs has revealed that the arterial baroreflex control of heart rate resets as maturation proceeds. This resetting towards a lower heart rate followed the normal rise in blood pressure

concomitant with maturation (32,59). The mechanisms that account for this developmental resetting of the arterial baroreflex were not investigated in these experiments. A possible mechanism, however, may involve the high levels of circulating ANG II in the newborn period (72), which could shift the baroreflex towards higher pressures. Administration of the AT_1 receptor antagonist, losartan, shifted the baroreflex to lower pressures (84), suggesting that early in life, ANG II exerts a tonic effect on the baroreflex control of heart rate. The maturation of AVP's modulation of the arterial baroreflex could also aid in developmental resetting in the postnatal period, offsetting ANG II effects. These postulates are speculative and require further investigation.

The current studies have found that the SBP at the midrange of the baroreflex curve is decreased in older compared to newborns lambs. In fact, older lambs have a resting SBP of 102±15 mmHg, which is above the SBP at the midrange (92±3 mmHg). Surprisingly, newborns have a resting SBP of 106±12 mmHg, which is below the SBP at the midrange (120±4 mmHg). These age-dependent differences can be interpreted to suggest that the older lamb is better able to compensate for decreases in blood pressure whilst the newborn lamb is better able to compensate for increases in blood pressure. The physiological relevance of this phenomenon is unknown at present.

In the present experiments, the sensitivity (gain) of the efferent limb of the baroreflex control of heart rate was not different with postnatal age however, a trend for an increase in gain with development was observed. These findings are in agreement with Segar et al (1997) who demonstrated that the sensitivity of the heart rate response to perturbations in SBP is similar in newborn lambs and 4 to 6 week-old lambs (84). Numerous studies have reported a decrease in heart rate response to changes in blood pressure in newborn lambs (31,32), suggesting the arterial baroreflex sensitivity increases with maturation. However, other investigators have found arterial baroreflex is greater in the newborn period and decreases with age (85).

The reasons for disagreement among previous studies and the current study in the reported maturational changes in sensitivity of the baroreflex control of heart rate was not examined but, several differences are apparent. Our studies constructed complete sigmoidal arterial baroreflex heart rate responses in conscious animals using bolus doses of phenylephrine and nitroprusside to increase and decrease the blood pressure, respectively. Others have only evaluated the baroreflex by increasing blood pressure using phenylephrine or aortic balloon inflations (31,85) and have applied linear regression to obtain the slope or sensitivity measurement of the curve.

In older lambs, administration of a 5.00 mg/kg dose of furosemide was associated with a shift in the relationship between HR: SBP towards higher pressures, as indicated by an increase in the SBP at the midrange of the curve and a delayed decrease in the gain. These results are not surprising since administration of a 5.00 mg/kg dose of furosemide results in a peak pressor response at 30 minutes (Chapter 3). This occurs whilst heart rate remains at control levels, suggesting that the arterial baroreflex has been reset towards higher pressures. It is well known that this transient hypertensive phase is correlated to increases in circulating ANG II levels (45,59). The current data support this finding, with PRA significantly elevated 30 minutes after furosemide administration (Chapter 3). This is evidence to suggest that ANG II may be playing a role to reset the arterial baroreflex control of heart rate following furosemide administration.

There is a discrepancy in the literature regarding the effects of ANG II on the arterial baroreflex gain. Studies in conscious dogs (86) and rabbits (17) have found that ANG II resets the arterial baroreflex control of heart rate without altering its sensitivity or gain. The current studies suggest that the gain is altered with furosemide administration to older lambs through the effect of ANG II on the arterial baroreflex. This theory is supported by the studies of Lee and Lumbers (1981) who reported a decrease in the arterial baroreflex gain in conscious adult sheep in response to angiotensin II administration (87). The

premise that furosemide alters the arterial baroreflex gain in six-week old lambs via an increase in ANG II levels requires further investigation.

Furosemide is known to stimulate renin production in conscious lambs within 5 minutes (59). Furosemide also stimulates AVP release within 35 minutes, with the levels further increased after 65 minutes (60). The fact that AVP shifts the arterial baroreflex control of heart rate toward lower pressures complicates the interpretation of the current findings. It has been shown that AVP increases the gain of the arterial baroreflex in the conscious rabbits (29) and baboons (88). Studies by Nuyt et al (1996) have shown that administration of AVP does not alter the baroreflex gain, at least in artificially ventilated, paralyzed adult sheep (28). Thus, it is postulated that, in older lambs, ANG II modulates the arterial baroreflex control of heart rate at 30 minutes but after 60 minutes AVP levels increase and aid in restoring the baroreflex to basal levels. It is unknown whether ANG II and/or AVP are responsible for the change in gain elicited by furosemide. Future studies involving pretreatment with AT_1 or V_1 receptor antagonists prior to furosemide administration could help elucidate the mechanisms underlying this phenomenon. In the future, it would also be of interest to conduct similar studies in edematous animals to determine if the arterial baroreflex control of heart rate is altered in a diseased state.

In newborn lambs, furosemide increases the maximum heart rate and decreases the minimum heart rate. The arterial baroreflex gain is increased whilst the SBP at the midrange is altered. These effects are in contrast to what is observed in older lambs, where the gain and maximum heart rate are decreased and SBP at the midpoint is only transiently changed. To date, there is a paucity of information regarding hormonal modulation of the baroreflex in the conscious newborn animal. Studies by Segar et al (1997) examined the effect of removal of ANG II by systemic administration of the AT_1 receptor antagonist, losartan, to one week-old paralyzed and artificially ventilated lambs. They reported a shift of the baroreflex towards lower pressures with an increase in the gain (35). These studies suggest that ANG II acts to reset the baroreflex towards higher pressures, via AT_1 receptors, in keeping with ANG II's effect on the baroreflex in the adult. These findings are in accordance with furosemide's effects on the baroreflex in older lambs (i.e. an increase of the SBP at midrange and a decrease in gain).

In other studies by the same laboratory, Intra-cerebral ventricle (I.C.V. lateral ventricle) administration of losartan to 1-week-old lambs shifted the baroreflex towards lower pressures with no change in gain but a decrease in the maximum heart rate. These studies provide evidence to suggest that central ANG II can reset the arterial baroreflex control of heart rate with a concomitant increase in heart rate maximum. This phenomenon occurs in newborn, but not older lambs,

in response to furosemide administration suggesting possible maturational differences of ANG II modulation of the baroreflex occurring at the level of the CNS. This postulate is further supported by evidence that I.C.V. (fourth ventricle) administration of losartan resets the arterial baroreflex control of heart rate in 4-8 week old lambs but not in 1-week old lambs. These data suggest that in newborn lambs ANG II is acting at central sites above the brain stem. This developmentally regulated response may be related to maturational changes in AT_1 receptor distribution or function. The exact mechanism(s) governing the interesting age-dependent baroreflex responses to furosemide have yet to be elucidated.

Baroreflex resetting induced by furosemide in newborn lambs is more pronounced and prolonged. The reason for this is unclear. Age-dependent differences in baroreflex modulation observed with furosemide could be attributed to maturational changes in other factors, in addition to ANG II. For example, furosemide stimulates AVP release in both newborn and adult animals (46,47,60). Nuyt et al (1997), recently reported that AVP plays no apparent role in modulating the baroreflex control of heart rate following I.V. infusion of AVP to sedated, paralyzed, and artificially ventilated newborn lambs (28). Thus, it is possible that the absence of AVP's modulation on the arterial baroreflex could accentuate ANG II's effects on the arterial baroreflex in the newborn lamb. This may account for the age-dependent alteration of the arterial baroreflex in

response to furosemide such that in newborn lambs, the alteration of the baroreflex control of heart rate is more pronounced and prolonged compared to older lambs.

The notion that furosemide could be stimulating other neuromodulators of the arterial baroreflex cannot be ruled out. It must be stated that furosemide administration indirectly induces other hormonal substances to be released in addition to ANG II and AVP. For example, ANG II is known to stimulate vasodilators such as nitric oxide. At this time, the effects of these substances on the arterial baroreflex are not well known (89), this is especially true in the newborn animal. Unpublished observations (Sener and Smith) have observed that administration of L-NAME, a blocker of nitric oxide synthesis, resets the arterial baroreflex control of heart rate towards lower pressures in newborn but not older lambs. This maturational effect of nitric oxide on the baroreflex could, in concert with ANG II, enhance the arterial baroreflex resetting observed with furosemide. The possibility that furosemide's effects on the baroreflex are due to other factors, in addition to ANG II or AVP, requires further investigation.

5.0 CONCLUSIONS

The data obtained from the present experiments are among the first definitive descriptions of the arterial baroreflex control of heart rate in conscious animals during postnatal maturation.

This study also provides the first measurement of arterial baroreflex responses to furosemide administration. These data illustrate that furosemide alters the arterial baroreflex control of heart rate and that these alterations are developmentally regulated. Furthermore, the present experiments provide new information that blood pressure, heart rate and renal blood flow responses to furosemide are altered during ontogeny.

We conclude that there are definite age-dependent changes in the hemodynamic and endocrine responses to furosemide, which may be correlated to the maturation of neuromodulating systems. We therefore accept our first hypothesis that furosemide alters the hemodynamic and renin responses to furosemide in an age-dependent fashion. Moreover, we accept our second hypothesis and conclude that furosemide alters the baroreflex control of heart rate during postnatal maturation in conscious lambs.

BIBLIOGRAPHY

1. Cannon, W.B. 1989. The Wisdom of the Body. Classics of Medicine Library, Birmingham.

2. Persson, P.B., H. Ehmke, and H.R. Kirchheim. 1989. Cardiopulmonary-arterial baroreceptor interaction in control of blood pressure. *News in Physiological Science* 4:56-59.

3. Bishop, V.S. 1994. Central nervous system regulation of cardiovascular homeostasis. *New Horizons* 2:415-418.

4. Guo, G.B., M.D. Thames, and F.M. Abboud. 1983. Arterial baroreflexes in renal hypertensive rabbits. Selectivity and redundancy of baroreceptor influence on heart rate, vascular resistance, and lumbar sympathetic activity. *Circulation Research* 53:223-234.

5. Briggs, J.P. and J.B. Schnermann. 1996. Whys and wherefores of juxtaglomerular apparatus function. *Kidney International* 49:1724-1726.

6. Gardiner, S.M. and T. Bennett. 1986. Endogenous vasopressin and baroreflex mechanisms. *Brain Research Reviews* 11:317-334.

7. Sved, A.F. and F.J. Gordon. 1994. Amino acids as central neurotransmitters in the baroreceptor reflex pathway. *News in Physiological Science* 9:243-246.

8. Spyer, K.M. 1981. Neural organization and control of the baroreceptor reflex. *Reviews in Physiological and Biochemical Pharmacology* 88:23-124.

9. Agarwal, S.K. and F.R. Calaresu. 1991. Monosynaptic connection form caudal to rostral ventrolateral medulla in the baroreceptor reflex pathway. *Brain Research* 555:70-74.

10. Stuesse, S.L.a.S.E.F. 1984. Projection to the cardioinhibitory region of the nucleus ambiguus of rat. *Journal of Comparative Neurology* 229:271-278.

11. Kent, B.B., J.W. Drane, B. Blumenstein, and J.W. Manning. 1972. A mathematical model to access changes in baroreceptor reflex. *Cardiology* 57:295-310.

12. Lumbers, E.R., D.I. McCloskey, and E.K. Potter. 1979. Inhibition by angiotensin II of baroreceptor-evoked activity in cardiac vagal efferent nerves in the dog. *Journal of Physiology* 294:69-80.

13. Reid, I.A. 1996. Angiotensin II and baroreflex control of heart rate. *News in Physiological Science* 11:270-274.

14. Cowely, A.W., E.W. Quillen, and M.M. Skelton. 1983. Role of vasopressin in cardiovascular regulation. *Federation Proceedings.* 42:3170-3176.

15. Reid, I.A. 1992. Interactions between ANG II, sympathetic nervous system, and baroreceptor reflexes in regulation of blood pressure. *American Journal of Physiology* 262:E763-E778.

16. Caine, A.C., E. Lumbers, and I.A. Reid. 1985. The effects and interactions of angiotensin and vasopressin on the heart of unanaesthetized sheep. *Journal of Physiology* 367:1-11.

17. Reid, I.A. and L. Chou. 1990. Analysis of the action of angiotensin II on the baroreflex control of heart rate in conscious rabbits. *Endocrinology* 126:2749-2756.

18. Kelly, R.A., C.S. Wilcox, W.E. Mitch, T.W. Meyer, P.F. Souney, C.M. Rayment, P.A. Friedman, and S.L. Swartz. 1983. Response of the kidney to furosemide. II. Effect of captopril on sodium balance. *Kidney International* 24:233-239.

19. Mc Kinley, M.J., R.M. McAllen, F.A.O. Mandelsohn, A.M. Allen, S.Y. Chai, and B.J. Oldfield. 1990. Circumventricular organs: neuroendocrine interfaces between the brain and the hemal milieu. *Frontiers in Neuroendocrinology* 11:91-127.

20. Bishop, V.S. and M. Hay. 1993. Involvement of the area postrema in the regulation of sympathetic outflow to the cardiovascular system. *Frontiers in Neuroendocrinology* 14:57-73.

21. Hegarty, A.A., L.F. Hayward, and R.B. Felder. 1996. Influence of circulating angiotensin II and vasopressin on neurons of the nucleus of the solitary tract. *American Journal of Physiology* 270:R675-R681.

22. Gehlert, D.R., S.L. Gackenheimer, and D.A. Schober. 1991. Autoradiographic localization of subtypes of angiotensin II antagonist binding in the rat brain. *Neuroscience* 44:501-514.

23. Matsukawa, S. and I.A. Reid. 1990. Role of the area postrema in the modulation of the baroreflex control of heart rate via angiotensin II. *Circulation Research* 67:1462-1473.

24. Wong, J., L. Chou, and I.A. Reid. 1993. Role of AT1 receptors in the resetting of the baroreflex control of heart rate by angiotensin II in the rabbit. *Journal of Clinical Investigation* 91:1516-1520.

25. Doris, P. 1984. Central cardiovascular regulation and the role of vasopressin: a review. *Clinical and Experimental Theory and Practice* 12:2197-2217.

26. Hook, J.B., A.H. Blatt, M.J. Brody, and H.E. Williamson. 1966. Effects of several saliuretic-diuretic agents on renal hemodynamics. *Journal of Pharmacology and Experimental Therapeutics* 154:667-673.

27. Luk, J., I. Ajaelo, V. Wong, J. Wong, D. Chang, L. Chou, and I.A. Reid. 1993. Role of V1 receptors in the action of vasopressin on the baroreflex control of the heart rate. *American Journal of Physiology* 265:(34)R524-R529.

28. Nuyt, A.m., J.L. Segar, A.T. Holley, M.S. O'Mara, M.W. Chapleau, and J.E. Robillard. 1996. Arginine vasopressin modulation of arterial baroreflex responses in fetal and newborn sheep. *American Journal of Physiology* 271:R1643-R1653.

29. Undesser, K.P., E.M. Hasser, J.R. Haywood, A.K. Johnson, and V.S. Bishop. 1985. Interactions of vasopressin with the area postrema in arterial baroreflex function in conscious rabbits. *Circulation Research* 56:410-417.

30. Johnston, G.D., D.P. Nicholls, W.J. Leahey, and M.B. Finch. 1983. The effects of captopril on the acute vascular responses to furosemide in man. *Clinical Science* 65:359-363.

31. Shinebourne, E.A., E.K. Vapaavuori, R.L. Williams, M.A. Heymann, and A.M. Rudolph. 1972. Development of baroreflex activity in unanesthetized fetal and neonatal lambs. *Circulation Research* 31:710-718.

32. Dawes, G.S., B.M. Johnston, and D.W. Walker. 1980. Relationship of arterial pressure and heart rate in fetal, new-born and adult sheep. *Journal of Physiology* 309:405-417.

33. Segar, J.L. 1997. Ontogeny of the arterial and cardiopulmonary baroreflex during fetal and postnatal life. *American Journal of Physiology* 273:R457-R471.

34. Downing, S.E. 1960. Baroreceptor reflexes in newborn rabbits. *Journal of Physiology* 150:201-213.

35. Segar, J.L., D.C. Merrill, and J.E. Robillard. 1994. Role of endogenous ANG II on resetting arterial baroreflex during development. *American Journal of Physiology* 266:H52-H59.

36. Consolim-colombo, F.M., M. Hay, T.C. Smith, M. Elizondo-Fournier, and V.S. Bishop. 1996. Subcellular mechanisms of angiotensin II and arginine vasopressin activation of area postrema neurons. *American Journal of Physiology* 271:R34-R41.

37. Grady, E.F., L.A. Sechi, C.A. Griffin, M. Schambelan, and J.E. Kalinyak. 1991. Expression of AT2 receptors in the developing rat fetus. *Journal of Clinical Investigation* 88:921-933.

38. Tufro-McReddie, A., J.K. Harrison, A.D. Everett, and R.A. Gomez. 1993. Ontogeny of type 1 angiotensin II receptor gene expression in the rat. *Journal of Clinical Investigation* 91:530-537.

39. Robillard, J.E., B.C. Schutte, W.V. Page, J.A. Fedderson, C.C. Porter, and J.L. Segar. 1994. Ontogenic changes and regulation of renal angiotensin II type 1 receptor gene expression during fetal and newborn life. *Pediatric Research* 36:755-762.

40. Millan, M., D.M. Jacobowitz, G. Aguilera, and K.J. Catt. 1991. Differential distribution of AT1 and AT2 angiotensin II receptor subtypes in the rat brain during development. *Proceeding of National Academy of Science USA* 88:11440-11444.

41. Seely, J.F. and J.H. Dirks. 1977. Site of action of diuretic drugs. *Kidney International* 11:1-8.

42. Stokes, W. 1964. A new effective diuretic-Lasix. *British Medical Journal* 2:910-914.

43. Wilcox, C.S., W.E. Mitch, R.A. Kelly, K. Skorecki, T.W. Meyer, P.A. Friedman, and P.F. Souney. 1983. Responses of the kidney to furosemide I. Effects of salt intake and renal compensation. *Journal of Laboratory and Clinical Medicine* 102:450-458.

44. Dikshit, K., J. Vyden, J. Forrester, K. Chatterjee, R. Prakash, and H. Swan. 1973. Renal and extrarenal hemodynamic effects of furosemide in congestive heart failure after acute myocardial infarction. *New England Journal of Medicine* 288:1087-1090.

45. Johnston, G.D., W.R. Hiatt, A.S. Nies, N.A. Payne, R.C. Murphy, and J.G. Gerber. 1983. Factors modifying the early nondiuretic vascular effects of furosemide in man. *Circulation Research* 53:630-635.

46. Vander, A.J. and J. Carlson. 1969. Mechanism of the effects of furosemide on renin secretion in anesthetized dogs. *Circulation Research* XXV:145-152.

47. Lammintausta, R., M. Anttila, and O. Viinamaki. 1980. Responses of renin, aldosterone and antidiuretic hormone to furosemide and furosemide-triamterene combination. *International Journal of Clinical Pharmacology, Therapy and Toxicology* 18:395-398.

48. Gerber, J. and A. Nies. 1980. Furosemide-induced renal vasodilation: The role of the release of arachidonic acid. *Advances in Prostaglandin and Thromboxane Research* 7:1079-1082.

49. Janssen, B.J.A., H.A.J. Struyker-Boudier, and J.F.M. Smits. 1989. Acute arteriolar vasoconstriction following furosemide in conscious spontaneously hypertensive rats. *European Journal of Pharmacology* 170:1-9.

50. Petersen, J.S. and G.F. DiBona. 1994. Reflex control of renal sympathetic nerve activity during furosemide diuresis in rats. *American Journal of Physiology* 266:R537-R545.

51. Johnston, G.D., D.P. Nicholls, and W.J. Leahey. 1984. The dose-response characteristic of the acute non-diuretic peripheral vascular effects of frusemide in normal subjects. *British Journal of Clinical Pharmacology* 18:75-81.

52. Ueno, Y., M. Arita, H. Suruda, O. Mohara, and Y. Masuyama. 1983. Contributions of central sympathetic neural activity to furosemide-induced increases in plasma renin activity and noradrenaline. *Japan Heart Journal* 24:259-267.

53. Francis, G.S., R.M. Siegel, S.R. Goldsmith, M.T. Olivari, T.B. Levine, and J.N. Cohn. 1985. Acute vasoconstrictor response to intravenous furosemide in patients with congestive heart failure. *Annuals in Investigative Medicine* 103:1-6.

54. Nelson, G.I.C., R.C. Ahuja, B. Silke, R.C. Okoli, M. Hussain, and S.H. Taylor. 1983. Haemodynamic effects of frusemide and its influence on repetitive rapid volume loading in acute myocardial infarction. *European Heart Journal* 4:706-711.

55. Lorenz, J.N., H. Weihprecht, J. Schnermann, O. Skott, and J.P. Briggs. 1990. Characterization of the macula densa stimulus for renin secretion. *American Journal of Physiology* 259:F186-F193.

56. Smith, F.G., A.M. Strack, and S. Wildt. 1996. Renal nerves do not modulate the renal and endocrine responses to furosemide in conscious lambs. *Canadian Journal of Physiology & Pharmacology* 74:614-620.

57. Schmitt, S., K. Taylor, R. Schmidt, D. Van Orden, , and . 1997. The role of volume depletion, antidiuretic hormone and angiotensin II in the furosemide-induced decrease in mesenteric conductance in the dog. *Journal of Pharmacology and Experimental Therapeutics* 219:407-414.

58. Gariépy, L., P. Larose, B. Bailey, and P. du Souich. 1992. Effect of lignocaine on arginine-vasopressin plasma levels: baseline or induced by frusemide. *British Journal of Pharmacology* 106:470-475.

59. Patel, A. and F.G. Smith. 1997. Dose dependent cardiovascular, renal and endocrine effects of furosemide in conscious lambs. *Canadian Journal of Physiology & Pharmacology* 75:1101-1107.

60. Siegel, S.R., R.E. Weitzman, and D.A. Fisher. 1979. Endogenous angiotensin stimulation of vasopressin in the newborn lamb. *Journal of Clinical Investigation* 63:287-293.

61. Aranda, J.V., J. Perez, D.S. Sitar, J. Collinge, A. Portuguez-Malavasi, B. Duffy, and C. Dupont. 1978. Pharmacokinetic disposition and protein binding of furosemide in newborn infants. *Journal of Pediatrics* 93:507-511.

62. Richardson, H. 1971. Furosemide in heart failure of infancy. *Arch.Dis.Child.* 46:520-524.

63. Chemtob, S., B.S. Kaplan, J.R. Sherbotie, and J.V. Aranda. 1989. Pharmacology of diuretics in the newborn. *Pediatric Clinics of North America* 36:1231-1250.

64. Kaplan, M.R., M.D. Plotkin, D. Brown, S.C. Hebert, and E. Delpire. 1996. Expression of the mouse Na-K-2Cl cotransporter, mBSC2, in the terminal inner medullary collecting duct, the glomerular and extraglomerular mesangium, and the glomerular afferent arteriole. *Journal of Clinical Investigation* 98:723-730.

65. Greene, T.P. 1987. The pharmacological basis of diuretic therapy in the newborn. *Clinics in Perinatology* 14:951-964.

66. Loggie, J.M.H., L.I. Kleinman, and E.F. Van Maanen. 1975. Renal function and diuretic therapy of infants and children. Part III. *Journal of Pediatrics* 86:825-832.

67. Smith, F.G. and J. Abraham. 1995. Renal and renin responses to furosemide in conscious lambs during postnatal maturation. *Canadian Journal of Physiology & Pharmacology* 73:107-112.

68. Smith, F.G. and A.M. Strack. 1995. Effects of renal denervation on cardiovascular response to furosemide in conscious lambs. *American Journal of Physiology* 269:H149-H152.

69. de Wildt, S.N. and F.G. Smith. 1997. Effects of angiotensin converting enzyme (ACE) inhibitor, captopril, on the cardiovascular, endocrine, and renal responses to furosemide in conscious lambs. *Canadian Journal of Physiology & Pharmacology* 75:263-270.

70. Siegel, S. and D.A. Fisher. 1977. The renin-angiotensin-aldosterone system in the newborn lamb: response to furosemide. *Pediatric Research* 11:837-839.

71. Van Acker, K.J., S.L. Scharpe, A.J. Deprettere, and H.M. Neels. 1979. Renin-angiotensin-aldosterone system in the healthy infant and child. *Kidney International* 16:196-203.

72. Mott, J.C. 1975. The place of the renin-angiotensin system before and after birth. *British Medical Bulletin* 31:44-50.

73. Bednarik, J.A. and C.N. May. 1995. Evaluation of a transit-time system for the chronic measurement of blood flow in conscious sheep. *American Journal of Physiology* 78:524-530.

74. Woo, W., C. Dupont, J. Collinge, and J.A. Aranda. 1978. Effects of furosemide in the newborn. *Clinical Pharmacology & Therapeutics* 23:266-271.

75. Weber, P.C., B. Scherer, and C. Larsson. 1977. Increase of free arachidonic acid by furosemide in man as the cause of prostaglandin and renin release. *European Journal of Pharmacology* 41:329-332.

76. Fujimura, A. and A. Ebihara. 1988. Role of angiotensin II in renal prostaglandin E_2 production after furosemide administration. *Hypertension* 11:491-494.

77. Mackay, I.G., A.L. Muir, and M.L. Watson. 1984. Contribution of prostaglandins to the systemic and renal vascular response to frusemide in normal man. *British Journal of Clinical Pharmacology* 17:513-519.

78. Kirchner, K.A. 1989. Role of medullary plasma flow in the attenuated furosemide response in indomethacin treated rats. *Journal of Pharmacology and Experimental Therapeutics* 249(3):757-761.

79. Haber, E., T. Koerner, B. Page, B. Kilman, and A. Purnobe. 1969. Application of radioimmunoassay for angiotensin I to the physiological measurements of plasma renin activity in normal human subjects. *Journal of Clinical Endocrinlogy and Metabolism* 29:61-79.

80. Siegel, S.R. and D.A. Fisher. 1980. Ontogeny of the renin-angiotensin-aldosterone system in the fetal and newborn lamb. *Pediatric Research* 14:99-102.

81. Matherne, G.P., K.T. Nakamura, and J.E. Robillard. 1988. Ontogeny of a-adrenoceptor responses in renal vascular bed of sheep. *American Journal of Physiology* 254:R277-R283.

82. Bland, R.D., D.D. McMillan, and M.A. Bressack. 1978. Decreased pulmonary transvascular fluid filtration in awake newborn lambs after intravenous furosemide. *Journal of Clinical Investigation* 62(3):601-609.

83. Hasser, E.M. and V.S. Bishop. 1990. Reflex effect of vasopressin after blockade of V1 receptors in the area postrema. *Circulation Research* 67:265-271.

84. Segar, J.L., A. Minnick, A.m. Nuyt, and J.E. Robillard. 1997. Role of endogenous ANG II and AT1 receptors in regulating the arterial baroreflex responses in newborn lambs. *American Journal of Physiology* 272:R1862-R1873.

85. Maloney, J.E., E. Cannata, M.H. Dowling, W. Else, and B. Ritchie. 1977. Baroreflex activity in conscious fetal and newborn lambs. *Biology of the Neonate* 31:340-350.

86. Brooks, V.L. and I.A. Reid. 1986. Interaction between angiotensin II and baroreceptor reflex in the control of ACTH secretion and heart rate. *Circulation Research* 58(6):829-838.

87. Lee, W.B. and E.R. Lumbers. 1981. Angiotensin and the cardiac baroreflex response to phenylephrine. *Clinical Experimental Pharmacology and Physiology* 8:109-117.

88. Barazanji, M.W. and K.G. Cornish. 1989. Vasopressin potentates ventricular and arterial reflexes in the conscious nonhuman primate. *American Journal of Physiology* 256:H1546-H1552.

89. Du, Z.-Y., G.J. Dusting, and O.L. Woodman. 1992. Baroreceptor reflexes and vascular reactivity during inhibition of nitric oxide synthesis in conscious rabbits. *European Journal of Pharmacology* 214:21-26.