A TECHNIQUE FOR THE STUDY OF FACTORS INFLUENCING SODIUM EXCRETION IN THE RABBIT AND ITS USE IN EXAMINING THE ROLE OF CEPHALIC SODIUM CONCENTRATION IN THE CONTROL OF RENAL SODIUM EXCRETION

by

Kimberly Lynn Barker

B.Sc.H., Queen's University, 1992

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

DEPARTMENT OF PHYSIOLOGY

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

June 1994

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Physiology

The University of British Columbia
Vancouver, Canada

Date May 25/94.

DE-6 (2/88)

ABSTRACT

The regulation of overall body fluid balance as well as fluid sodium concentration and osmolality involves a complex and interrelated sequence of similar receptor and effector mechanisms. In a recent study in conscious dogs, intracarotid infusions of hypertonic saline were performed concurrent with a jugular vein infusion of water at a rate such that only the areas of the brain supplied by the carotid arteries experienced an increase in plasma sodium concentration (Emmeluth et al., 1992). The results of these infusions were then compared to the effects of an identical salt and water load infused as isotonic saline at the same rate into the carotid arteries and jugular vein. It was concluded from these studies that a mechanism regulating sodium excretion in the distribution of the carotid arteries exists in the conscious dog, that the efferent pathway of this mechanism likely involves the release of urodilatin (a natriuretic peptide), and that this occurs in response to changes in osmolality smaller than those required to stimulate the release of vasopressin. In order to further examine these conclusions, we used similar intracarotid infusions of hypertonic and isotonic saline in an anaesthetized rabbit preparation. The preparation used in these experiments was developed so that renal perfusion pressure could be controlled without interrupting renal blood flow at any time during the surgical procedure. This preparation was examined with respect to the state of hydration of the animals, renal function and renal nerve function, all of which were determined to be normal. Results of experiments done using this preparation do not support the concept of a specific mechanism regulating sodium excretion in the

distribution of the carotid arteries that acts independently of renal perfusion pressure. The intracarotid infusion of hypertonic saline did not cause a greater increase in sodium excretion or a greater decrease in renal vascular resistance than the intracarotid infusion of isotonic saline. Immunoreactive atrial natriuretic peptides do not appear to play a role in mediating sodium excretion in this study and, the release of vasopressin and an increase in sodium excretion appeared to occur in response to the same degree of osmotic change.

TABLE OF CONTENTS

Abstract		p. ii
Table of Contents		p. iv
List of Figures		p. vii
Acknowledgements		p. viii
INTRODUCTION		
1.)	The Homeostasis of Fluid Volume, Sodium and Osmolality	p. 1
2.)	Receptor Mechanisms	p. 3
	a.) Volume Receptors	p. 3
	b.) Osmoreceptors and Sodium Receptors	p. 8
3.)	Effector Mechanisms	p. 19
	a.) Renal Mechanics	p. 19
	b.) Factors Regulating Water and Sodium Excretion:	p. 22
	i.) Renal Autoregulation	p. 22
	ii.) Renal Perfusion Pressure	p. 23
	iii.) Renal Nerves	p. 24
	iv.) Endocrine and Paracrine Factors Affecting Sodium Excretion	p. 25
4.)	Summary and Rationale for Performing these Studies	p. 33
5.)	Goals of these Studies	p. 34
6.)	Hypotheses	p. 35
METHODS		p. 36
1.)	Introduction	p. 36
2.)	Anaesthesia and General Management	p. 36
3.)	Surgical Procedures	p. 38
4.)	Electronic Recording	p. 52
5.)	Chemical Analysis	p. 53
6.)	Radioimmunoassays	p. 58
7.)	Calculations	p. 64
8.)	Experimental Protocols	p. 65
9.)	Statistics	p. 73
10.)	Format and Presentation of Results	p. 74

RESULTS

Part 1)	Protocols 1 & 2 - Validation of the Preparation	p. 76
a.)	State of Hydration of the Animals	p. 76
b.)	Renal Function	p. 76
c.)	Endocrine Parameters	p. 78
d.)	Renal Nerve Function	p. 79
Part 2)	Protocol 3 - The Effects of Increasing Sodium Concentration in Blood Perfusing the Brain	p. 89
a.)	State of Hydration of the Animals	p. 89
b.)	Renal Effects	p. 90
c.)	Cardiovascular Effects	p. 94
d.)	Endocrine Effects	p. 96
e.)	Validation of the Hypertonic Saline Infusions	p. 98

DISCUSSION

Part 1)	Protocols 1 & 2 - Validation of the Preparation	p. 115
a.)	State of Hydration of the Animals	p. 115
b.)	Renal Function	p. 116
c.)	Endocrine Parameters	p. 118
d.)	Renal Nerve Function	p. 119
e.)	Summary	p. 120
Part 2)	Protocol 3 - The Effects of Increasing Sodium Concentration in Blood Perfusing the Brain	p. 120
a.)	State of Hydration of the Animals	p. 120
b.)	Effects of the Control and Experimental Procedures on GFR, the Filtered Fractions of Sodium and Potassium and Osmolar Clearance	p. 122
c.)	Effects of the Control and Experimental Procedures on Renal Blood Flow and Vascular Resistance	p. 123

DISCUSSION (cont'd)

d.) Effects of the Control and Experimental Procedures on Plasma IR-ANP, Right Atrial Pressure, IR-ANP Excretion and the Filtered Fraction of IR-ANP p. 124

e.) Effects of the Control and Experimental Procedures on Sodium Excretion p. 125

f.) Different Effects of the Control and Experimental Procedures p. 127

g.) Vasopressin Levels in the Control and Experimental Procedures p. 130

h.) Validating the Sodium Infusion p. 131

CONCLUDING COMMENTS p. 133

REFERENCES p. 137

List of Figures

METHODS

Fig. 1 - Placement of Double Lumen Cannula in the Descending Aorta p. 47
Fig. 2 - Placement of Cannulae in the Carotid Artery p. 51
Fig. 3 - Experimental Protocol #1 p. 68
Fig. 4 - Experimental Protocol #2 p. 69
Fig. 5 - Experimental Protocol #3 p. 71

RESULTS

Part 1: Validation of the Preparation

Fig. 6 - Plasma Osmolality and Hematocrit p. 80
Fig. 7 - Plasma Protein and Plasma Sodium p. 81
Fig. 8 - Urine Volume Produced in 15 minutes p. 82
Fig. 9 - Sodium Excretion in 15 minutes p. 83
Fig. 10 - Glomerular Filtration Rate Normalized to weight p. 84
Fig. 11 - Filtered Fraction of Sodium and Potassium p. 85
Fig. 12 - Plasma IR-ANP Concentration p. 86
Fig. 13 - IR-ANP Excreted in 15 minutes and Filtered Fraction of IR-ANP p. 87
Fig. 14 - Renal Nerve Function p. 88

Part 2: The Effects of Increasing Sodium Concentration in Blood Perfusing the Brain

Fig. 15 - Plasma Osmolality and Hematocrit p. 99
Fig. 16 - Plasma Protein and Plasma Sodium p. 100
Fig. 17 - Urine Flow p. 101
Fig. 18 - Sodium Excretion in 15 minutes p. 102
Fig. 19 - Glomerular Filtration Rate Normalized to Weight p. 103
Fig. 20 - Filtered Fraction of Sodium and Potassium p. 104
Fig. 21 - Osmolar Clearance p. 105
Fig. 22 - Free Water Clearance p. 106
Fig. 23 - Arterial Blood Pressure p. 107
Fig. 24 - Right Atrial Pressure p. 108
Fig. 25 - Renal Blood Flow p. 109
Fig. 26 - Vascular Resistance p. 110
Fig. 27 - IR-ANP Excreted in 15 minutes & Plasma IR-ANP p. 111
Fig. 28 - IR-ANP Excreted in 15 minutes and Filtered Fraction of IR-ANP p. 112
Fig. 29 - Plasma Arginine Vasopressin Concentration p. 113
Fig. 30 - Validation of the Carotid Artery Infusions p. 114

ACKNOWLEDGEMENTS

The experiments described in this thesis could not have been accomplished without the assistance and support of a number of people. I would like to thank Carolyn Redekopp for all of her kindnesses, her assistance with radioimmunoassays and for keeping me well supplied with cookies and flowers. I would also like to thank Jeannie Sharp-Kehl for her technical expertise, for treating these animals with the same care as her patients in intensive care and above all for her friendship. I appreciate the advice and assistance of Gabriele Weichert who has helped me in so many ways (not to mention proofreading above and beyond the call of duty) and I would also like to thank all of the other graduate students for their encouragement and support. I appreciate the comments and suggestions of faculty members who have read this thesis and I would like to thank B.C. Tel for helping me to keep in touch with my family and friends in the east. Finally and above all I would like to thank Dr. Ledsome for his ongoing encouragement and support, for his unfailing ability to answer any question and for sharing with me his enthusiasm for research. It has been a privilege and a pleasure to work in his laboratory over the last two years.

INTRODUCTION

1.) THE HOMEOSTASIS OF FLUID VOLUME, SODIUM AND OSMOLALITY

The regulation of overall body fluid balance as well as fluid sodium concentration and osmolality involves a complex and interrelated sequence of similar receptor and effector mechanisms (McKinley, 1992). Aside from their related receptor and effector mechanisms, these three factors alone (i.e. sodium, osmolality and overall body fluid volume) are also interdependent. Sodium, as the most abundant cation in the extracellular fluid (McKinley, 1992), is primarily responsible for the osmolality of all extracellular fluid. Due to the attraction of sodium ions and water, sodium levels also affect fluid volume levels through solvent drag as do other osmotic particles in the extracellular fluid (Schauf et al., 1990). Finally, an increase in consumed fluid results in a dilution of plasma osmolality while dehydration results in an increase in plasma osmolality (Brenner and Rector, 1991a). In spite of the complexity of these regulatory systems, body fluid and osmolar balance basically depend on two factors: The appropriate intake of water (determined primarily by thirst receptors) and the appropriate excretion of water by the kidneys (primarily controlled by arginine vasopressin (AVP)).

An increase in thirst as well as an increased AVP secretion (causing antidiuresis) both occur in response to increases in plasma osmolality of only 1-2% (Robertson, 1976; Thompson et al., 1986). This precise regulation of plasma osmolality and fluid balance,

affecting both the intake and excretion of water, underlies the importance of maintaining plasma osmolality within a narrow range. Unfortunately, a number of pathological conditions do occur where the kidney is unable to adequately regulate sodium and fluid excretion. For example, in all forms of chronic hypertension, a risk factor for heart disease, the kidney does not increase fluid and sodium excretion appropriately to maintain normal blood pressure (Hall, 1990). Although there is some controversy over whether this dysfunction of the kidney is a cause or a consequence of hypertension (Omvik et al., 1980), the fact that renal fluid and sodium excretion is impaired during hypertension emphasizes the importance of fully understanding the regulation of body fluid osmolality and volume.

In the vast majority of cases, plasma osmolality will increase concurrent with a decrease in body fluid volume, and thirst and antidiuresis will work together to restore these variables to normal physiological levels. However, experiments have been done to determine the prevailing stimulus if plasma osmolality and fluid volume change so as to cause conflicting compensatory mechanisms. In 1982, Cowley demonstrated that in situations where hyponatremia exists concurrently with hypovolemia, the regulation of blood volume overrides that of osmolality (i.e. "dilutional hyponatremia is a lesser evil than circulatory collapse"). Fortunately, receptor and effector mechanisms controlling fluid osmolality and volume rarely work in opposition. Normally, volume receptors, osmoreceptors and sodium receptors work collectively and their stimulation results in the activation of many of the same effector mechanisms. Although this redundancy adds to

the complexity of this regulatory system, it further enables the body to precisely regulate the osmolality and volume of body fluid.

2.) RECEPTOR MECHANISMS

a.) Volume Receptors

Receptors sensitive to changes in body fluid volume are located within the vascular system. These receptors assess the fullness of the vascular system and defend against excessive extracellular fluid (ECF) volume expansion (Brenner and Rector, 1991a). The location of these receptors can be divided into those receptors located in the arterial vasculature ("high-pressure" volume receptors) and those located in the venous system ("low-pressure" volume receptors).

i.) High-Pressure Receptors

High-pressure baroreceptors are located in the carotid sinuses and the aortic arch. In response to an increase in arterial pressure, these stretch receptors elicit an increase in urine output (Brenner and Rector, 1991a). Guyton (1952) was the first to demonstrate this relationship between carotid baroreceptor stimulation and natriuresis and in 1974, Keeler demonstrated that this increase in urinary sodium excretion was not caused by an increase in glomerular filtration rate (GFR) or an increase in renal perfusion pressure (RPP). Keeler concluded that at least part of the natriuretic response seen due to stimulation of the carotid baroreceptors was mediated via a blood-borne agent. In 1988, Courneya et al. showed that, in an anaesthetized rabbit preparation,

when the carotid sinus pressure is changed across the range of 40 - 160 mmHg, plasma AVP levels do not change. However, if the aortic baroreceptor afferents (the aortic depressor nerves) are sectioned, a decrease in carotid sinus pressure results in an increase in AVP release. This indicates that the carotid and aortic baroreceptors can tonically inhibit AVP release. More recently, it has also been shown that at increased plasma osmolalities of 309 and 323 mosm/kg H_2O, plasma AVP increases more at low carotid sinus pressures than when plasma osmolality remains normal (289 mosm/kg H_2O) (Scott et al., 1994). This indicates a further variable involved in controlling the effects of the carotid and aortic baroreceptors on renal function. The inhibition of sympathetic activity, stimulation of the parasympathetic nervous system and the reduction in renal nerve activity caused by baroreceptor activation are also factors involved in the natriuresis and diuresis that result from baroreceptor activation (Brenner and Rector, 1991a).

The renal arteries also act as high-pressure receptors and respond to an increase in blood pressure through the mechanisms of pressure natriuresis and diuresis. In these pathways, the sensor and effector limbs of the pathway are located together in the kidney (Brenner and Rector, 1991a). The increase in sodium and water excretion in response to an increase in arterial pressure is primarily a hemodynamic effect. However, there is some controversy regarding the relative contributions of an increased GFR and a decrease in the tubular reabsorption of sodium and water in this diuretic and natriuretic response (Hall, 1990). Regardless of the efferent mechanisms, the

autoregulation of the kidney through these mechanisms is essential for the long-term control of arterial blood pressure (Guyton et al., 1974) and therefore overall body fluid balance.

ii.) Low-Pressure Receptors

Work done by Henry et al. in 1956 first demonstrated the presence of low-pressure volume receptors in the atria. This work was performed in dogs in which localized distention of the left atrium was caused by inflating a balloon in the left atrium and partially obstructing the mitral valve. As a result of the increased stretch in the atria, urine flow was significantly increased. These atrial stretch receptors are located primarily at the junction of the pulmonary veins and left atria and affect renal function through nervous impulses sent through the vagus nerves (Henry and Pearce, 1956). In 1985, Ledsome showed that stimulation of the atrial volume receptors by balloon inflation at the pulmonary vein-left atrial junction caused an inhibition of AVP release and a decrease in renal sympathetic nerve activity. This therefore demonstrates the mechanisms through which these receptors cause diuresis. Atrial stretch might be expected to further mediate natriuresis through the release of atrial natriuretic peptide (ANP), and thereby provide another effector mechanism in this reflex. However, there is no evidence that atrial volume receptors affect ANP secretion (Peterson and Benjamin, 1992).

The exact role of atrial stretch receptors in controlling blood volume has become

more controversial over recent years. It now appears that atrial receptors may be involved to different extents in renal function depending on the type of volume stimulus administered (eg. water immersion vs. atrial balloon distension, etc.) (Peterson and Benjamin, 1992). Also, species differences appear to exist with respect to the control atrial receptors have over the tonic inhibition of AVP release. For example, atrial receptors in monkeys have been shown to be much less sensitive than those in the dog (Peterson and Benjamin, 1992). The rate of change of blood volume has also been shown to affect the function of atrial receptors in rabbits. In conscious rabbits undergoing a slow haemorrhage, cardiac receptors play a larger role in causing the release of AVP than in anaesthetized rabbits undergoing a faster haemorrhage (Courneya et al., 1989 and Courneya et al., 1992). Although it will be important in future studies to clarify the exact degree to which these atrial receptors are affecting renal function, these studies all indicate that atrial receptors play an important role in regulating body fluid volume through effects on renal function.

Along with the presence of low-pressure volume receptors in the atria, it now seems apparent that low-pressure volume receptors may also exist in the ventricles. Stimulation of unmyelinated afferent nerves from the right ventricle has been shown to cause an increase in the urinary excretion of sodium (Brenner and Rector, 1991a). Ventricular receptors have also been shown to have effects on AVP and renin secretion as well as on sympathetic nerve activity (Peterson and Benjamin, 1992). Wang et al. (1988) examined the effects of ventricular denervation in conscious dogs undergoing

haemorrhage. The secretion of AVP in response to haemorrhage was markedly attenuated in ventricular denervated dogs compared to sham-operated animals. Wang et al. concluded from these studies that ventricular receptors initiate a reflex responsible for increasing AVP secretion in response to haemorrhage and that these receptors are not essential for increasing renin and aldosterone release during haemorrhage. Although the atrial receptors are expected to play a larger role in controlling renal function in response to an increased volume load than the ventricular receptors, it is clear from these studies that ventricular receptors are involved in the regulation of body fluid volume. Further studies are required to determine the exact extent of this involvement and the specific pathways responsible for the renal effects that occur due to ventricular receptor stimulation.

There are two more low-pressure receptors that are involved in the regulation of fluid volume. Cardiopulmonary receptor afferent fibers travel through cardiopulmonary sympathetic afferents and have been shown to inhibit or excite renal nerves (Weaver, 1977). During volume expansion in the same study, activation of these cardiopulmonary sympathetic afferents was shown to depress spontaneous renal nerve activity. Although the kidney has been shown to function well in the absence of cardiopulmonary afferent nerves (Goetz et al., 1975), these receptors may represent another example of the redundant control in the regulation of overall body fluid volume.

Hepatic sensors may represent another reflex system through which the body

controls fluid balance. Levy and Wexler demonstrated in 1987 that dogs with mild intrahepatic hypertension (achieved through low-grade thoracic supradiaphragmatic caval constriction) retained sodium in contrast to sham-operated controls which did not. When the liver was denervated in these dogs with intrahepatic hypertension, the dogs excreted sodium in a similar manner to the control animals. This was taken as evidence that hepatic baroreceptors are involved in the regulation of fluid volume through renal effects on sodium excretion.

b.) Osmoreceptors and Sodium Receptors

i.) Peripheral Osmoreceptors and Sodium Receptors

Osmoreceptors sensitive to changes in body fluid osmolality are a second type of receptor mechanism through which body fluid balance and osmolality can be regulated. Osmoreceptors mediating the release of AVP are primarily thought to be located in the brain. However, splanchnic osmoreceptors have recently been identified in the mesentery of the upper small intestine and portal vein area (Choi-Kwon and Baertschi, 1991). These splanchnic osmoreceptors appear to mediate AVP release through afferent pathways to the major splanchnic nerves and the spinal cord. The location of these osmoreceptors would enable them to detect changes in the osmolality of fluids absorbed from the gut. These studies confirmed earlier experiments done by Passo et al. in 1972 that demonstrated that hypertonic sodium chloride infused into the hepatic portal vein of anaesthetized cats with bilateral vagotomy caused an increase in urine flow and sodium excretion.

ii.) The Central Osmoreceptor Theory

In 1949, Verney demonstrated the presence of cerebral receptors sensitive to changes in plasma osmolality. In conscious dogs undergoing a water diuresis, Verney demonstrated that an intracarotid infusion of hypertonic sodium chloride significantly attenuated the water diuresis whereas an infusion of isotonic sodium chloride had no effect on the course of the diuresis. This attenuation of the diuresis was thought to be caused by AVP release. Verney concluded that these osmoreceptors, located somewhere in the vascular bed supplied by the internal carotid artery, were surrounded by a membrane impermeable to sodium chloride and sucrose. These solutes when infused into the carotid artery are hypothesized to work by raising the osmolality of the plasma which would in turn cause an osmotically induced flow of water across the membrane. This loss of water from the osmoreceptors would result in a subsequent shrinkage of the osmoreceptors which in turn would elicit the release of AVP to cause antidiuresis. This theory was verified by the fact that urea, a substance freely permeable across this membrane, caused no antidiuretic effects when infused into the carotid artery. In subsequent experiments, Jewell and Verney (1957) determined through tying off various branches of the internal carotid artery that the osmoreceptors are located in the anterior hypothalamus. The inhibition of diuresis in response to an intracarotid infusion of hypertonic sodium chloride has since been demonstrated in the goat (Erikson et al., 1971, Andersson et al., 1972, Olsson, 1972a) and in the monkey (Wu and Gilmore, 1987).

The "membrane" described by Verney surrounding the osmoreceptors has been

determined to be the blood brain barrier and some controversy has evolved regarding whether the osmoreceptors are located within the blood brain barrier, as hypothesized by Verney, or outside the blood brain barrier, most likely in one of the seven circumventricular organs (Gross and Weindl, 1987). Cell membranes are normally permeable to urea, galactose and glucose and are not permeable to sodium ions, fructose and sucrose (Schauf et al., 1990). With respect to the permeability of the blood brain barrier, it is generally accepted that fructose, sucrose, glycerol, sodium (Olsson, 1972a) and mannitol (Choi-Kwon and Baertschi, 1991) are not able to cross the blood brain barrier. Glucose and galactose are able to cross the blood brain barrier although this transfer is via a rate-limiting, carrier-mediated transport mechanism and may occur more slowly than the osmotic effects resulting from the presence of these solutes in hyperosmolar concentrations outside of the blood brain barrier (Thrasher et al., 1980b, Olsson, 1972a). Finally, urea is not able to cross the blood brain barrier according to Verney (1947), and Olsson, (1972a) and Thornton et al. (1985) although Thrasher et al. (1980b), and Leng et al. (1982) indicate that this does occur. Due to these inconsistencies, studies performed using infusions of urea must be verified with similar infusions of molecules whose actions on the blood brain barrier and cell membranes are known. The effects of galactose and glucose infusions should be examined with respect to the time course over which these effects occur.

iii.) The Central Sodium-Sensitive Receptor Theory

A further controversy that has evolved since Verney's original description of the cerebral osmoreceptor is the possibility that a receptor sensitive to sodium exists in the brain and that this receptor may be involved in controlling body fluid and osmolality. This theory evolved from studies by Eriksson and colleagues in which attempts were made to determine if Verney's osmoreceptor was located inside or outside of the blood brain barrier (Erikson et al., 1971; Andersson et al., 1972; Olsson, 1972a). This was accomplished through the examination of the differing effects of intracarotid and intraventricular infusions of hypertonic solutions. Intracarotid infusions of hypertonic sodium chloride, sucrose and fructose were all shown to inhibit diuresis in the goat while intracarotid infusions of glucose and galactose did not inhibit diuresis (Erikson et al., 1971, Andersson et al., 1972, Olsson, 1972a). The inhibition of diuresis was abolished after the induction of diabetes insipidus indicating these effects were mediated via the release of AVP (Erikson et al., 1971). In contrast, a hypertonic infusion of fructose into the third ventricle had no effect on diuresis while an equi-osmolar sodium chloride solution infused into the third ventricle significantly inhibited diuresis (Eriksson et al., 1971). Eriksson made two different conclusions from these results. Eriksson concluded from these studies that the osmoreceptors are located outside the blood brain barrier, otherwise infusions of glucose and galactose, which penetrate the blood brain barrier slowly and cell membranes quickly, should inhibit diuresis. Secondly, Eriksson concluded that because intraventricular infusions of fructose do not affect AVP secretion while an equi-osmolar intraventricular infusion of hypertonic sodium chloride stimulates AVP

release, that an intraventricular sodium-sensitive receptor may be involved in the central regulation of fluid volume.

The experiments by Eriksson were repeated in the rhesus monkey by Swaminathan in 1980. Swaminathan demonstrated similar effects of the intraventricular and intracarotid infusions and concluded that sodium-sensitive receptors likely exist in the anterior part of the third ventricle. Swaminathan also measured plasma AVP levels by bio-assay during the intracarotid and intraventricular infusions and demonstrated that AVP levels increased when the free water clearance decreased. In dehydrated sheep, intraventricular infusion of hypertonic mannitol increased cerebral spinal fluid (CSF) osmolality while causing a decrease in CSF sodium concentration due to increased water movement out of cells (Leskell et al., 1981). In response to this infusion, plasma AVP levels decreased and water diuresis occurred despite the fact that the animals were dehydrated. These results further support the role of a central sodium- sensitive receptor in controlling fluid balance.

In contrast to the above experiments demonstrating the presence of a sodium sensitive receptor inside the blood brain barrier, Thorton et al. (1985) concluded that the presence of sodium receptor was unlikely. Thorton et al. examined the drinking responses and changes in CSF sodium concentration in the conscious goat in response to intracarotid infusions of hypertonic saline (shown to cause drinking) and hypertonic urea (not shown to affect drinking). In response to both urea and sodium chloride

infusions, CSF osmolality and sodium chloride concentration both increased, presumably due to cellular dehydration. Plasma osmolality also increased but only the sodium chloride caused a significant increase in drinking behaviour. Because the urea and sodium chloride infusions caused the same increase in CSF sodium concentration, if a sodium sensitive receptor inside the blood brain barrier was mediating the drinking effects resulting from these infusions, then both infusions should have elicited drinking behaviour.

Lack of evidence for a sodium sensitive receptor on either side of the blood brain barrier was demonstrated by Thrasher et al. (1980a). Intravenous infusions of hypertonic sodium chloride, sucrose, glucose and urea all caused significant and similar increases in the osmolality and sodium concentration of the CSF in conscious dogs. However, only hypertonic sodium chloride and sucrose infusions stimulated drinking and elevated immunoreactive plasma AVP and only hypertonic sodium infusions elevated plasma sodium concentrations. This refutes the possibility of a sodium sensitive receptor on either side of the blood brain barrier and concurrent with Thornton et al. (1985) necessitates that an osmoreceptor be located in an area lacking a blood brain barrier. Although the changes in CSF sodium concentrations were very small in these experiments (as would be expected due to intravenous infusions) these changes were apparently significant and within the precision of the instrument used to measure sodium levels. In order to further investigate the apparent lack of a sodium sensitive receptor, further experiments were done by Thrasher where infusions of hypertonic solutions of

sodium chloride, sucrose, glucose or urea (all of a final osmolar concentration of 500 mosm/l) were made into the third ventricle. Sodium chloride and sucrose stimulated water intake and AVP secretion, results that are once again incompatible with a sodium sensitive receptor and compatible with an osmosensitive receptor located in an area with no blood brain barrier (Thrasher et al., 1980b). Circumventricular organs fit all of the criteria of these osmoreceptors and it is postulated in these studies that these organs be examined as a possible anatomic locus of central osmoreceptors.

iv.) Conclusions Regarding the Central Receptors Regulating Fluid Balance

Recent evidence seems to indicate that a cerebral sodium receptor may work in conjunction with a cerebral osmoreceptor in the overall control of body fluid balance and osmolality. The possibility that a sodium receptor exists is certainly valid even with respect to Verney's initial experiments. Verney did not take into consideration effects that intracarotid infusions may be having via the choroid plexes and the CSF (Andersson, 1977). In 1972 Olsson examined the importance of CSF sodium concentration on the effects of intracarotid infusions of hypertonic sodium chloride. Olsson demonstrated a reversal of water diuresis in conscious hydrated goats in response to an intracarotid infusion of hypertonic saline. This is exactly what Verney saw in the conscious dog (Verney, 1947). However, when these experiments were repeated with hypotonic and isotonic injections of sodium chloride into the CSF of the lateral ventricles (thereby preventing an increase in CSF sodium concentration through osmotic effects via the choroid plexes) this reversal of water diuresis was eliminated. Olsson concluded from

these experiments that the ionic composition of the CSF is of greater importance in the central control of fluid balance than are strictly osmotic factors. Further studies have confirmed the importance of the sodium concentration of the CSF in the central control of fluid balance (Olsson,1972c; McKinley et al., 1974; Olsson and Kolmodin, 1974).

In 1978, McKinley et al., hypothesized that a dual osmoreceptor-sodium sensor system may participate in regulating AVP secretion in response to intracarotid hypertonic sodium chloride infusions. In these experiments, McKinley validated the results shown by Verney and demonstrated that intracarotid, hyperosmolar infusions of sodium chloride and sucrose did in fact cause a significant increase in CSF sodium concentration of 3.0 and 5.2 mmol/l respectively. This effect was mediated presumably through the osmotic withdrawal of water through the choroid plexus and into the blood. These effects were not seen with the intracarotid infusions of hypertonic glucose, galactose or isotonic sodium chloride. Separate intraventricular infusions reported in this paper provided further support for the presence of central sodium receptors. It should be noted that significant differences were found in these experiments between sodium levels differing by as little as 1.2 mmol/l. Despite the fact that not only were the standard errors for these measurements stated as 0.7 and 0.8 mmol/l, it is questionable whether an instrument exists that can measure sodium concentrations with such precision. Therefore, although these experiments demonstrate some potentially interesting findings and elicit an interesting theory for a dual osmoreceptor-sodium sensing system, their real significance remains in question.

In 1990, Rundgren et al. also measured the changes in CSF sodium concentration in response to intracarotid hypertonic infusions. They demonstrated a similar change in CSF sodium concentration as did McKinley et al. (1978) and indicated that this change, although small, was within the limits of their ability to accurately measure sodium concentration. Rundgren et al. also measured thirst in response to the intracarotid and intraventricular hypertonic infusions and demonstrated that simultaneously increased CSF and plasma sodium concentrations (and therefore osmolalities) caused a larger intake of water in conscious sheep than a separate increase in sodium concentration to the same degree in either compartment alone. The presence of a ventricular sodium receptor was further supported by McKinley et al. (1974) who showed that intraventricular injections of hypertonic sodium chloride in artificial CSF produced a much larger drinking response than hypertonic sucrose or glucose in artificial CSF. Evidence therefore supports the concept of two receptor mechanisms, one sensing changes in the composition of the CSF and one sensing changes in the composition of the blood.

It should be noted that rarely in these studies involving the intraventricular and intracarotid infusions of various solutions are the blood pressure of the animals in question measured. An increased blood pressure or an increased sympathetic tone may affect renal sodium and water excretion. This increase in blood pressure was hypothesized to be the cause of the increase in sodium excretion seen in response to intraventricular, hypertonic sodium chloride infusion in anaesthetized cats (Chiu and

Sawyer, 1974) and a potential reason for the lack of inhibition of diuresis seen due to intracarotid, hypertonic sodium chloride infusion in anaesthetized dogs (Bie, 1976).

Finally, it should be noted that central osmoreceptors may also cause other efferent activity aside from AVP release which may effect renal function. For example, Schad and Seller (1975) found that hypertonic sodium chloride infusions into the carotid arteries or the cerebral ventricular system in baroreceptor denervated cats caused a significant increase in renal sympathetic nerve activity. Also, Emmeluth et al., 1992, hypothesized that the increased sodium excretion seen in response to an intracarotid infusion of hypertonic sodium chloride was the result of the concurrent increase in urinary urodilatin and not due to changes in AVP secretion. The myriad effector mechanisms that are involved in the regulation of sodium and water excretion from the kidney and the role that these effector mechanisms may be playing in the responses seen due to osmoreceptor (or sodium receptor) stimulation should therefore be considered in evaluation of these studies.

v.) The Location of the Central Osmoreceptors

As mentioned earlier, Eriksson et al. (1971) proposed that, contrary to Verney's findings, the central osmoreceptors appeared to be located outside of the blood brain barrier. Thrasher et al. (1980a) also concluded that these osmoreceptors are located outside the blood brain barrier and hypothesized that the circumventricular organs would make ideal osmoreceptors (Thrasher et al., 1980b). Three of the seven circumventricular

organs, the subfornical organ (SFO), the organum vasculosum of the lamina terminalis (OVLT) and the area postrema (AP), have since been shown to be involved in the regulation of body water and sodium (Gross and Weindl, 1987). In 1985, Zeidonis et al. demonstrated that intracarotid hypertonic saline infusions resulted in an increase in plasma AVP accompanied by an increase in blood flow to the neural lobe, another circumventricular organ. Ablation of the anterior wall of the third ventricle and therefore destruction of the OVLT has been shown to greatly reduce water drinking in response to intracarotid infusions of hypertonic sodium chloride (McKinley et al., 1982). Similar studies in dogs demonstrated that ablation of the OVLT not only inhibited drinking in response to intracarotid hypertonic sodium chloride infusions but inhibited AVP release as well (Thrasher et al., 1982a). Ablation of the SFO in dogs did not affect osmotically stimulated water intake (Thrasher et al., 1982b) although ablation of the SFO in sheep (McKinley et al., 1986) and rats (Mangiapane et al., 1984) caused reduced drinking in response to hypertonic stimuli. Further evidence for the role of the SFO in osmoreception was demonstrated by the finding that projections from the SFO to the supraoptic nucleus (SON) and paraventricular nucleus (PVN) in anaesthetized rats, undergo increased firing in response to intracarotid infusions of hypertonic sodium chloride (Gutman et al., 1988). The SON and PVN contain magnocellular neurosecretory neurons responsible for the secretion of AVP. It therefore seems clear that the SFO contains osmoreceptors responsible for the increased AVP secretion seen as a result of intracarotid hypertonic saline infusions. The OVLT, AP and neural lobe also seem likely to contain osmoreceptors responsible for regulating fluid balance. Other

possible central osmoreceptive areas include the preoptic area in the cat (Hubbard et al., 1985) and the SON itself (Leng et al., 1982).

vi.) The Location of the Central Sodium Sensitive Receptors

Due to the location of the ventricular infusions that have resulted in the demonstration of a sodium sensitive receptor, this receptor is most commonly thought to be located in the vicinity of the third ventricle. This is really a matter of speculation as the path of solutions infused into the ventricular system and the potential receptor sites they may be stimulating are unknown. A great deal of work needs to be done to clarify not only the existence of these sodium sensitive receptors, but their actual location and structure as well.

3.) EFFECTOR MECHANISMS

a.) Renal Mechanics

The only way to maintain long-term fluid and osmolar balance is through the appropriate ingestion and excretion of salt and water every day. Normally, salt and water enter the body via the digestive system and are excreted through breathing (water), sweating (salt and water) and renal excretion (salt and water) (Schauf et al., 1990). Of these three mechanisms of excretion, the renal excretion of salt and water is by far the most adaptable.

A change in water or sodium excretion from the kidney occurs through only two

possible mechanisms despite the vast number of neural and endocrine agents that affect water and sodium excretion. Either there is an alteration in GFR or there is a change in the tubular absorption of sodium and water in the nephron. The GFR is the rate at which plasma is filtered into the glomerulus and is calculated by multiplying the glomerular filtration coefficient by the filtration pressure gradient (Brenner and Rector, 1991b). The glomerular filtration coefficient depends on the permeability and surface area of the kidney and is affected by the levels of many hormones including renin, angiotensin II (AII) and aldosterone (Brenner and Rector, 1991b). The filtration pressure gradient represents the sum of the hydrostatic and osmotic forces that affect filtration. Specifically, the filtration pressure gradient equals the Glomerular Capillary Hydrostatic Pressure - the Bowman's Space Hydrostatic Pressure - the Intracapillary Colloid Osmotic Pressure + the Bowman's Space Colloid Osmotic Pressure (Brenner and Rector, 1991b). Bowman's Space Colloid osmotic pressure is essentially equal to zero because protein does not filter through the glomerulus into Bowman's Space in the normally functioning kidney. An increase in capillary hydrostatic pressure, caused by a dilation of afferent arterioles or constriction of efferent arterioles, causes an increase the filtration pressure gradient and therefore an increase in GFR and a resulting increase in sodium and water excretion (Schauf et al., 1990). In contrast, an increase in Bowman's Space Hydrostatic Pressure, possibly caused by occlusion of the ureters would decrease the filtration pressure gradient and therefore decrease GFR and sodium and water excretion (Schauf et al., 1990). If plasma proteins are diluted, then there is less inhibition of the filtration pressure gradient, GFR will increase and natriuresis and

diuresis will result. In 1991, Cowley and Skelton showed that the diuresis and natriuresis resulting from isotonic volume expansion in conscious dogs is primarily due to a reduction of colloid osmotic pressure.

Sodium is freely filtered at the glomerulus of the kidney although less than 1% of the filtered sodium is excreted in the urine produced by a normally functioning kidney (Seldin and Giebisch, 1990). The remainder of the sodium is reabsorbed along the entire length of the nephron (with the exception of the thin limbs of the loop of Henle) (Brenner and Rector, 1991b). There are three primary mechanisms through which sodium is absorbed into cells lining the lumen of the nephron (Seldin and Giebisch, 1990). Sodium channels are found primarily in the distal convoluted tubule and collecting duct and sodium enters renal tubule cells by following a concentration gradient into the cells. Sodium may also enter renal tubule cells via sodium-symporters or sodium-antiporters. These two mechanisms of sodium transport are found throughout the nephron although they exist primarily in the proximal tubule and the thick ascending limb of the loop of Henle. Sodium-symporters allow the entry of sodium into cells coupled to the entry of a solute or solutes (eg. glucose, phosphate, amino-acids, chloride and potassium). In contrast, sodium-antiporters allow the entry of sodium into cells in exchange for another ion or solute (eg. hydrogen ion). All three of these mechanisms utilize the electrochemical gradients set up by the sodium-potassium-ATPase pump. This pump is located on the basolateral membrane of renal tubule cells and secretes three sodium ions out of the cell in exchange for two potassium ions entering the cell

(Schauf et al., 1990). Through this mechanism, this energy dependent sodium-potassium pump lowers intracellular sodium and creates a transmembrane electric gradient. In cells permeable to water, this movement of ions into the cells causes the movement of water across the cells and therefore affects fluid reabsorption as well (Seldin and Giebisch, 1990). The proximal convoluted tubule is responsible for the reabsorption of 70% of the filtered load of sodium and the thick ascending limb of the Loop of Henle reabsorbs 20% of the filtered load of sodium (Schauf et al., 1990). This reabsorption of sodium is obligatory and occurs at about the same rate regardless of the state of sodium balance in the body. The reabsorption of sodium in the distal tubule and collecting ducts accounts for the remainder of the reabsorption of sodium. The reabsorption of 2% of the remaining 10% of the filtered load of sodium may be influenced (primarily through endocrine mechanisms) to meet the changing needs of the body.

b.) FACTORS REGULATING WATER AND SODIUM EXCRETION

i.) Renal Autoregulation

The kidney has its own intrinsic system through which it regulates GFR (Brenner and Rector, 1991b) and therefore sodium and water excretion. In response to changes in renal blood flow, afferent and efferent arterioles dilate or constrict resulting in a constant rate of glomerular filtration (Schauf et al., 1990). The renin-angiotensin system (RAS) is also thought to be involved in this tubuloglomerular feedback through vascular effects on the efferent arterioles (Brenner and Rector, 1991b).

ii.) Renal Perfusion Pressure

An increase in renal perfusion pressure has been shown to cause natriuresis and diuresis; effects commonly referred to as pressure natriuresis and diuresis. This is an especially important mechanism in the long-term control of blood pressure (Hall et al., 1986b) and has been shown to be defective in all forms of hypertension (Hall et al., 1990). At first glance, it would seem obvious that pressure natriuresis and diuresis are due to an increased capillary hydrostatic pressure and therefore an increase in GFR. Although small increases in GFR do play a role in these mechanisms, GFR does not increase enough to result in the natriuretic and diuretic effects shown (Hall et al., 1986b). Recent evidence has indicated that decreases in the tubular reabsorption of sodium and water are also responsible for these effects (Hall et al., 1986b). Increased renal perfusion pressure is commonly associated with an increase in renal interstitial hydrostatic pressure (RIHP). When renal perfusion pressure is increased without this increase in RIHP, pressure natriuresis is severely attenuated (Granger, 1991). Therefore an increase in RIHP is thought to inhibit sodium reabsorption possibly through altering tight junction permeability to sodium in the proximal tubule and/or through the release of prostaglandins (Granger, 1991). Hall et al. (1986b) also showed that changes in the renin-angiotensin system and aldosterone formation amplify renal pressure natriuresis and diuresis during chronic changes in arterial pressure. These results provide some hypotheses regarding the mechanisms through which increases in renal perfusion pressure mediate pressure natriuresis and diuresis.

iii.) Renal Nerves

The role of renal nerves in the physiological regulation of water and sodium excretion is quite controversial. Although a decrease in renal nerve activity appears to consistently result in natriuresis and diuresis (Colindres et al., 1980; DiBona, 1990), this does not necessarily indicate a cause and effect mechanism. This is complicated by the fact that the total denervation of renal nerves is often very difficult to prove (Bricker et al., 1957). Also, there are many efferent mechanisms which may work to control renal sodium and water secretion and therefore mask the effects of renal denervation. If renal nerves are important in the regulation of sodium excretion, it would be expected that an increase in efferent renal sympathetic nerve activity (ERSNA) would accompany a decrease in ingested sodium in order to increase the reabsorption of sodium and decrease sodium excretion. A number of studies have been performed, on many different experimental animals and in humans, which have investigated the role of renal nerves in the regulation of sodium excretion in response to different dietary intakes of sodium. These studies were summarized in a review by DiBona (1990) and ERSNA was shown to be directionally appropriate to regulate sodium excretion in response to dietary intake in the majority of these studies. There was also a general agreement that intact renal nerves are not essential in regulating sodium excretion during normal or modest reductions in dietary sodium intake. However, during more severe reductions in dietary sodium intake, renal nerves appear to be required for normal renal conservation of sodium. Therefore, renal nerves appear to serve as a further mechanism regulating sodium excretion.

iv.) Endocrine and Paracrine Factors Affecting Sodium Excretion

a.) Renal Prostaglandins

There is some evidence that prostaglandins target renal medullary interstitial cells and renal arteries and arterioles in the renal cortex (Brenner and Rector, 1991b). Prostaglandins are thought to have a protective effect during sodium depletion by causing vasodilation of the microcirculation (Brennor and Rector, 1991b) although this vasodilation would have to be selective to the efferent arterioles in order to be effective. Prostaglandins may also alter sodium excretion by attenuating AII and norepinephrine induced vasoconstriction as well as by decreasing renin release although these effects have not been definitively proven (Brennor and Rector, 1991b).

b.) Nitric Oxide

Nitric oxide has been shown to modulate autoregulatory vasoconstriction in the kidney and is said to undergo enhanced formation and inhibit renin release during increases in renal perfusion pressure (Romero et al., 1992). Romero et al. (1992) also indicate that an impairment of nitric oxide synthesis in the systemic circulation results in a lack of sodium excretion which may leave the body susceptible to a salt-sensitive hypertension. Nitric oxide may be involved in the physiological regulation of sodium excretion although the ubiquitous and ephemeral nature of this vasodilator make it an unlikely candidate for playing a major role in regulating sodium excretion.

c.) Dopamine

There is substantial evidence supporting the fact that dopamine (DA) decreases the renal tubular reabsorption of sodium, and therefore causes a natriuresis (Meister and Aperia, 1993). Dopamine prevents the reabsorption of sodium in renal tubule cells by acting at DA-1 and DA-2 receptors to inhibit the action of the sodium-potassium ATPase pump on the basolateral surface of renal tubule cells. This occurs in proximal convoluted tubule cells (Meister and Aperia, 1993) and dopamine has recently been shown to cause an even greater inhibition of sodium reabsorption in distal nephron cells (Takemoto et al., 1992). In 1991, Chen and Lokhandwala demonstrated that in response to acute iso-osmotic volume expansions, the renal excretion of DA increased in amounts proportional to the degree of volume expansion. Natriuretic and diuretic responses to volume expansion in anaesthetized rats have also been shown to be attenuated by administration of a DA-receptor antagonist (Lokhandwala et al., 1990). DA therefore appears to play a relatively well-defined role in the regulation of sodium excretion through its actions on renal tubule cells. Evidence also indicates that DA may play a permissive role in the function of ANP in the kidney (Lokhandwala et al., 1990).

d.) The Renin-Angiotensin II Aldosterone System

The Renin-AII Aldosterone system is one of the most well-defined and influential endocrine systems involved in the regulation of sodium and water excretion (Drummer et al., 1992). Renin is a protease that is released from the juxtaglomerular apparatus in response to a decrease in renal plasma sodium concentration, a decrease in renal

perfusion pressure or an increase in sympathetic activity (Schauf et al., 1990). Renin catalyzes the cleavage of angiotensinogen, a plasma protein, to angiotensin I. Angiotensin I is further cleaved by a converting enzyme to form AII, a hormone with important physiological effects. AII is further broken down in the adrenal cortex to form angiotensin III, a potent stimulator of aldosterone secretion (Schauf et al., 1990). AII causes an increase in renal sodium reabsorption through a direct enhancement of proximal tubule sodium-bicarbonate coupled reabsorption (Hall et al., 1986a). AII also mediates sodium and fluid reabsorption through its vasoconstrictor actions on the efferent arteriole (Hall et al., 1986a) and through stimulation of the sympathetic nervous system (Brenner and Rector, 1991b). Recent studies have also demonstrated that AII can influence the pressure natriuresis mechanism (Hall et al., 1986), and modulate the sensitivity of tubuloglomerular feedback (Navar and Mitchell, 1990).

Aldosterone is a mineralocorticoid that is secreted from the adrenal cortex in response to sympathetic nerve activation as well as in response to angiotensin III (Schauf et al., 1990). Receptors for aldosterone are found throughout the collecting ducts although the principal site of action of aldosterone appears to be in the cortical collecting duct (CCD). Aldosterone binds to cytoplasmic receptors in CCD's and causes the transcription of mRNAs and the synthesis of many aldosterone-induced proteins. These proteins are primarily involved in changing the transport machinery in the cells with a net result of increasing sodium reabsorption (Seldin and Giebisch, 1990). Some of the specific changes that are attributed to aldosterone include the insertion and

activation of sodium channels in the apical membrane and the synthesis and insertion of more sodium-potassium-ATPase enzyme units in the basolateral membrane (Stanton, 1985). Aldosterone has also been shown to cause the synthesis of several mitochondrial enzymes, thereby providing the energy required for the action of the increased number of sodium-potassium-ATPase pumps (Seldin and Giebisch, 1990). Due to the location of its receptors, aldosterone can affect the potential reabsorption of 2% of the filtered load of sodium. This 2% of the filtered load of sodium is highly significant and represents the most potent endocrine effect on sodium reabsorption in the kidney.

e.) AVP

AVP is a peptide hormone with important actions in the regulation of fluid balance. AVP is synthesized in the paraventricular and supraoptic nuclei of the hypothalamus and is secreted through the posterior pituitary into the bloodstream (Schauf et al., 1990). The osmolality of extracellular fluid is the biggest stimulus for AVP release and the release of AVP is also influenced by the activity of high and low pressure baroreceptors (Scott et al., 1994; Cowley, 1982), and levels of AII and dopamine (Brenner and Rector, 1991b). In the kidney, AVP acts on V2 receptors and causes an increase in water permeability of the collecting duct by actually opening pores for water in the apical membrane of the epithelial cells (Schauf et al., 1990). Although AVP is clearly important in regulating fluid balance, the role of AVP in the regulation of sodium excretion remains to be clearly defined. AVP may indirectly cause sodium reabsorption through the phenomenon of solvent drag but AVP has also been shown to

cause an increase in sodium reabsorption in the collecting duct independent of water reabsorption. This mechanism appeared to be mediated by the adenylate cyclase second messenger system (Seldin and Giebisch, 1990). A relatively weak decrease in sodium excretion has been seen in humans in response to AVP administration however, the mechanism behind this potential antinatriuretic action of AVP requires further investigation (Bie, 1990).

f.) Endogenous Digitalis

There is some evidence that supports the existence of an endogenous endocrine agent which inhibits sodium-potassium-ATPase and therefore causes natriuresis. Digitalis, an inotropic agent, has been shown to inhibit sodium-potassium-ATPase and it is hypothesized that an endogenous digitalis exists which regulates sodium excretion through renal actions on this enzyme system at the digitalis-binding site (Goto et al., 1991). In 1984, Haupert et al. demonstrated the presence of a bovine hypothalamic factor which mediated a high-affinity reversible inhibition of sodium-potassium-ATPase. The structure of endogenous digitalis and its exact role in the regulation of sodium excretion are still in question (Goto et al., 1991).

g.) ANP

ANP is a 28 amino acid peptide that is released from the atria of the heart in response to atrial stretch (Goetz, 1988). ANP has potent and well-documented natriuretic and diuretic properties and acts on ANP receptors found throughout the

collecting ducts (Brenner and Rector, 1991b). ANP exerts a direct effect on sodium excretion through causing a decrease in the open-time of amiloride-sensitive cation channels (Light et al., 1988). Furthermore, a decrease in sodium flux across conductive cation channels has been demonstrated in response to ANP (Light et al., 1989). ANP also inhibits the reabsorption of sodium through indirect actions. ANP inhibits aldosterone, AVP and renin secretion and is thought to interfere with transepithelial driving forces through its vasodilating properties (Brenner and Rector, 1991b). ANP has also been shown to increase GFR (Awazu and Ichikawa, 1993) and increases the slope of pressure natriuresis at elevated renal perfusion pressures, partly through modulation of the renin-angiotensin II system (Mizelle et al., 1989). Finally, there is also evidence that ANP may be mediating the actual excretion of sodium, as opposed to just the reabsorption of sodium, through a basolateral sodium-potassium-chlorine cotransporter (Sonnenberg et al., 1986).

Although the role of ANP as a natriuretic and diuretic hormone is apparent, the role of ANP in the physiological regulation of sodium excretion is under debate. Some authors have theorized that ANP may be involved in the long-term regulation of blood pressure through its vasodilative properties and that urodilatin, rather than ANP, may be the primary natriuretic agent responsible for the regulation of sodium excretion (Goetz, 1991). These conclusions are based on studies where ANP infusions of 3-12 times normal ANP levels had only modest renal effects, and where the natriuretic effect of a volume load was shown to occur even if ANP levels were caused to decrease

(Goetz, 1990a). Finally, a number of studies have demonstrated a lack of correlation of circulating ANP levels with renal sodium excretion (Goetz, 1990a; Goetz, 1990b). However, if factors that modify the effect of ANP such as sodium status and renal perfusion pressure are taken into account, many of the studies opposing a physiological role for ANP in the regulation of sodium excretion can be challenged (Richards, 1990). In conclusion, ANP does appear to play a role in the physiological regulation of sodium excretion although this role must be further clarified.

h.) Urodilatin

Urodilatin is a 32 amino acid natriuretic peptide that was discovered in the urine by Schulz-Knappe et al. in 1988. Urodilatin has a similar structure to ANP except for a four amino acid N-terminal extension. Further research has shown that urodilatin is produced and processed by the kidney and secreted into the lumen of the distal convoluted tubule and collecting duct in the nephron where it is thought to cause natriuresis via a paracrine action at ANP receptors (Schulz-Knappe et al., 1988; Feller et al., 1989). Urodilatin is thought to be more important than ANP in mediating natriuresis because it does not appear to be susceptible to degradation by neutral endopeptidase 24.11 (Gagelmann et al., 1988), an enzyme located in the brush-border membrane of the proximal tubule thought to be responsible for ANP metabolism (Chiu et al., 1991; Erdos and Skidgel, 1989). The secretion of urodilatin further along the nephron may also enable it to escape potential degradation by neutral endopeptidase 24.11. Urodilatin binds with high affinity to ANP receptors and has been shown to be

a more effective natriuretic and diuretic agent than ANP (Hildebrant et al., 1992).

The role of urodilatin as a physiological regulator of sodium excretion has been evaluated by a number of studies. Urodilatin is involved in the circadian regulation of sodium excretion (Drummer et al., 1991). Urodilatin and renal sodium excretion have been shown to follow very similar patterns (Goetz, 1991) and Drummer et al. (1992) have shown that urodilatin and the renin-angiotensin system appear to be the most important endocrine factors involved in the long-term renal response to an acute saline infusion. In a recent study, intracarotid infusions of hypertonic saline were performed concurrent with a jugular vein infusion of water at a rate such that only the areas of the brain supplied by the carotid arteries experienced an increase in plasma sodium concentration (Emmeluth et al., 1992). Results of these studies demonstrated that an intracarotid infusion of hypertonic sodium chloride causes a much larger increase in sodium excretion than a comparable isotonic infusion (into the carotid arteries and the jugular vein). The larger increase in renal sodium excretion in response to the hypertonic sodium chloride infusion was accompanied by an increase in urinary excretion of urodilatin. Urodilatin did not increase in response to the isotonic sodium chloride infusion. Because these infusions resulted in comparable increases in diuresis and free water clearance, the hypertonic stimulus was also determined to be below the threshold required to cause AVP release. In summary, these experiments showed that an intracarotid hypertonic sodium chloride infusion caused a significant increase in renal sodium excretion that was accompanied by an increase in urinary urodilatin excretion.

This increase in urodilatin occurred in response to changes in plasma osmolality smaller than those required to stimulate the release of AVP. This study therefore further emphasizes the important role that urodilatin seems to play in the physiological regulation of sodium excretion.

4.) SUMMARY AND RATIONALE FOR PERFORMING THESE STUDIES

The regulation of body fluid and sodium homeostasis involves a complex mechanism of interacting and overlapping receptors and effectors. Although the receptors involved in these regulatory processes can be roughly divided into volume, osmolality and sodium receptors, there is still some controversy regarding the differences between osmolality and sodium receptors. In recent years, the focus of research into the regulation of sodium and fluid excretion has shifted from the receptor mechanisms themselves to the exact mechanisms through which stimulation of these receptors effect the regulation of sodium and water excretion. It is apparent from the above discussion that there are a number of efferent mechanisms that may be involved in these regulatory processes. These efferent mechanisms have not as yet been fully explored and appear to offer many redundancies in the regulation of sodium and water excretion.

Emmeluth et al. (1992) concluded that the release of one of these effectors of sodium excretion, urodilatin, is controlled through an as yet unknown pathway between the brain and the kidney. In response to an osmotic (or possibly sodium) stimulus in the brain so small that not it does not elicit the release of AVP, this pathway is activated to

cause the release of urodilatin. In order to further verify the conclusions of this study, we performed similar experiments in an anaesthetized rabbit preparation. Renal perfusion pressure was controlled during these experiments and plasma AVP levels and changes in carotid plasma sodium concentrations were measured during these experiments. These measurements of plasma AVP and carotid artery plasma sodium concentration were not performed in the study done by Emmeluth.

5.) GOALS OF THESE STUDIES

The goals of the following studies were two-fold. Primarily, we wanted to develop a preparation of perfused rabbit kidneys in which we could control renal perfusion pressure and in which the animals maintain a relatively constant state of hydration, normal renal function and normal renal nerve function. Secondly, we wanted to use this preparation to examine the cardiovascular, renal and endocrine effects of an intracarotid infusion of hypertonic sodium chloride concurrent with a jugular vein infusion of water at rates such that only the areas of the brain supplied by the carotid arteries experienced an increase in plasma sodium concentration. The cardiovascular, renal and endocrine effects of these infusions would then be compared to the cardiovascular, renal and endocrine effects of a similar saline load infused through the carotid arteries and jugular vein as isotonic saline.

6.) HYPOTHESES

Using the preparation and protocols described:

1.) That an intracarotid infusion of hypertonic saline will cause a greater increase in sodium excretion than an intracarotid infusion of isotonic saline.

2.) That an intracarotid infusion of hypertonic saline will cause a greater decrease in renal vascular resistance than an intracarotid infusion of isotonic saline.

3.) That sodium excretion will increase in response to a smaller osmotic stimulus than that which causes release of AVP.

METHODS

1.) Introduction

The experiments to be described were done in three groups. In the first two series of experiments, the feasibility of performing a new surgical procedure and its effects on an anaesthetized rabbit preparation were established. This new procedure allowed for the control and manipulation of renal perfusion pressure and was implemented without interrupting blood flow to the kidneys or damaging the renal nerves. In the third series of experiments to be described, this new preparation was utilized to maintain renal perfusion pressure constant while the cardiovascular and renal effects of a saline load were examined. This saline load was infused as isotonic saline into the carotid arteries and left jugular vein in a control group of animals, and in a second group of animals the same saline load was infused as water into the left jugular vein and hypertonic sodium chloride into the carotid arteries.

2.) Anaesthetic and General Management

Male New Zealand White Rabbits obtained from a local breeder were used in these experiments. All surgeries and experiments were performed according to the stated guidelines of the Canadian Council of Animal Care. Rabbits were housed in The University of British Columbia Animal Care Facility and four to five days prior to an experiment animals were housed in the Physiology Department. The rabbits were fed Purina Rabbit Chow and water ad libitum and acclimatized to a photoperiod between

0800 and 2000. Animals weighing between 3.0 and 4.5 kg were used in these experiments.

On the day of an experiment, two animals were transported to the laboratory. One animal was used for the experimental procedure (the "experimental rabbit") and the second animal (the "donor rabbit") was used for a supply of blood to replace blood samples taken from the experimental animal. Rabbit red blood cells do not demonstrate blood group antigens and previous work in the laboratory has shown that rabbit blood can be injected without incident into other rabbits (Scott et al., 1994).

The animals were weighed and anaesthetized with a mixture of α-chloralose (BDH Chemicals, UK, 100 mg/kg) and urethane (1g/kg, Sigma Chemical Co, St. Louis, MO). Briefly, 1 gram of α-chloralose was dissolved in 100 ml of a 0.9% saline solution through heating to 65 ° C. This solution was then filtered into 33 ml of 32% urethane and the final solution kept at 38 ° C until the time it was used. Rabbits were anaesthetized with 35 ml of the α-chloralose/urethane solution through an E-Z Set Infusion Set (Catalog No: 38-5312-1, Deseret Medical Inc., UT) inserted in an ear vein. This infusion set was left in the ear vein and taped onto the ear for use in future injections. Depth of anaesthesia was examined at the beginning of surgery (or blood removal in the case of the donor rabbit) with a pedal reflex response. An extra 5 - 10 ml of anaesthetic was administered to any animal exhibiting a pedal reflex.

Sterile techniques were not used in any of the experiments to be described as all of the experiments were acute. At the end of the experimental period or after blood removal (in the case of the donor rabbit), the animals were euthanized with 10 ml of 20% Potassium Chloride Solution (Potassium Chloride from Nichols Chemical Co. Ltd., Montreal, Quebec) injected through the ear vein (experimental rabbit) or directly into the heart (donor rabbit). Unless otherwise noted, all solutions to be injected or infused were made up using 0.9% sodium chloride as the solvent (Sodium Chloride from Fisher Chemicals, Fair Lawn, New Jersey).

3.) Surgical Procedures

a) Donor Rabbit

After the rabbit had been anaesthetized, the animal was placed in the supine position and hair was shaved from the chest area with an electric razor (Oster, Model A2-22, Milwaukee, WI). A cardiac puncture was performed with an 18.5 gauge needle (Precision Glide Needles, Becton Dickinson, NJ) attached to a 20 ml syringe (Becton Dickinson, Rutherford, NJ) coated with 2 ml of heparinized saline (1,000 units Lithium Heparin/ml) (Lithium Heparin from Sigma Diagnostics Inc., Catalog #HO8-78, St. Louis, MO). When the syringe was filled with blood, it was detached from the needle, and emptied into a 250 ml beaker coated with 15 ml of heparinized saline. 1-2 ml of the heparinized saline solution was added to the syringe before it was reattached to the needle and filled up again. The procedure was repeated until 100 - 120 ml of blood had been collected. It was possible to remove approximately 100 to 120 ml of blood from

the donor rabbit through elevating the animal's hind limbs.

b) Experimental Rabbit

1.) Surgical Procedures Common to All Experiments

The following pages contain a detailed, methodological description of the common surgical procedures used in these experiments. In summary, a complete bowel resection was performed through an abdominal incision to provide access to the descending aorta and ureters. The descending aorta was cannulated below the renal arteries with a specially constructed double-lumen cannula with a longer inner cannula. Blood flowing down the descending aorta entered the inner cannula above the renal arteries, flowed through an extra-corporeal circuit and then was pumped back into the descending aorta below the renal arteries through the outer cannula. Renal perfusion pressure was controlled through a servo-control mechanism in the extra-corporeal circuit. Each experimental animal was also volume loaded with an intravenous infusion of hypotonic saline to maintain urine flow and the ureters were cannulated for the collection of urine. A constant state of hydration was maintained by replacing the volume of urine produced with an intravenous infusion of isotonic Krebs Ringer Solution. All experimental animals were mechanically ventilated at appropriate rates.

In more detail: After the rabbit was anaesthetized, the back, neck, chest and entire abdominal region of the rabbit were shaved with an electric razor. The back was shaved to produce a better ground to the surgical table and the neck, chest and abdomen

were shaved to facilitate the surgery. In experiments where the ear and/or brachial arteries were to be cannulated, the ears and left forelimb of the rabbit were also shaved as required.

The animal was placed in the supine position on a heated surgical table maintained at 40° C (Telethermometer, Yellow Springs Instrument Co., OH). The trachea was exposed through a surgical incision made using a scalpel and a tracheotomy was performed. An endotracheal tube was tied into the trachea and attached to a respirator (Harvard Apparatus Respirator Model 665, South Natick, MA) set at 30 breaths per minute and a tidal volume of 10 ml/kg body weight. The rabbit remained connected to the respirator and breathing 100% oxygen (Medigas, Praxair, Mississauga, ON) mixed with room air throughout the remainder of the experimental procedure. During the preliminary surgery, a 1 ml blood sample was taken from an arterial cannula to measure pH, P_{CO2}, and P_{O2} using a blood gas analyzer (168 pH/Blood Gas Analyzer, CIBA-Corning Canada Inc., Richmond Hill, ON). If the rabbit's P_{CO2} was outside the range of 25-30 mmHg, the animal's acid-base state was corrected by slightly increasing the respirator ventilation rate (if the P_{CO2} >30mmHg), or decreasing the respirator ventilation rate (if the P_{CO2} <25 mmHg). If the rabbit had a negative base excess of less than -2.0 mmol/l it was corrected by i.v.injection of 1 mole/l sodium bicarbonate solution (Sodium Bicarbonate from Sigma Chemical Co., St. Louis, MO). The volume of sodium bicarbonate to be injected was calculated as the volume of extracellular fluid in the rabbit (20% of total body weight in ml) multiplied by the base excess. Blood

gases were re-analyzed after sodium bicarbonate injection, and these procedures were repeated if the P_{CO2} and base excess values were not within the acceptable range. This method of maintaining acid-base balance has been shown to be effective in anaesthetized rabbits in previous experiments done in this laboratory (Scott et al., 1994).

After the rabbit was connected to the ventilator, 1 mg/kg Succinylcholine Chloride solution (1 mg/ml, Succinylcholine Chloride from Sigma Chemical Co., St. Louis, MO) was injected through the ear vein to paralyse the animal during use of an electric cautery (Model 754, The Birtcher Corp., Los Angeles, CA). Succinylcholine is rapidly metabolized and its effects last for approximately 30 minutes (Miller, 1989). This injection of Succinylcholine would therefore not affect experimental results. Electric cautery was used to open the neck of the rabbit from the most ventral point of the jaw bone to the clavicle. Electric cautery was also used to open the abdomen from the xiphoid process to the base of the bladder. Skin and abdominal muscles were cut to expose the gastro-intestinal tract. Use of the cautery served to prevent excess bleeding during these initial procedures as well as future bleeding that would result after the animal was heparinized.

In all experiments, the left jugular vein was exposed just superior to the left clavicle and cannulated with polyethylene tubing (Intramedic, Becton-Dickinson PE-190, Parsippany, NJ). The end of this cannula was connected to wider diameter silicone tubing (Masterflex 6411-3, Cole-Parmer, Chicago, IL) and this tubing was fed through

a roller pump (Masterflex Model 7520-25, Cole-Parmer, Barrington, IL). Through this cannula, a number of different solutions were infused into the animal depending on the protocol. However, in all experiments, immediately following cannulation of the left jugular vein, a 0.45% saline infusion was started at 2.0 ml/min to increase urine flow and maintain adequate urine production for chemical analysis. After two hours, the rate of this infusion was decreased to 1.0 ml/min, a rate that was maintained until the beginning of the experimental protocol. At this point, the 0.45% saline infusion was replaced with a solution of 165 ml Krebs Ringer Solution, 165 ml water and 30 ml of the α-chloralose/urethane anaesthetic solution. The Kreb's Ringer Solution was made fresh on the day of the experiment with the following concentrations: 0.124 M NaCl, 0.005 M KCl, 0.026 M $NaHCO_3$, 1.24 mM $MgSO_4$, 1.24 mM KH_2PO_4, 1.66 mM $CaCl_2$ (BDH Chemical Co., Toronto, ON) and 0.011 M Dextrose (Dextrose, $MgSO_4$, and KH_2PO_4 from Fisher Scientific, Nepean, ON). 5% CO_2 and 95% O_2 (Medigas, Union Carbide Canada Ltd., Vancouver, BC) was bubbled through the Kreb's Solution to dissolve the $CaCl_2$. This Krebs/anaesthetic solution was infused at 1.0 ml/min through the duration of the experimental protocol. Previous work in the laboratory (Scott et al., 1994) showed that this infusion maintained a constant plasma osmolality between 280-290 mOsm/kg H_2O.

In each protocol, the volume of urine lost during each 15 minute period was replaced in the subsequent 15 minute period with an infusion of 0.6% Krebs Ringer solution (500 ml Krebs Ringer Solution and 250 ml H_2O) infused through the left jugular

vein. This infusion was connected to the infusion already going into the left jugular vein through a T-joint in the left jugular vein cannula. A roller pump (Masterflex Model 7520-25, Cole-Parmer, Barrington, IL) fitted with silicone tubing (Masterflex 6411-3, Cole-Parmer, Chicago, IL) was used to replace this volume of urine.

In all experiments, the right jugular vein was exposed just superior to the right clavicle and was cannulated with a 10 cm length of polyethylene tubing (PE-190). This tubing was fed through the jugular vein and secured in the jugular vein when the tip of the cannula was in the right atrium (approximately 8 cm from the point of entry). This cannula was connected to a pressure transducer (Model P23 Db, Statham Instruments, Puerto Rico) for the measurement of right atrial pressure.

The bowel was resected between the duodenum and the rectum to expose the descending aorta and ureters for cannulation. All mesenteric vessels were divided between ligatures to prevent bleeding. The spleen was resected and the duodenum was isolated just distal to the stomach and cut between ligatures. The small and large intestines were then dissected free by cutting connective tissue and tying off small mesenteric vessels. The superior mesenteric artery was dissected for a length of 0.5 cm from its branching point on the descending aorta and cut between ligatures at this point. The arterial supply to the gut was intentionally cut off before the venous supply to allow venous drainage from the gut and thereby minimize the blood loss occurring as a result of the bowel resection. The resection of the bowel was completed by cutting the rectum

between ligatures, cutting any connective tissue still attaching the bowel to the abdominal cavity and tying off any remaining mesenteric vessels attaching the bowel to the abdominal cavity.

With the bowel removed, the descending aorta could be visualized and was dissected from just below the renal arteries for a length of 2.5 cm. Two sets of posterior spinal arteries coming off of the descending aorta were cut between ligatures at this point to free up the required 2.5 cm. length of descending aorta. Two ligatures were placed under the descending aorta at this point. The descending aorta was also dissected free immediately above the mesenteric artery. A single ligature was placed around the descending aorta at this point.

If the bladder, which was now exposed, contained more than approximately 5 ml of urine, the urine was removed from the bladder by puncturing the bladder with a needle and withdrawing the urine through a syringe. This urine was discarded. This removal of urine prevented any possible stimulation of stretch receptors in the bladder which may have affected renal function (Schauf et al., 1990).

The ureters were exposed as a result of the bowel resection and were gently dissected free from the bed of fatty tissue in which they were imbedded. The ureters were each cannulated approximately 6 cm from their origin at the kidney with a 20 cm length of polyethylene tubing (PE-190). The end of each ureter cannula was placed in

a small beaker for urine collection.

At this point, a bolus injection of 1.0 ml/kg of the heparinized-saline solution was injected i.v.. For the remainder of the experimental period, every 30 minutes following the bolus injection of Heparin, 1 ml of the heparinized-saline was injected i.v.. This heparinization of the rabbit was done to prevent clotting of cannulae to be placed subsequently in veins and arteries.

Once the animal was adequately heparinized, the 0.5 cm stump of the superior mesenteric artery that remained after the bowel resection was cannulated with a 3.0 cm length of polyethylene tubing (PE-190). This cannula was inserted in the mesenteric artery until the tip of the cannula was just at the junction of the mesenteric artery and the descending aorta. The end of this cannula was connected to a pressure transducer (Model P23 Db, Statham Instruments, Puerto Rico) which in turn was connected to the feedback port of the servo-controlled roller pump. This cannula was therefore responsible for measuring the renal perfusion pressure.

The descending aorta was cannulated distal to the renal arteries with a specially constructed, double-lumen, stainless steel cannula. This double-lumen cannula consisted of an inner small lumen cannula of a length approximately 3.0 cm longer than its surrounding outer lumen cannula. The diameter of the inner cannula was approximately 2.0 mm and the diameter of the outer cannula was approximately 5.0 mm. A small

groove was filed around the tip of the inner cannula approximately 3 mm from the end of the cannula. A small groove was also filed around the outer cannula approximately 3 mm from its end. These two small grooves on the cannula served as anchoring points for the two ligatures to be tied around the descending aorta (Fig.1). The lumen of the inner cannula was connected to the lumen of the outer cannula through an extra corporeal circuit of silicone tubing (Masterflex 96400-16, Cole-Parmer, Chicago, IL). This tubing was fed through a servo-controlled roller pump (H-R Flow Inducer Type MHRE, Watson-Marlow Ltd., UK) so that blood flowing into the inner cannula would be pumped out at a controlled pressure around the circuit and out through the outer cannula. Aside from the silicone tubing, this extra-corporeal circuit also contained: a custom-made damping chamber/water bath, to heat the blood to body temperature before it reentered the body and prevent any air bubbles in the blood from reentering the circulation; a flow probe (Transonic Systems Inc., Probe #2N419, Ithaca, NY) to measure renal blood flow; and a filter (Syringe Filter Holder, CANLAB Catalog No.: F2858-57, Baxter Diagnostics Corp., Burnaby, B.C.) complete with a 149 micron diameter nylon mesh (Small Parts Inc., Miami, FL) to prevent any small emboli in the blood from reentering the circulation. These components of the extra-corporeal perfusion circuit were connected in series. The total dead space of the extra-corporeal ciruit was approximately 15 ml. The circuit was primed with macrodex-saline (Pharmacia Canada Inc., Doval, PQ) before use and tubing at both ends of the circuit was clamped to prevent air from entering the circuit.

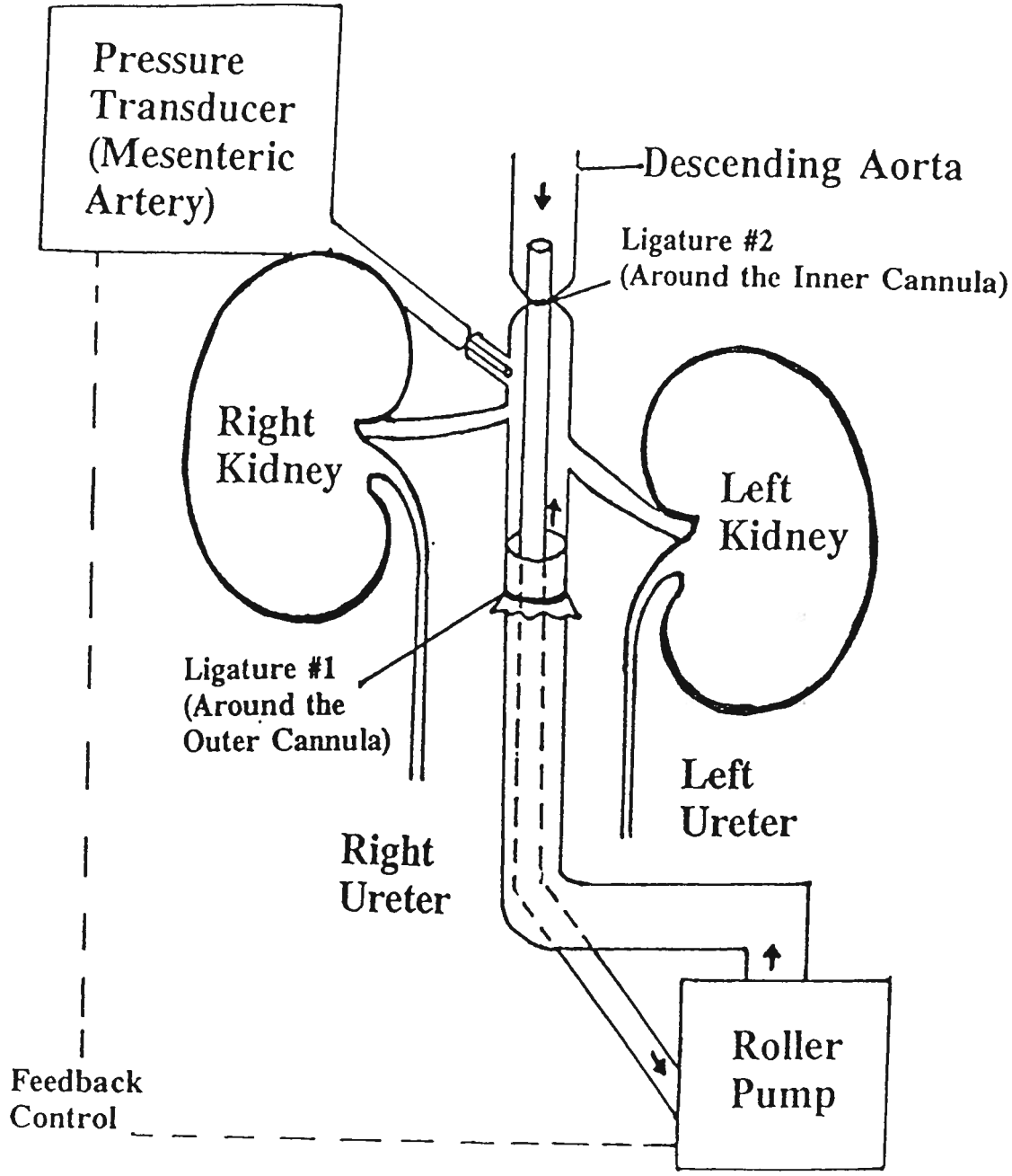

Fig. 1) A Diagrammatic Sketch Showing the Placement of the Double Lumen Cannula in the Descending Aorta.
(Arrows Represent Direction of Blood Flow)

The descending aorta was clamped just below the renal arteries and the double-lumen cannula was inserted in the aorta approximately 2.0 cm below the renal arteries. A loose ligature was tied around the inner cannula, the aorta was unclamped and the cannula was advanced until the outer cannula was about 0.5 cm into the aorta. Once the outer cannula was advanced into the descending aorta, a ligature was tied into the groove on the outer cannula, anchoring the outer cannula to the descending aorta (Fig. 1 - Ligature #1). The ligature on the inner cannula was then removed and the cannula was advanced until the tip of the inner cannula was proximal to the junction of the superior mesenteric artery and the descending aorta. At this point, the roller pump was started with a flow of 30 ml/min. Once the extra-corporeal circuit was functioning, the ligature around the descending aorta (above the superior mesenteric artery) was tied to the groove on the inner cannula (Fig. 1 - Ligature #2) and the servo-control was set to maintain renal perfusion pressure at the desired level.

At the completion of the surgery, 20 ml of whole blood from the donor rabbit was injected into the ear vein to compensate for blood lost during the surgical procedure.

2.) Surgical Procedures Unique to Protocols 1 and 2 (Validation of Experimental Technique)

In this protocol, after the right and left jugular veins were cannulated and before the bowel was resected, the left carotid artery was isolated and cannulated with a 7 cm length of polyethylene tubing (PE-190). This cannula was connected to a three-way

stopcock attached to a pressure transducer (Model P23 Db, Statham Instruments, Puerto Rico) and used for the purposes of collecting blood samples and measuring arterial pressure.

3.) Surgical Procedures Unique to Protocol 3 (The Effect of Increasing Sodium Concentration in Blood Perfusing the Brain)

In this protocol, after the right and left jugular veins were cannulated and prior to commencing the bowel resection, both carotid arteries were dissected to expose their proximal branches and the bifurcation of the common carotid artery. A ligature was placed underneath each carotid artery. Six sutures were placed in the skin around the neck incision and these sutures were tied to an iron ring suspended approximately 3.5 cm above the surface of the rabbit. This set-up opened up the neck area of the rabbit and facilitated the dissection of the branches of the external carotid arteries. There were usually three main branches of each external carotid artery. Each branch of both carotid arteries was carefully dissected free and a ligature placed underneath it. The left brachial artery was isolated through an incision at the shoulder joint of the rabbit and a ligature was placed underneath the artery. In the first five experiments completed using this protocol, the central ear artery of each ear was also dissected free and a ligature was placed underneath the artery. These vessels were isolated prior to heparinization of the animal to minimize blood loss which could result from isolating these vessels in a non-heparinized preparation.

Following the bowel resection, heparinization and set-up of the extra-corporeal perfusion circuit; the isolated left brachial artery was cannulated with a 7 cm length of polyethylene tubing (PE-50). This cannula was connected to a three-way stopcock attached to a pressure transducer (Model P23 Db, Statham Instruments, Puerto Rico) and was used for the measurement of arterial pressure as well as for collecting blood samples. A small ventral branch of each of the external carotid arteries that was in line with the common carotid artery (the facial artery), was cannulated with a 6 cm length of polyethylene tubing (PE-10) until the tip of the cannula lay proximal to the bifurcation of the common carotid artery (Fig. 2). These two cannulae were each connected to a 30 cm length of polyethylene tubing (PE-50) which in turn were each connected to a 20 ml syringe set in an infusion pump (Infusion/Withdrawal Pump, Model 901, Harvard Apparatus Co. Inc., Millis, MA). These cannulae and syringes were primed with either 0.9% saline or 677 mmol/l saline depending on the solution to be infused during the protocol. The dead space in this infusion system was approximately 1.0 ml in each cannula.

In the first five experiments completed using this protocol, the central ear artery of each ear was cannulated using a 5 cm length of polyethylene tubing (PE-50). These cannulae were used for the collection of blood samples assumed to be representative of blood perfusing the brain.

In order to examine the effects of the intracarotid infusions of saline on plasma

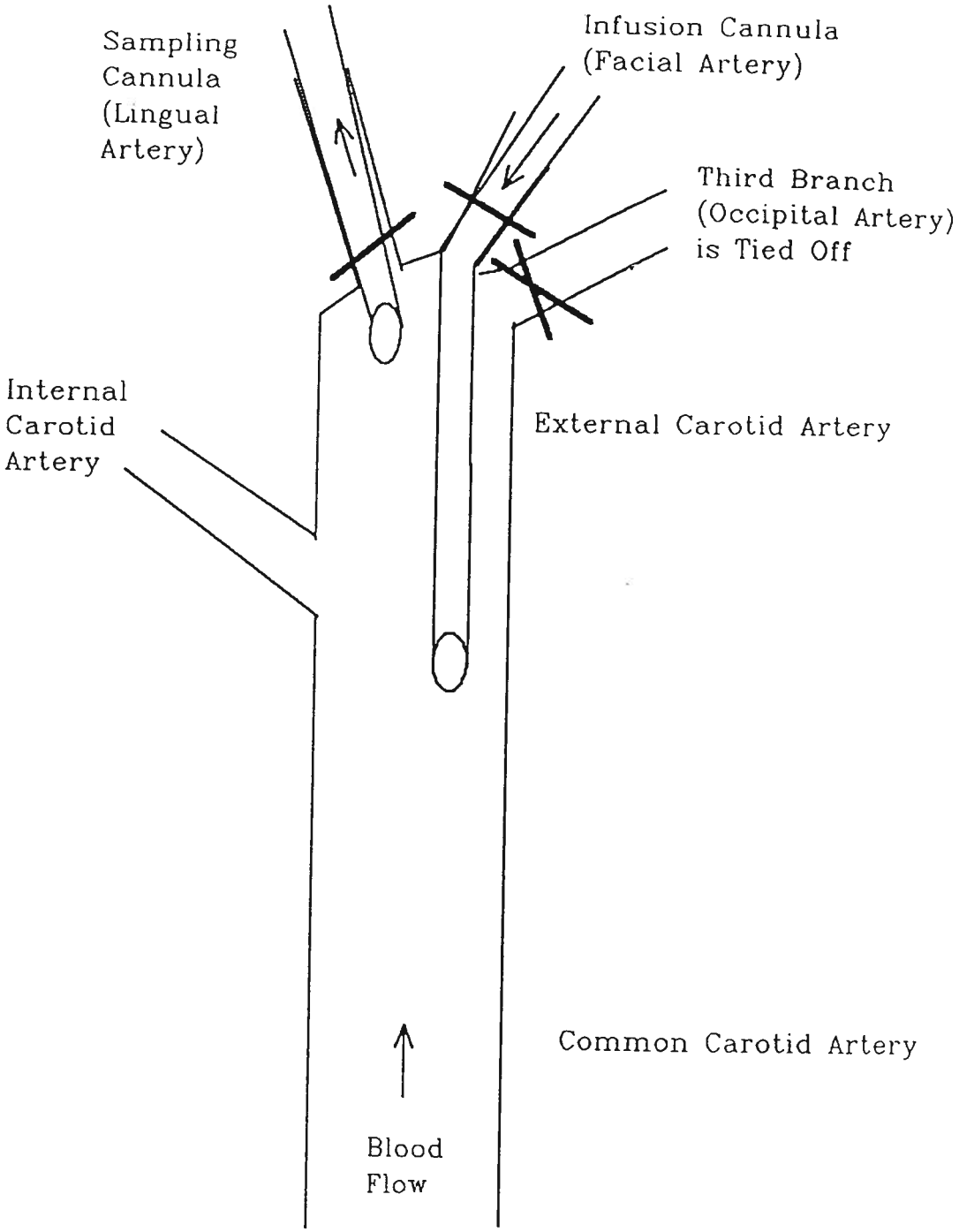

Fig. 2) A Diagrammatic Sketch showing the Placement of the Infusion Cannula and the Sampling Cannula in the Left Carotid Artery.

sodium concentrations directly in the carotid arteries and to ensure that the entire infused volumes were entering the internal carotid arteries, following the fifth experiment completed using this protocol, and in all subsequent experiments, the ear arteries were not cannulated. In replacement, a second branch of each of the external carotid arteries (the lingual artery), was cannulated with a 5 cm length of polyethylene tubing (PE-50) and blood samples were taken through these cannulae. The tip of each of these two cannulae was positioned so that it was just inside the external carotid artery and proximal to the infusion cannula and the bifurcation of the common carotid artery (Fig. 2). The careful positioning of these sampling cannulae ensured that the sampling cannula did not interfere with the functioning of the infusion cannula and did not block the entrance to the internal carotid artery. All other branches of the external carotid were tied off to prevent the infused solution from escaping through routes other than the internal carotid artery (Fig. 2).

4.) Electronic Recording

The three pressure transducers set up during the surgical procedure were connected to a thermal array electronic recorder (Astro-Med Model MT 95000, Astro-Med Inc., Rhode Island). This recorder was calibrated prior to the beginning of each experimental protocol at 0 and 160 mmHg on two channels for the measurement of renal perfusion pressure (from the mesenteric artery cannula) and arterial pressure. The recorder was also calibrated to 0 and 25 cmH_2O on a third channel for the measurement of right atrial pressure (from the right jugular vein cannula). A fourth channel of the

electronic recorder was calibrated to 0 and 100 ml/min to record changes in renal blood flow. This channel was electrically connected to the flow meter contained in the extra-corporeal perfusion circuit. Calibrations were checked for accuracy at one point during each experimental protocol and at the end of the experimental protocol. These 4 variables were recorded continuously throughout each experimental protocol.

5.) Chemical Analysis

a) Sodium and Potassium Measurements

Sodium and Potassium were analyzed in urine and plasma samples through use of a flame photometer (Model IL943, Instrumentation Laboratory Co., Italy). The flame photometer required sample volumes of 20 μl for both urine and plasma samples. Samples presented for analysis were aspirated and automatically diluted with a cesium chloride solution (1.5 mmol cesium chloride/l) (Instrumentation Laboratory Co., Lexington, MA) at a fixed dilution of 100:1. This instrument measured sodium and potassium by aspirating the diluted sample through a propane flame. The combustion of the propane dehydrated the sample droplets and atomized the resulting solute particles. The emission wavelengths of sodium and potassium were detected in the light emitted from these burning solute particles. The intensity of light of these specific wavelengths incident on a photocell was then converted to an electrical signal representing the amount of sodium or potassium in the sample. The Flame Photometer was calibrated with a plasma standard containing 140 mmol/l Sodium and 5 mmol/l Potassium (Instrumentation Laboratory Co., Lexington, MA) and a urine standard of 100

mmol/l Sodium and 100 mmol/l Potassium (Instrumentation Laboratory Co., Puerto Rico). The Flame Photometer checked that the standards and cesium diluent were within a preset tolerance limit and automatically requested a re-calibration every 15 minutes during operation. The coefficient of variation (%) of measurements made with the Flame Photometer at 90% confidence limits was 1.0% for sodium measurements and 1.5% for potassium measurements. Duplicate measurements of sodium and potassium performed on different days resulted in an average difference from the original measurements of ± 1.2 mmol/l for sodium measurements and ± 0.03 mmol/l for potassium measurements.

b) Osmolality Measurements

The osmolality of plasma and urine samples were determined using an Advanced Digimatic Osmometer (Model 3DII, Advanced Instruments Inc., Needham Heights, MA). This fully automated instrument required a sample size of 200 μl of plasma or urine. The Digimatic Osmometer measures osmolality by means of a freezing-point depression measurement. This measurement is based on the principle that the freezing point of a solution will decrease proportional to the molar amount of solute dissolved in the solvent. The Digimatic Osmometer supercools the sample in a cooling bath to a specific temperature below the freezing point of the solvent. The sample is then vibrated with a stir wire for 1 second. This causes the sample to freeze and the heat of fusion produced by the sample as it freezes brings the temperature of the sample to the actual freezing point of the solution. The Digimatic Osmometer then displays the osmolality

corresponding to the actual freezing point of the sample. The Digimatic Osmometer was calibrated before each series of measurements in triplicate with 100 and 500 mOsm/kg H_2O standards (Precision Systems Inc., Natick, MA). Standard samples with a mean osmolality greater than ± 2 mosm/kg H_2O outside of the expected value were repeated after adjusting the calibration. Duplicate measurements of osmolality performed on different days showed an average difference of ± 4.3 mosm/kg H_2O from the original measurement.

c) Creatinine Measurements

Creatinine was measured in both plasma and urine samples for the purposes of calculating glomerular filtration rate (GFR). Creatinine measurements were performed using a Creatinine Reagent Kit (Sigma Diagnostics Inc., Catalog Number: 555-A, St. Louis, MO). This kit uses a method for measuring creatinine based on the Jaffe reaction where a yellow-red colour is produced when creatinine interacts with alkaline picrate. This colour produced by the creatinine fades faster under acidic conditions than the colour produced by interfering chromogens in the sample (Slot, 1965). These principles are incorporated into this manual method for measuring creatinine where the creatinine concentration was calculated based on the difference in colour intensity of the sample before and after acidification. Colour intensity was measured at 500 nm on a Spectrophotometer (Model SP6-450 UV/VIS, Pye Unicam Ltd., UK). The sample size required for analysis was 0.3 ml. Plasma samples were analyzed without dilution. If very concentrated, urine samples were diluted 1:5 before analysis. (At most, only the first

three urine samples from a protocol ever required diluting.) All urine and plasma samples were centrifuged (Refrigerated Centrifuge Model TJ-6, Beckman Instruments Inc., Palo Alto, CA) at 3000 rpm for 5 minutes to remove any remaining blood cells from the samples before analysis. A standard curve of 5 samples (from 0 - 10.0 mg creatinine/dl) was set up for each creatinine assay performed from a standard included in the kit. The standard curves produced were linear between creatinine concentrations of 0 and 10.0 mg/dl and all points on the standard curves had a standard deviation of ± 0.01 absorbance units. Duplicate measurements of creatinine performed on different days showed an average difference from the original measurements of 0.24 mg/dl in the plasma samples and 0.68 mg/dl in the urine samples. This resulted in an average difference in the calculated GFR of ± 1.56 ml/min from the original calculated GFR levels.

d) Protein Measurements

Plasma protein concentration was measured using a Total Protein Reagent (Sigma Diagnostics Inc., Catalog Number: 541-2, St. Louis, MO). This reagent allows for the determination of plasma protein concentration based on the biuret method in which a tartrate-complexed copper and alkali solution will produce a violet colour in the presence of protein due to the reaction of copper ions with peptide bonds (Doumas et al., 1981). This violet colour has an absorbance maximum of 540 nm and the intensity of this colour reaction was measured by spectrophotometry at this wavelength. Plasma samples of 60 μl were used in these measurements. Plasma protein concentrations were

determined from a standard curve created from standards of known protein concentration. Protein standards of 2,4,6,8, and 10 g/dl total protein (Sigma Diagnostics Inc., Catalog Number: P 6529, St. Louis, MO) were used to create a standard curve to accompany each set of plasma samples to be assayed. Increasing protein concentrations resulted in a linear increase in absorbance from 0 to 10 g/dl of protein. The average standard deviation for the five protein standards was ± 0.014 absorbance units. An absorbance change of 0.06 absorbance units corresponds to 1 g/dl total protein. Plasma samples were centrifuged at 3000 rpm for 5 min. before assaying to remove fibrin strands or any remaining blood cells from the plasma before analysis. Duplicate measurements of plasma protein performed on different days and after freezing and thawing samples, showed an average difference of 0.69 g/dl total protein from the original measurements.

e.) Hematocrit Measurements

The hematocrit levels of blood samples were determined in duplicate for each blood sample taken. Microhematocrit Heparinized Capillary Tubes (75 mm., Fisher Scientific, Pittsburgh, P.A.) were filled with blood taken from the 5 ml blood sample and centrifuged in a capillary tube centrifuge (Model CL32587M-3, International Equipment Co., Needham Heights, MA) for 15 min. at top speed. Hematocrits were determined by dividing the length of the packed red blood cells in the capillary tube from the entire length of the blood sample in the capillary tube in centimeters. The average between the two duplicate samples taken for each blood sample was determined to be the hematocrit. The average difference between duplicate hematocrits of 63 random

samples was 1.2%.

6.) Radioimmunoassays

a) Radioimmunoassay of ANP

The buffer used in the RIA of ANP contained 81 mM Na_2HPO_4, 19 mM NaH_2PO_4, 0.01% NaN_3 (all from Fisher Scientific, Nepean, Ont.), 50 mM NaCl, 0.01% BSA (ICN Biomedicals, Mississauga, Ont.), 0.1% Triton-x (Sigma Chemical Co., St. Louis, MO) and 1.5% Trasylol (Sigma Chemical Co., St. Louis, MO). The buffer was prepared by titrating the dibasic phosphate with the monobasic phosphate to a pH of 7.4 and then adding the NaCl, NaN_3 and Triton-X. The BSA and Trasylol were added on the day the assay was performed at which time the buffer was also diluted 1:1, resulting in a working buffer of 50 mM phosphate solution. 100 μl of buffer was added to each tube in the assay with the exception of the NSB tubes which contained 200 μl of buffer and the maximum binding tubes which contained 100 μl of buffer. All reagents used in the performance of this assay were refrigerated prior to use and all manipulations were carried out on ice in plastic 12 X 75 mm test tubes.

The anti-serum used in the RIA of ANP was rabbit anti-α ANP (rat) serum (RAS 9103, Peninsula Laboratories, Belmont, CA). The antibody was reconstituted with Triton X-100, 0.1%, and further diluted 1:3 with assay buffer giving a titer of 1:810,000. 100 μl of this anti-serum was used in each tube of the assay with the exception of the NSB tubes which contain no anti-serum. This anti-serum is 100% cross-reactive with α-ANP

(rat), α-ANP 1-28 (human, dog), ANF 8-33 (rat), Atriopeptin III (rat, rabbit, mouse), ß-ANP 1-28 (human), urodilatin and Auriculin A (rat).

Eight ANP standards were made up from purified, synthetic rat ANP 1-28 (Peninsula Laboratories, Belmont, CA). Rabbit ANP has been shown to have the same amino acid sequence as the rat (Oikawa et al., 1985). This lyophilized peptide was dissolved in 0.1 M acetic acid to a concentration of 1 mg/ml, then diluted with assay buffer to make a 5 ng/100 μl solution. This stock solution was further diluted into aliquots of the following ANP concentrations: 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0 and 100 pg/100 μl. These eight standards were used to create a standard curve. The standard curve was done in triplicate with 100 μl of the appropriate standard used in each tube.

Plasma and urine samples (2.0 ml) taken for ANP analysis were extracted using C18 silica gel extraction columns (Burdick and Jackson Solid Phase System, Baxter Canlab, Mississauga, ON) having average extraction efficiencies of 93.7%. These columns were washed with 5 ml of 100% methanol (Fisher Scientific Ltd., Ottawa, ON) followed by 5 ml of distilled water. The plasma or urine sample to be extracted was acidified with 100 μl of 1 mol/l HCl per millilitre of plasma and allowed to pass through the column. The column was then rinsed with 5 ml distilled water. The ANP adsorbed from the columns was eluted with 4.0 ml of 90% methanol - 0.5% trifluoroacetic acid (Sigma Chemicals, St. Louis, MO). The resulting extract was lyophylized and reconstituted in buffer for the assay. Extraction of plasma and urine samples in this

manner allows for the RIA of both ANP and AVP on the same sample (Adapted from Hartter and Woloszczuk, 1986). Samples were assayed in duplicate using 100 μl volumes in each tube.

After the buffer, anti-serum and sample or standard were added to the appropriate tubes in the assay, the tubes were vortexed and stored at 4 ° C for two days. On the second day, 100 μl of 2,000 cpm/100μl ^{125}I-Tyr^{28}ANP (rat) (Dupont Canada Ltd., Mississauga, ON) with a specific activity of 690 μCi/μg was added to each tube. The tubes were agitated and incubated at 4 ° C for 24 hours.

After the incubation period, the free antigen was separated from the bound antigen through use of Polyethylene Glycol (PEG) (PEG, Carbowax 8000, Fisher Scientific Ltd. Ottawa, ON). Initially 100 μl of 8 mg/ml bovine gamma globulin (ICN Biomedicals, Mississauga, ON) was added to each tube. The tubes were agitated, 600 μl of PEG was added to each tube and the tubes were agitated again. The tubes were centrifuged in a Sorval RC-3B refrigerated centrifuge (Dupont Co, Wilmington, DE) for 1 hour at 2,600 rpm and the supernatant was aspirated off of the pellet and discarded. The pellets remaining in the tubes contained the bound antigen-antibody complexes. These pellets were counted for 10 minutes using an automated gamma counter (RiaGamma 1274, LKB-Wallac, Wallac Oy, Finland) and the amount of ANP in each sample was determined from the standard curve.

The intra-assay and inter-assay variabilities of this ANP RIA expressed as an average of coefficients of variation (%) were 3.5% and 5.0% respectively. A 50% depression of maximum binding was achieved at 1.94 pg ANP/tube.

b) Radioimmunoassay of Vasopressin

The buffer used in the RIA of AVP was identical to the buffer used in the ANP RIA with the exception that the phosphate concentration was 0.1 mol/l and BSA made up 1% of the buffer. 300 μl of buffer was placed in each tube to be assayed with the exception of the NSB tubes which contained 450 μl of buffer and the maximum binding tubes which received 400 μl of buffer. As with the RIA for ANP, all reagents used in the performance of this assay were refrigerated prior to use and all manipulations were carried out on ice in plastic test tubes.

The anti-serum used in the RIA of AVP was raised in New Zealand White Rabbits by injecting a synthetic AVP (#V-0377, Sigma Chemicals, St. Louis, MO) conjugated to bovine thyroglobulin (Sigma Chemicals, St. Louis, MO). This conjugate was produced using the coupling agent ethyl carbodiimide (Sigma Chemicals, St. Louis, MO) using methods developed by Skowsky and Fisher (1972). The cross-reactivity of this antibody to various peptides was expressed as the mass ratio of AVP to the peptide in question that resulted in 50% inhibition of iodinated AVP from the antibody. The cross-reactivity of this antibody with lysine vasopressin, oxytocin, vasotocin and angiotensin II were determined to be 49.6%, 1.28%, 0.003% and $<0.001\%$ respectively.

These values give an indication of the high specificity for AVP of the antibody used in this assay. The antiserum was used in the assay in a dilution of 1:70000 and 50 μl of antiserum was used in each tube with the exception of the NSB tubes which received no antibody.

The synthetic AVP used to raise the aforementioned antibodies was also used to create the standard curve for the AVP RIA. This synthetic AVP stock solution was diluted with buffer to concentrations from 0.1 to 50 pg AVP/ml for the creation of the standard curve. A standard curve was created for every assay. The standard curve was done in triplicate and 100 μl of the appropriate standard was used in each tube of the standard curve.

Plasma samples for AVP analysis were extracted using the same method as plasma samples for ANP analysis. Plasma samples were all done in duplicate and 100 μl plasma was used in each tube of the assay.

After the buffer, anti-serum and sample or standard were added to the appropriate tubes in the assay, the tubes were well-agitated and stored at 4 $^{\circ}$C for 2 days. After this time, 50 μl of AVP-^{125}I was added to each tube. This AVP was iodinated using a modification of the method developed by Greenwood, Hunter and Glover (1963). Briefly, 10 μl of 0.5 mol/l phosphate buffer, 5 μl of 0.5 mCi Na^{125}I (Amersham Radiochemicals, Oakville, ON); and 5 μl of 1 μg/μl carrier-free chloramine

T (Sigma Chemicals, St. Louis, MO) were added to 3 μg of 0.3 μg/μl AVP. Afer 30 seconds, 50 μl of 0.25 μg/μl BSA was added to this solution to stop the reaction. The resulting mixture was then run through a prepared CM-Sephadex C-25 column (Pharmacia, Sweden) equilibrated with 0.6 mol/l acetate buffer (pH 4.8). The specific activity of the resulting AVP-^{125}I was determined to be approximately 1600 μCi/μg according to the self-displacement method of Morris (1976). Fractions of the eluted AVP-^{125}I peak were aliquoted and stored at -20 ° C for use up to 6 weeks. Once the AVP-^{125}I had been added to each tube, the tubes were well-agitated and incubated at 4° C for 3 days.

After the incubation period, the AVP bound to the antibody was separated from free AVP through use of PEG using the same method used in the RIA for ANP. Tubes were agitated and centrifuged at 4 ° C for 45 min. at 1500g. The supernatant was aspirated off of the pellet and discarded. The pellet remaining in each tube contained the bound antigen-antibody complexes. These pellets were counted for 10 min using an automated gamma counter and the amount of ANP in each sample was determined from the standard curve.

The intra-assay and inter-assay variabilities of this AVP RIA expressed as an average of coefficients of variation (%) were 2.6% and 9.2% respectively. A 50% depression of maximum binding was achieved at 1.58 pg AVP/tube.

7.) Calculations

For the expression of results, the following variables were calculated as follows:

a.) Urine Flow = Total Volume of Urine/15 min.
= ml/min

b.) GFR = $\frac{\text{(Urine Creatinine, mg/dl) * (Urine Flow, ml/min)}}{\text{(Plasma Creatinine, mg/dl)}}$

c.) GFR/kg = GFR/mass of rabbit

d.) Sodium Excretion in 15 minutes:

= Urine Sodium Concentration, mmol/l)*(urine volume in 15 min., ml)
= μmol

e.) ANP Excretion in 15 minutes:

= (Urine ANP Concentration, pg/ml)*(urine volume in 15 min., ml)

= pg

f.) Filtered Fraction of Sodium:

$$= \frac{\text{(Sodium Excretion in 15 min./15 min., } \mu\text{mol/min)}}{\text{(GFR, ml/min) * (Plasma Sodium, mmol/l)}} * 100$$
= %

g.) Filtered Fraction of Potassium:

$$= \frac{\text{(Potassium Excretion in 15 min./15 min., } \mu\text{mol/min)}}{\text{(GFR, ml/min) * (Plasma Potassium, mmol/l)}} * 100$$
= %

h.) Filtered Fraction of ANP:

$$= \frac{\text{(ANP Excretion in 15 min./15 min., pg/min)}}{\text{(GFR, ml/min) * (Plasma ANP, pg/ml)}} * 100$$
= %

i.) Osmolar Clearance:

$$= \frac{\text{(Urine Osmolality, mOsm/l) * (Urine Flow, ml/min)}}{\text{(Plasma Osmolality, mOsm/l)}}$$

j.) Free Water Clearance:

= (Urine Flow, ml/min) - (Osmolar Clearance, ml/min)

k.) Vascular Resistance:

= (Renal Perfusion Pressure, mmHg)/(Renal Blood Flow, ml/min)

8.) Experimental Protocols

a.) Treatment of Samples

Urine was collected from each ureter in plastic vials over 15 minute periods and stored on ice. Urine samples were centrifuged in a refrigerated centrifuge for 15 min. at 3000 rpm to remove any blood cells. The total volume of each urine sample, collected from both ureters and after centrifugation, was measured in plastic graduated cylinders. The 10 ml, 25 ml, 50 ml and 100 ml graduated cylinders used had accuracies of ± 0.05, 0.5, 0.5 and 0.5 ml respectively. After volume measurements, 2 ml of urine was aliquoted and frozen for RIA analysis of ANP and 1 ml was aliquoted and frozen for creatinine analysis. Sodium, potassium and osmolality measurements were done the day of each experiment. Samples were kept on ice until analysis.

Blood samples were taken from the carotid artery (Protocol 1 and 2) or from the brachial artery (Protocol 3). A 5 ml volume of blood was taken for each sample into a sterile 5 ml syringe (Becton Dickinson and Co., Rutherford, NJ) and placed in a cold 15

ml plastic test tube on ice. Blood samples were centrifuged at 4 ° C for 15 min. at 3,000 rpm. The plasma was pipetted off of the blood cells and placed in a 5 ml plastic test tube. Plasma (2.0 ml) was aliquoted and frozen for RIA analysis of ANP and AVP, and 1 ml was aliquoted and frozen for creatinine analysis. Sodium, potassium and osmolality measurements were done the day of each experiment. Samples were kept on ice until analysis. All blood samples taken were replaced with an equal volume of blood from the donor rabbit. This replacement blood was injected via an arterial cannula as soon as possible after taking a blood sample.

In Protocol 3, blood samples were also taken from the ear arteries or branches of the carotid arteries. These samples were taken for the express purpose of sodium analysis and a 0.5 ml volume was taken for each sample. Samples were taken by allowing the blood to drip slowly into a 0.65 ml microcentrifuge tube (Island Scientific, Catalog No.: 13, Bainbridge Island, WA). These tubes were stored on ice and centrifuged at 4 ° C for 15 min. at 3000 rpm. The plasma was pipetted off of the blood cells, and placed in microcentrifuge tubes on ice for sodium analysis. All sodium analysis of these blood samples was done the day of the experiment.

b) Protocol 1 - Validation of the Preparation (Evaluating the State of Hydration and Renal Function)

This series of 10 experiments was performed to ensure that the surgical procedure used was valid and resulted in a normal renal response to increased renal perfusion

pressure. After the surgery was completed and the electronic recording set up, the perfusion pressure in the kidney was set to 80, 110 or 140 mmHg. The starting perfusion pressure was determined randomly. The rabbit was left to equilibrate for 15 minutes at this starting pressure at which point the protocol was begun. The protocol consisted of three consecutive 45 minute periods each at one of the three different renal perfusion pressures (Fig 3). These perfusion pressures were applied in random order by changing the perfusion pressure set-point on the servo-controlled roller pump. Urine samples were collected from the two ureter cannulae over 15 minute periods resulting in 9 urine samples per protocol. Blood samples were taken from the carotid artery at the mid-point of each urine collection period resulting in 9 blood samples per protocol.

c.) Protocol 2 - Validation of the Preparation (Renal Nerve Function)

This series of 7 experiments was performed to ensure that the renal nerves remained intact and functional in this preparation. After the surgery was completed and the electronic recording set-up, renal perfusion pressure was set at 90 mmHg. The rabbit was left to equilibrate for 15 minutes at this pressure at which point the protocol was begun. The Protocol consisted of 3 sequential 30 minute periods (Fig. 4). A 30 minute control period was followed by a 30 minute period during which the right carotid artery was clamped. At the end of this 30 minute period, the carotid artery was unclamped and 15 mg/kg phenoxybenzamine (Smith Kline and French, Montreal, PQ) was injected into the ear vein. The effects of this injection were observed during the last 30 minute period. Urine samples were collected from the 2 ureter cannulae over 15

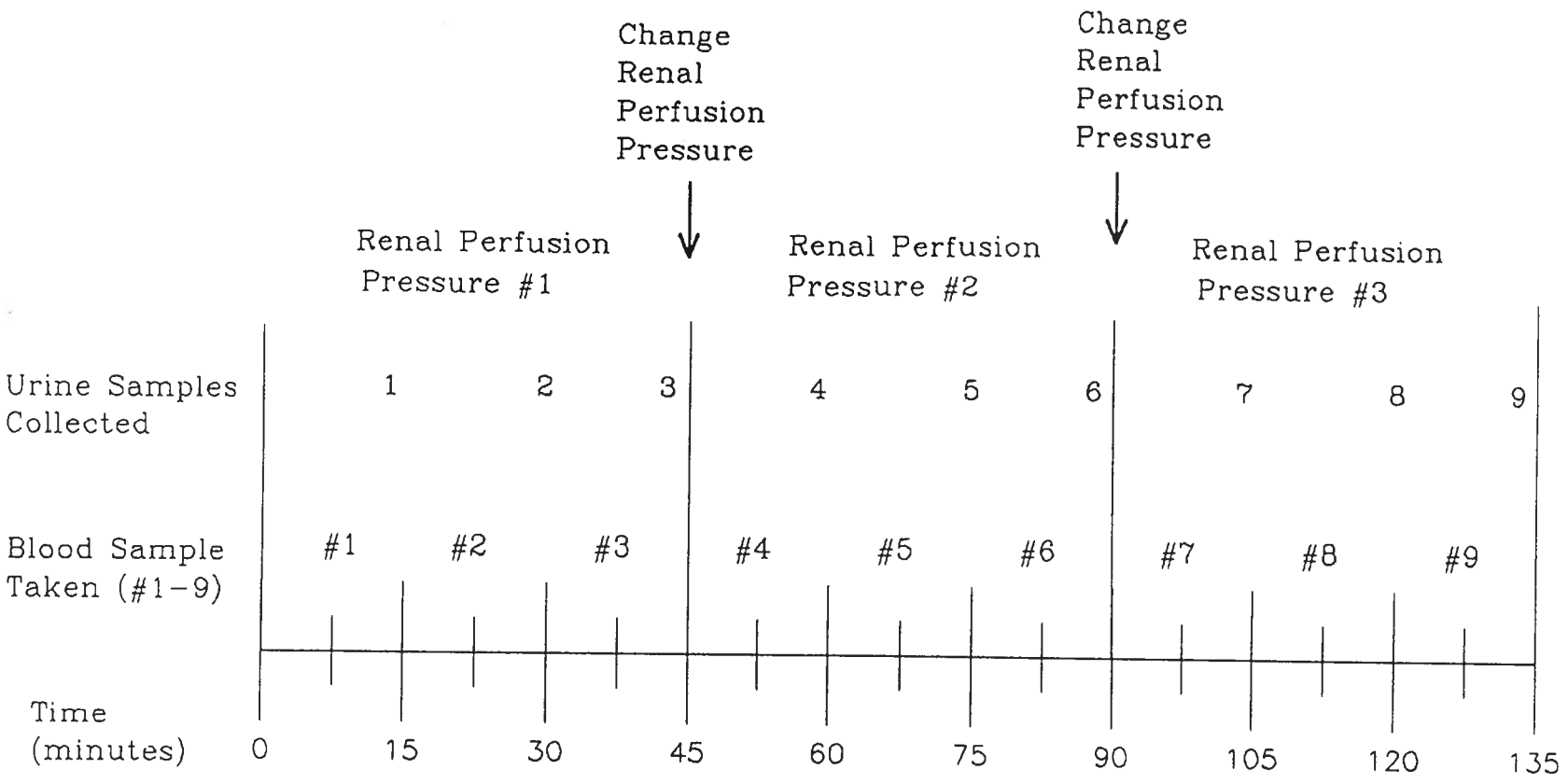

Fig. 3) Experimental Protocol #1 – Validating the Preparation through examining the effects of different Renal Perfusion Pressures on Cardiovascular and Renal Function.

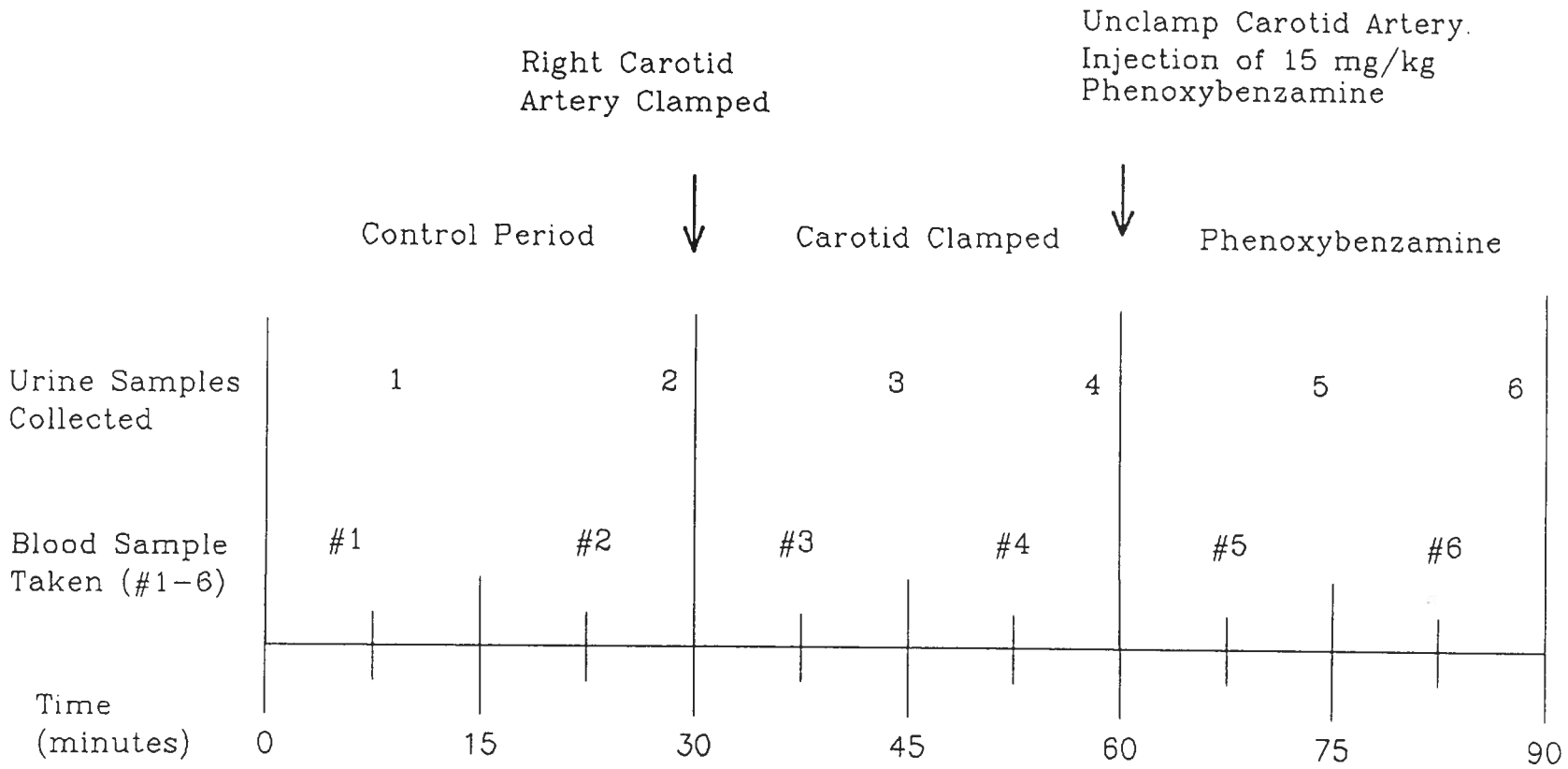

Fig. 4) Experimental Protocol #2 – Validating the Renal Nerve Function in the Preparation through examining the Effects of Clamping a Carotid Artery and Phenoxybenzamine Injection on Renal Blood Flow and Sodium Excretion.

minute periods during this protocol, resulting in 6 urine samples per protocol. Blood samples were taken from the carotid artery at the mid-point of each urine collection period resulting in 6 blood samples per protocol.

d) Protocol 3 - Comparison of an I.V. Saline Load vs. a Carotid Artery Saline Load

In this series of experiments 6 control and 6 experimental procedures were performed to compare the cardiovascular and renal effects of a saline load given intravenously with an identical saline load infused as H_2O into the venous system and hypertonic saline into the carotid arteries. After the surgery was completed and the electronic recording was set up, renal perfusion pressure was set at 110 mmHg and the preparation was left to equilibrate for 15 minutes at which point the protocol was begun. This protocol consisted of three consecutive 45 minute periods; an initial 45 minute period (Baseline), a 45 minute infusion period (Infusion) and a post-infusion period (Post-Infusion) during which the infusions were turned off (Fig. 5).

In the 6 control experiments, during the 45 minute Infusion Period, 0.9% (isotonic) saline was infused at 0.2 ml/min into each of the carotid arteries through the cannulae set up during the surgical procedure. This infusion was concurrent with an infusion of 0.9% saline at 1.5 ml/min into the left jugular vein. This infusion into the left jugular vein was achieved through silicone tubing (Masterflex 6411-3, Cole-Parmer, Chicago, IL) fed through an infusion roller pump (Masterflex Model 7520-25, Cole-Parmer, Barrington, IL). This infusion system was connected through a T-joint to the

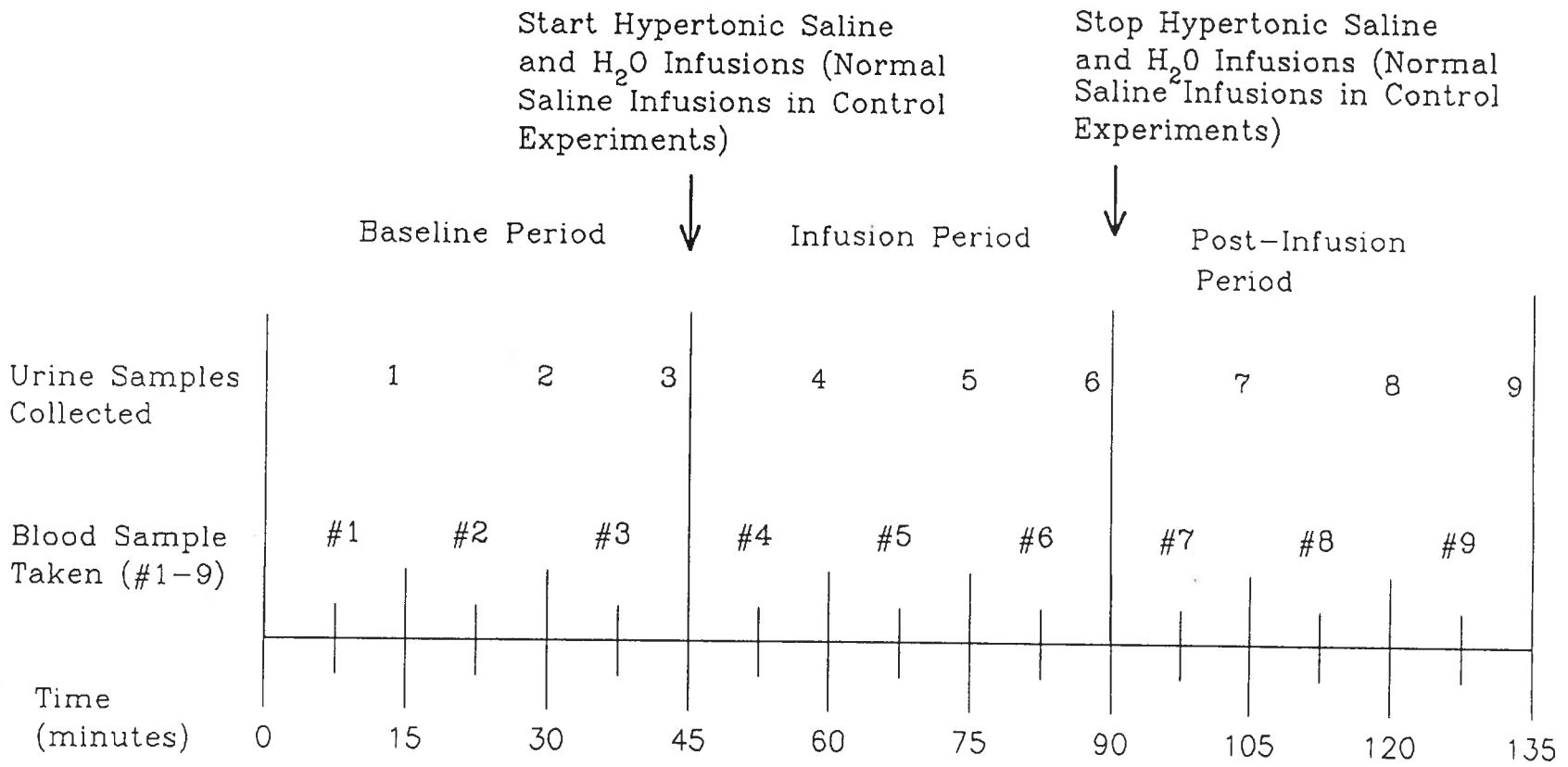

Fig. 5) Experimental Protocol #3 – Examining the Effects of a Hypertonic Saline infusion into the Brain on Renal and Cardiovascular Function.

2 infusions already going into the left jugular vein. These infusions of isotonic saline into the carotid arteries and left jugular vein are referred to as the "Control Procedure".

During the 45 minute Infusion Period in the 6 experimental animals, a 677 mmol/l saline solution was infused at 0.2 ml/min into each carotid artery through the infusion cannulae set up during the surgical procedure. This infusion was begun concurrent with an infusion of distilled water at 1.5 ml/min. into the left jugular vein. This infusion was achieved by means of an infusion roller pump through a T-joint attaching the distilled water infusion to the two infusions already going into the left jugular vein. The sum of these two infusions of 677 mmol/l saline and distilled water was isotonic. These infusions of hypertonic saline into the carotid arteries concurrent with the infusion of water into the left jugular vein are referred to as the "Experimental Procedure". The volumes and concentrations that were administered per unit body weight were the same as those used by Emmeluth et al. (1992) and were expected to produce an increase of 3 mmol Na^+/kg H_2O in carotid artery plasma. This was based on an assumption of a blood flow of 8 ml/min/kg body weight in each carotid artery. This blood flow in the carotid arteries was verified in an anaesthetized rabbit using a flow probe (Probe #2SB581, Transonic Systems Inc., Ithaca, NY) placed around a carotid artery and was found to be 10 ml/min/kg body weight. A larger volume of blood was therefore diluting the carotid artery infusions and a slightly smaller increase in carotid artery plasma sodium concentration was expected in response to the experimental procedure infusion than was calculated by Emmeluth et al. (1992). The 6 control and

6 experimental procedures were carried out in alternating order.

In this Protocol, urine samples were collected from the two ureter cannulae over 15 minute periods resulting in 9 urine samples per protocol. Blood samples were taken from the brachial artery at the mid-point of each urine collection period resulting in 9 blood samples per protocol. At the same time, blood samples were also taken from the ear arteries in the first 5 experiments in this protocol (2 control and 3 experimental) to determine the exact concentration of the plasma sodium reaching the brain. In the final 7 experiments in this series (4 control and 3 experimental) these small blood samples taken for sodium analysis were taken from 2 cannulae, each in a branch of a carotid artery.

9.) Statistics

In the validation of the experimental technique (Protocols 1 and 2) all variables were analyzed by ANOVA using a randomized block design. Statistical significance ($p < 0.05$) was tested using the post hoc Neuman-Keuls Test. $N = 10$ for all of the results obtained using Protocol 1 and $N = 7$ for results obtained using Protocol 2.

With the exception the Ear/Carotid Plasma Sodium Concentrations (Fig. 30), differences within control or experimental procedures in Protocol 3 were analyzed by ANOVA using a randomized block design. $N = 6$ for the control and experimental procedures and statistical significance ($p < 0.05$) was tested using the post hoc Neuman-

Keuls Test (unless otherwise noted). A Paired T-Test was used to determine if any differences existed between "Control" (see below) and Infusion Periods in Ear/Carotid Artery Plasma Sodium Concentrations (Fig. 30).

Possible differences between the 6 control and 6 experimental animals in Protocol 3 were examined by Repeated Measures ANOVA. If Repeated Measures ANOVA indicated a significant difference between the control and experimental groups, an unpaired T-Test was performed on the control and experimental data for each of the three treatment periods (Baseline, Infusion, and Post-Infusion) to determine at which point(s) the control and experimental periods varied.

10.) Format and Presentation of Results

For Protocols 1 and 3, in the analysis of results, the first 15 minutes of each 45 minute period was considered to be a time of transition and data from the initial urine and blood sample was not included in the results. Data taken during the last 30 minutes of each 45 minute collection period was averaged and these averages are presented in the results ± the standard error of the mean (SE).

In the calculation of Ear/Carotid Artery Plasma Sodium Concentrations (Fig. 30), the Base-Line and Post-Infusion Period Data was averaged to produce a "Control" value. Figure 30 shows these "Control" values compared to the concentrations of Ear/Carotid Plasma Sodium during the infusion period of Protocol 3.

In Fig. 12 (Protocol 2), values shown for the Control, Carotid Clamped and Phenoxybenzamine Periods represent the average values of the parameter in question over the entire 30 minute time period ± SE.

In all figures, unless otherwise noted, " * " indicates a significant difference between the point indicated and 80 mmHg perfusion pressure (Figures from Protocol 1, i.e. Fig. 6-13); between the point indicated and the Control Period (Fig. 14 - Protocol 2); or between the point indicated and Baseline (Figures from Protocol 3, i.e. Fig. 15-30). "+" indicates a significant difference between the point indicated and 110 mmHg perfusion pressure (Figures from Protocol 1) or a significant difference between the control and experimental groups at that point (Figures from Protocol 3). "δ" indicates a significant difference between the post-infusion and infusion periods within a protocol in Figures from Protocol 3.

RESULTS

Part 1) Protocol #1 and #2 - Validation of the Preparation

a.) State of Hydration of the Animals

As an index of the state of hydration of the preparation during this experimental protocol, a number of variables were measured from blood samples taken throughout the experimental protocol. These variables included hematocrit (Fig. 6a), plasma osmolality (Fig. 6b), plasma sodium concentration (Fig. 7a) and plasma protein concentration (Fig. 7b). These four variables did not change during the experimental protocol indicating that the animals were maintaining a relatively constant state of hydration despite varying levels of renal perfusion pressure (RPP) and renal excretion.

b.) Renal Function

The volume of urine produced by these animals in a 15 minute period was calculated at the three different levels of renal perfusion pressure (Fig. 8). Urine production was shown to increase significantly ($p < 0.05$) with each increase in RPP. These increases in urine production represented an increase of 142% from a RPP of 80 mmHg to 110 mmHg and a 234% increase in urine production from a RPP of 80 mmHg to 140 mmHg.

Concurrent with an increase in urine production, the amount of sodium excreted in a 15 minute period was also shown to increase as RPP increased (Fig. 9). The

amount of sodium excreted in 15 minutes increased significantly ($p<0.05$) both when increasing the RPP from 80 to 110 mmHg and when increasing the RPP from 110 mmHg to 140 mmHg. These changes represented increases in sodium excretion of 175% when increasing the RPP from 80 to 110 mmHg and a 338% increase when increasing the RPP from 80 to 140 mmHg.

The glomerular filtration rate (GFR) was also determined in animals from this series as an index of renal function (Fig. 10). GFR was normalized to the body weight of the animals because GFR varies with size. There was no significant change in GFR in this series despite the changes in RPP.

The effect of different RPP's on the percentage of the filtered load of sodium and potassium to be excreted in the urine (i.e. the filtered fraction of sodium and potassium) was calculated as a further index of renal function in this series of experiments. The filtered fraction of sodium was shown to increase significantly ($p<0.05$) with each change in RPP (Fig. 11a). These changes represented increases of 78% and 163% respectively as RPP was changed from 80 to 110 mmHg and from 80 to 140 mmHg. The filtered fraction of potassium did not change in this experimental protocol despite changes in RPP (Fig. 11b).

The actual urinary sodium concentrations at 80, 110, and 140 mmHg RPP were 57.7 ± 7.75, 72.8 ± 8.33, and 95.5 ± 6.31 mmol/l respectively. These values indicate that

there was a significant increase in the urinary concentration of sodium ($p<0.05$) as RPP changes from 80 to 140 mmHg and from 110 to 140 mmHg.

The actual urinary potassium concentrations at 80, 110 and 140 mmHg RPP were 29.6 ± 6.28, 18.0 ± 3.98, and 11.7 ± 1.86 mmol/l respectively. These values indicate that there was a significant decrease in the urinary concentration of potassium ($p<0.05$) as RPP changes from 80 to 110 mmHg and from 80 to 140 mmHg.

c.) Endocrine Parameters

The effect of different RPP's on the plasma level of immunoreactive atrial natriuretic peptide (IR-ANP) were examined in this series of experiments (Fig. 12). Plasma IR-ANP did not change despite changes in RPP. The average right atrial pressure at the different RPP's also did not change significantly. At 80, 110, and 140 mmHg RPP, the average right atrial pressures were determined to be 3.1, 3.1 and 2.3 mmH_2O respectively.

The filtered fraction of IR-ANP did not change when RPP was increased from 80 to 110 mmHg (Fig. 13a). However, the filtered fraction of IR-ANP was significantly increased ($p<0.05$) when RPP was changed from 80 to 140 mmHg and when RPP was changed from 110 to 140 mmHg. The total amount of IR-ANP excreted in 15 minutes was shown to increase significantly ($p<0.05$) when RPP was changed from 80 to 110 mmHg and when RPP was changed from 80 to 140 mmHg (Fig. 13b). There was no

change in the amount of IR-ANP excreted when RPP was changed from 110 to 140 mmHg.

d.) Renal Nerve Function

Clamping the right carotid artery resulted in a significant decrease ($p<0.05$) in renal blood flow (Fig. 14b). A 15 mg/kg I.V. injection of phenoxybenzamine caused a significant increase ($p<0.05$) in sodium excretion (Fig. 14a). Both of these results are indicative of intact and functional renal nerves. Sodium excretion was not affected by clamping the carotid artery and the average renal blood flow between 15 and 30 minutes post-phenoxybenzamine injection was not significantly increased. However, immediately after the phenoxybenzamine injections, renal blood flow did peak briefly at a significant level before kidney autoregulation decreased renal blood flow.

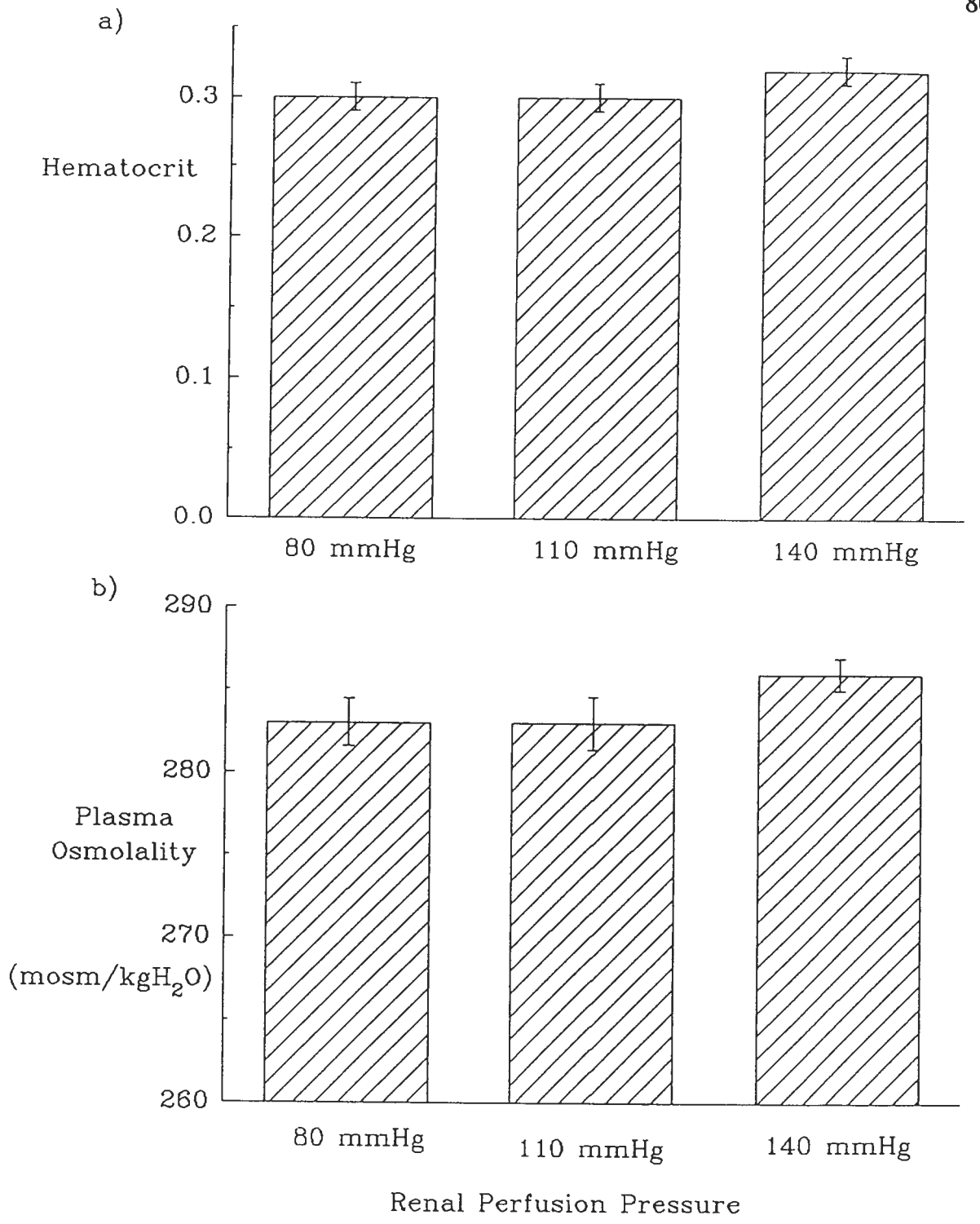

Fig. 6) The effect of different renal perfusion pressures on Hematocrit (Fig. a) and Plasma Osmolality (Fig. b). Each bar represents the average value of the parameter in question over the last 30 minutes of a 45 minute time period. Different perfusion pressures were implemented in random order.

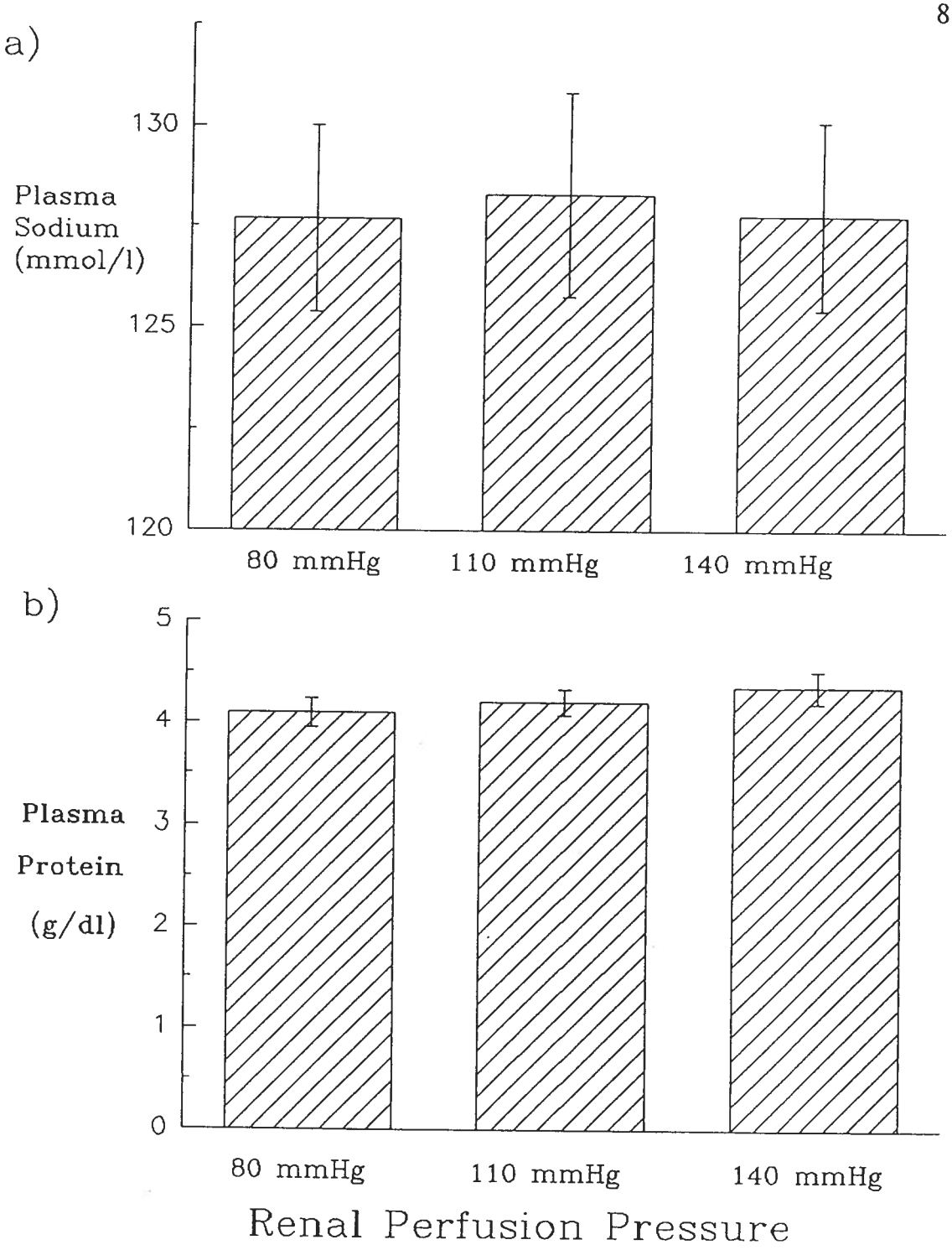

Fig. 7) Effects of different renal perfusion pressures on Plasma Sodium (Fig. a) and Plasma Protein (Fig. b). Each bar represents the average value of the parameter in question over the last 30 minutes of a 45 minute time period. Different renal perfusion pressures were implemented in random order.

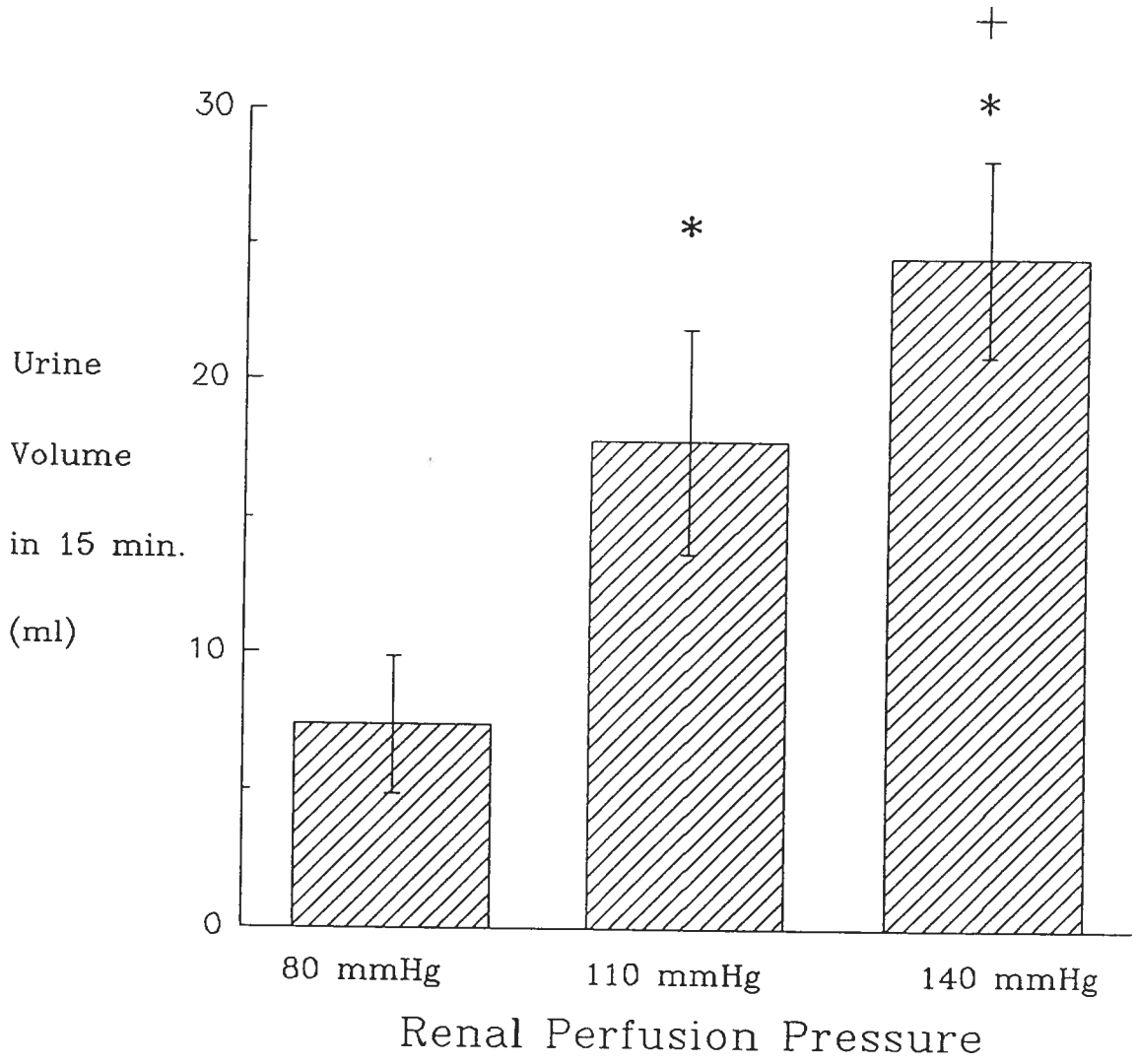

Fig. 8) The effects of different renal perfusion pressures on the volume of urine produced in 15 minutes. Each bar represents the average volume of urine produced in 15 minutes taken over the last 30 minutes of a 45 minute collection period. Different renal perfusion pressures were implemented in random order.

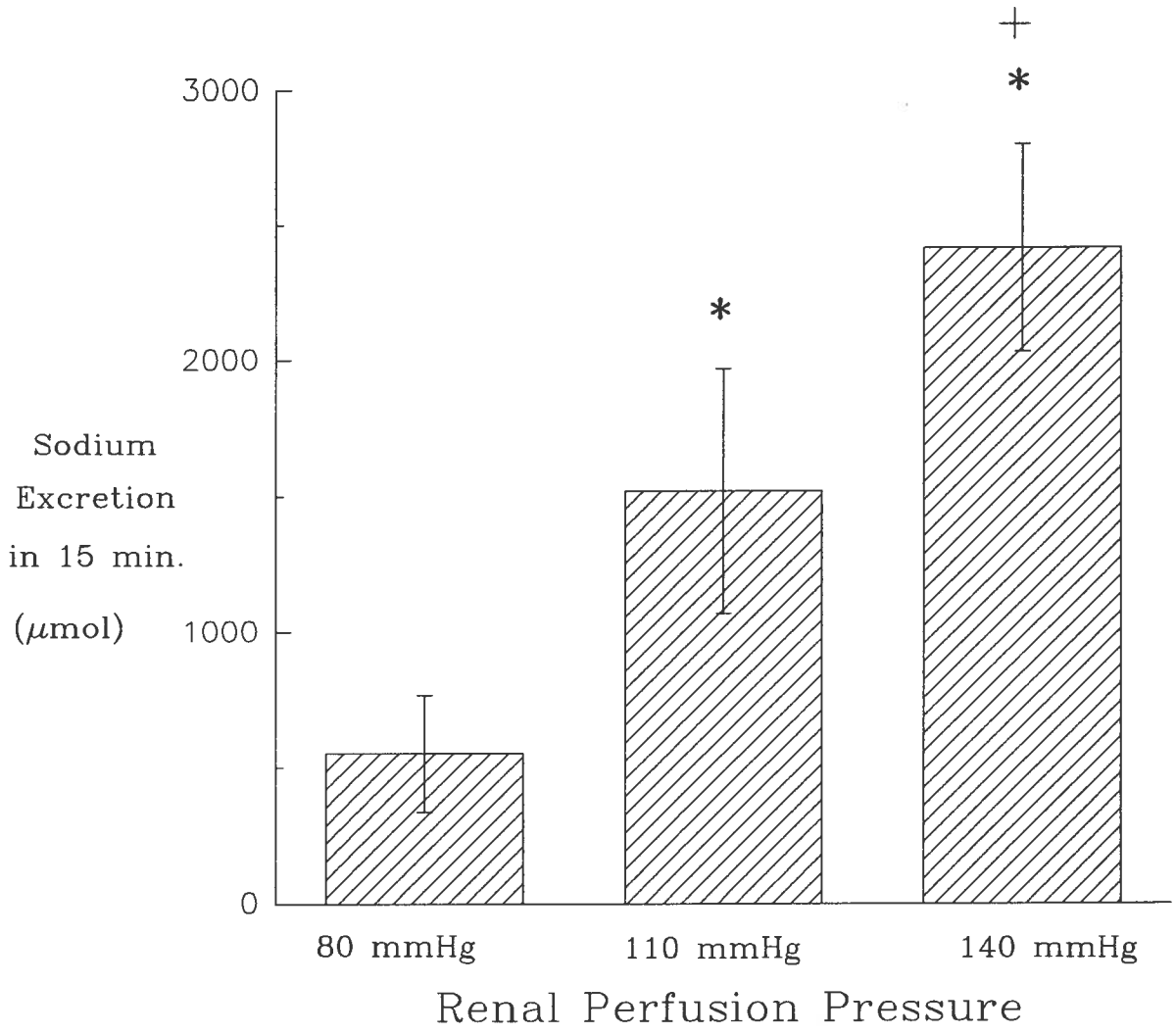

Fig. 9) The effects of different renal perfusion pressures on the amount of sodium excreted in a 15 minute period. Each bar represents the average amount of sodium excreted in 15 minutes taken over the last 30 minutes of a 45 minute collection period. Different renal perfusion pressures were implemented in random order.

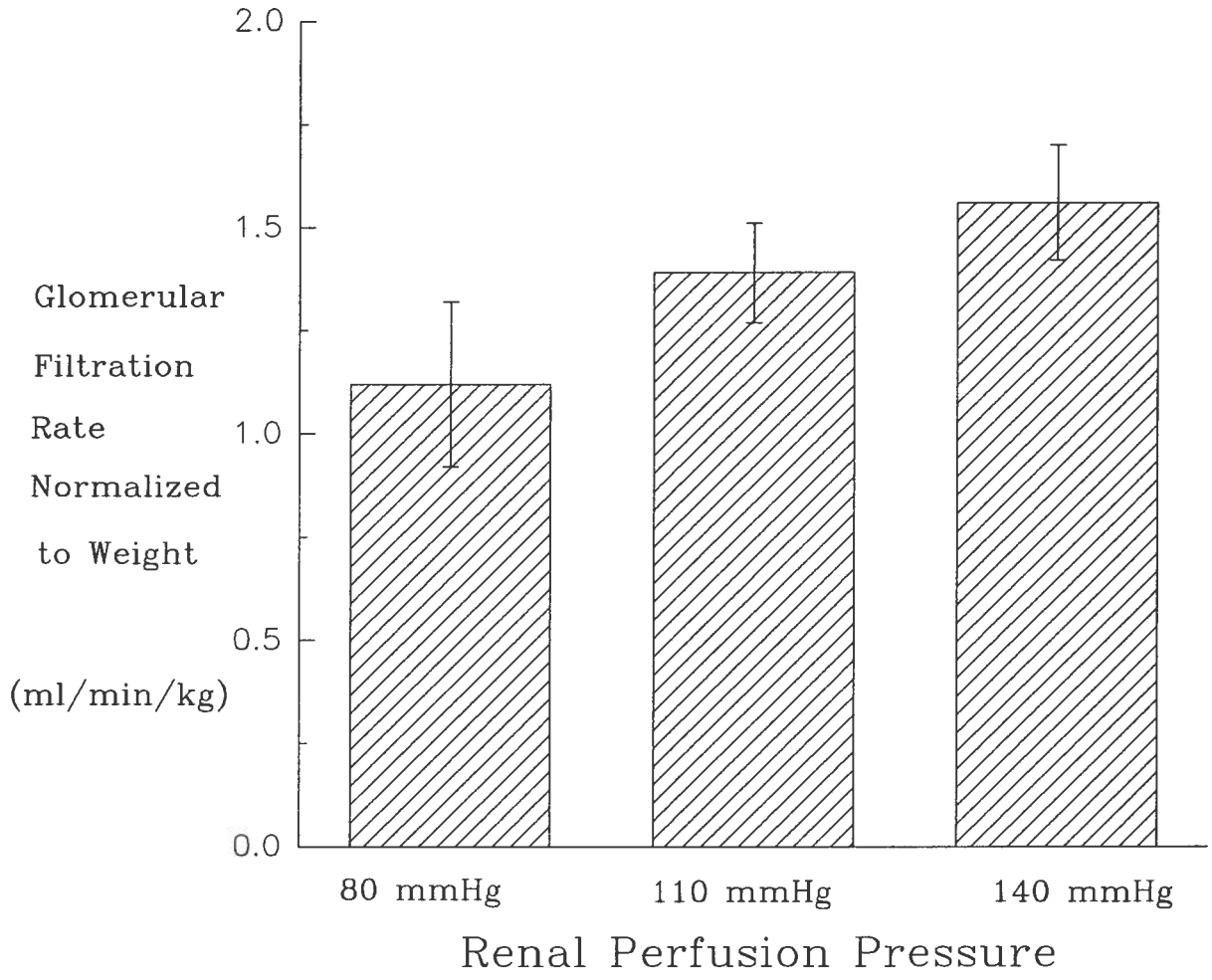

Fig. 10) The effects of different renal perfusion pressures on the Glomerular Filtration Rate (GFR) normalized to weight. Each bar represents the average GFR/kg over the last 30 minutes of a 45 minute time period. Different renal perfusion pressures were implemented in random order.

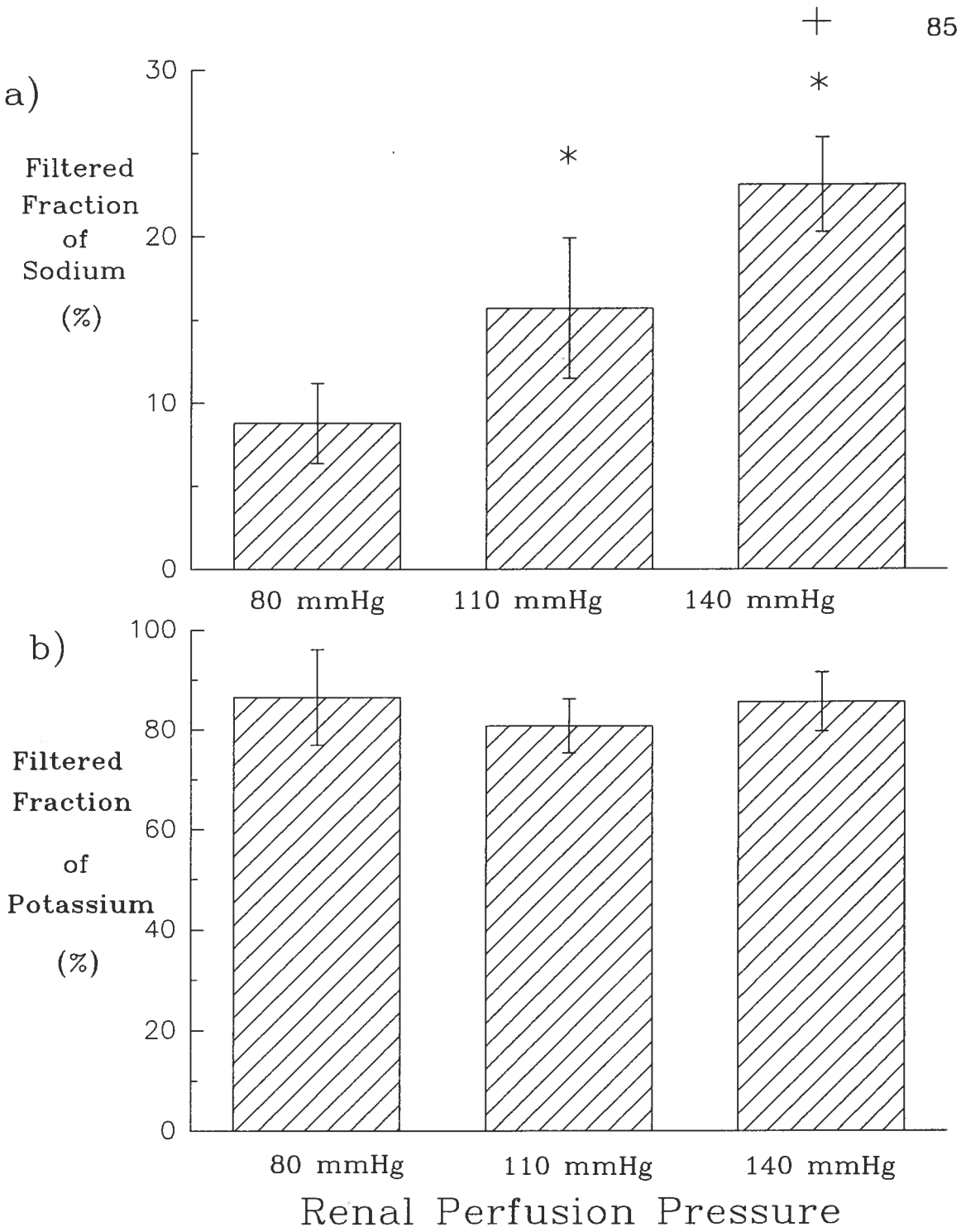

Fig. 11) Effects of different renal perfusion pressures on the percentage of the filtered load of Sodium (Fig.a) and Potassium (Fig. b) excreted in the urine. Each bar represents the average value of the parameter in question taken over the last 30 minutes of a 45 minute period. Different perfusion pressures were implemented in random order.

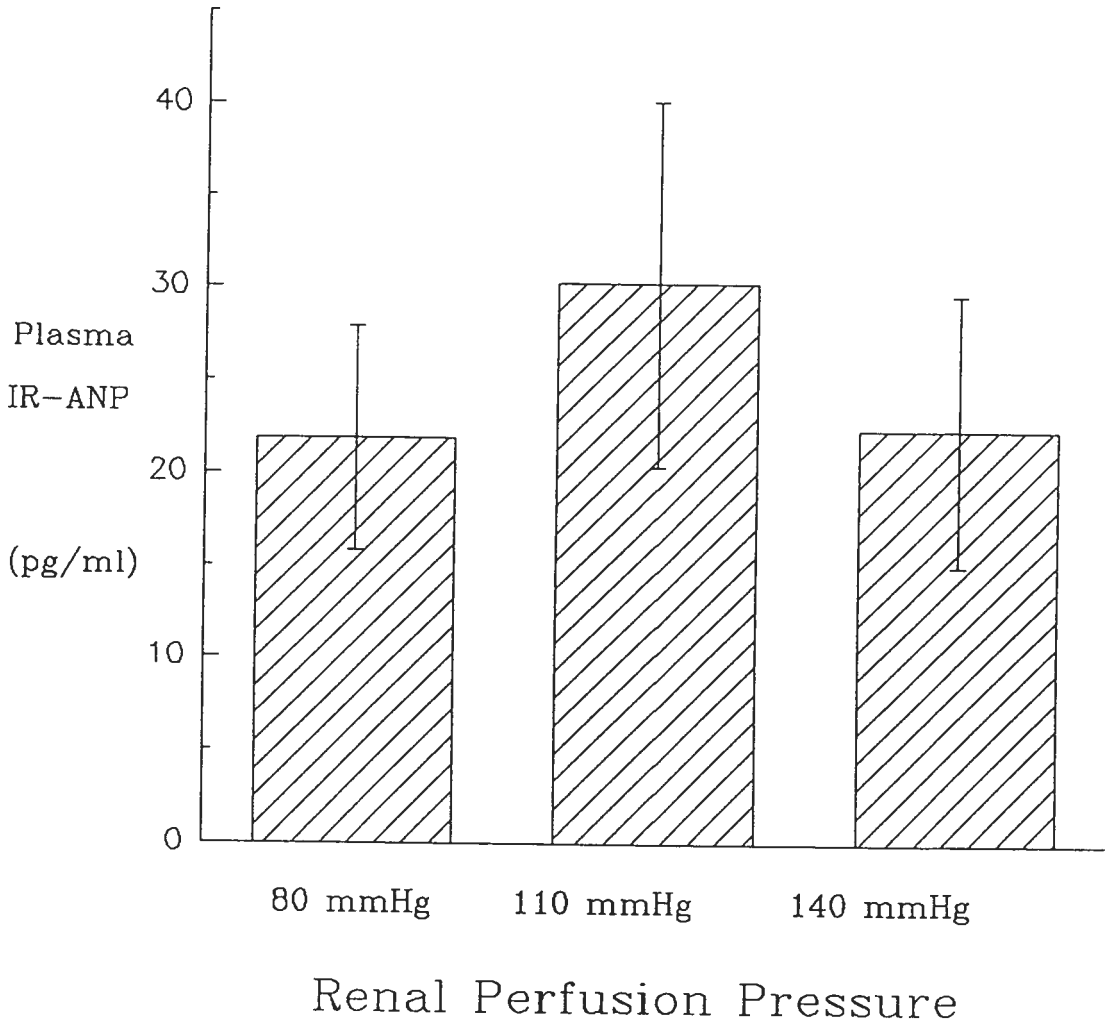

Fig. 12) Effects of different renal perfusion pressures on the level of Immunoreactive ANP (IR-ANP) in the Plasma. Each bar represents the average Plasma IR-ANP taken over the last 30 minutes of a 45 minute period. Different perfusion pressures were implemented in random order.

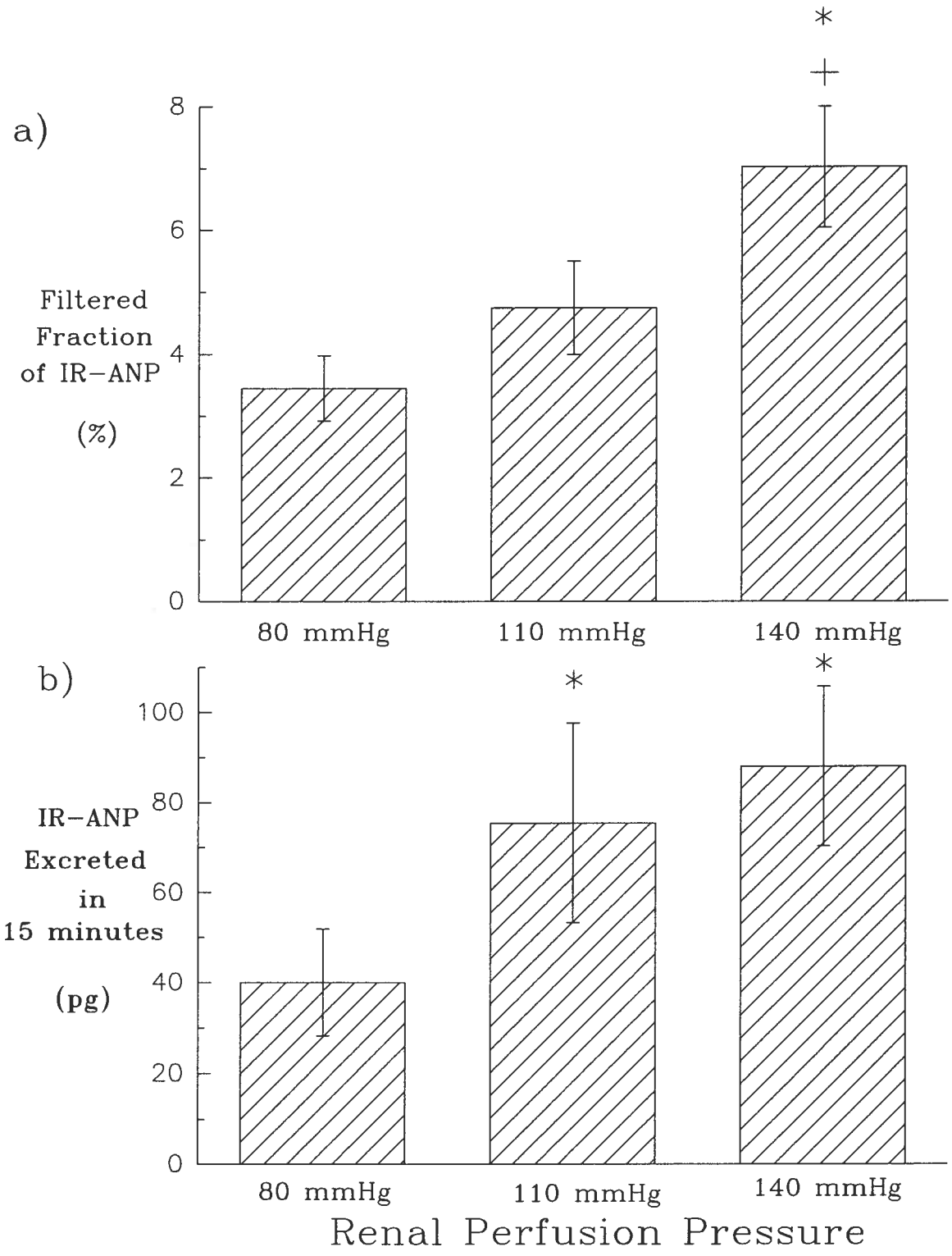

Fig. 13) Effects of different renal perfusion pressures on the Percentage of the filtered load of IR-ANP excreted in the urine (Fig.a) and on the total amount of IR-ANP excreted in 15 minutes (Fig. b). Each bar represents. the average value of the parameter in question taken over the last 30 minutes of a 45 minute period. Different renal perfusion pressures were implemented in random order.

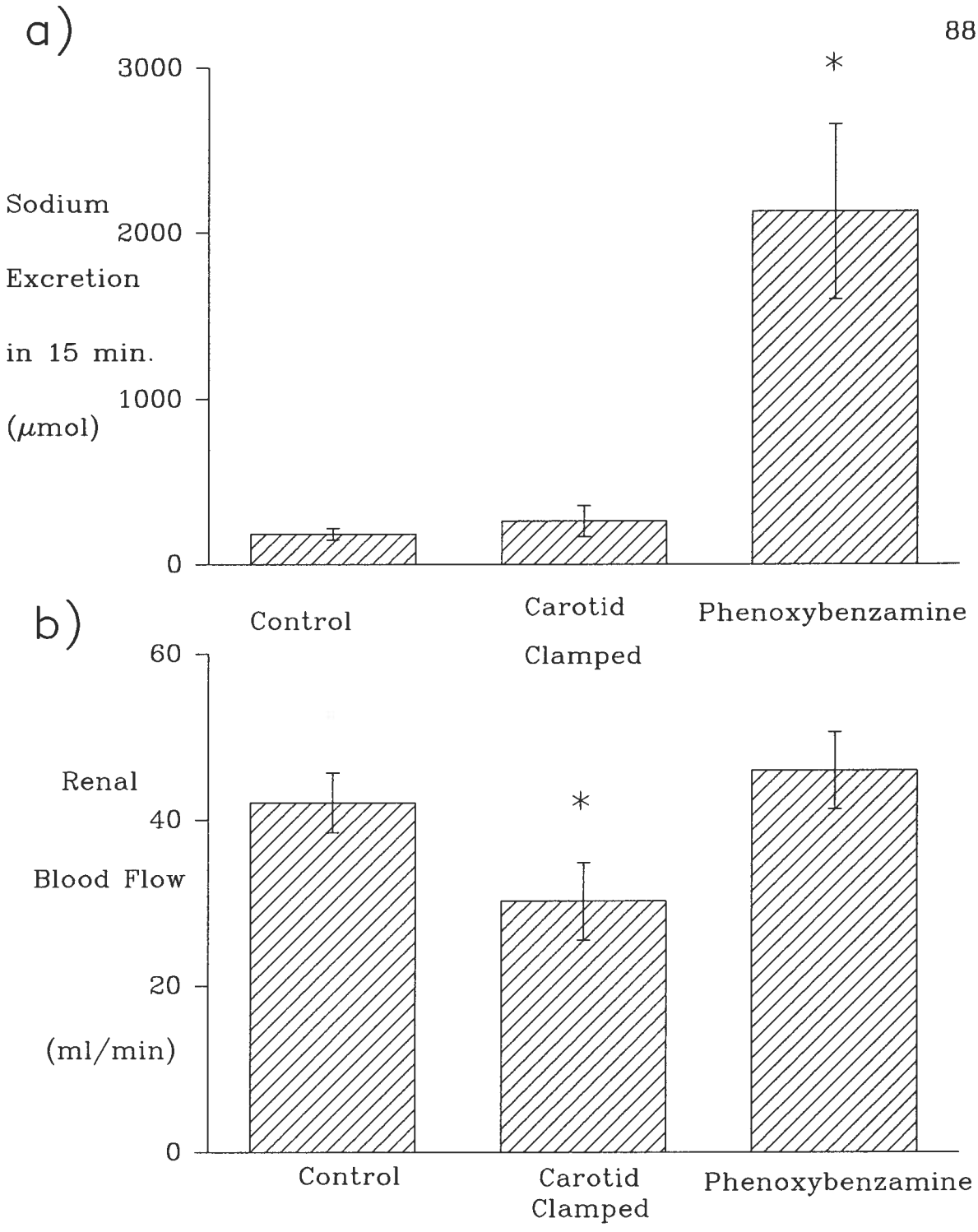

Fig. 14) Effects of Clamping the Right Carotid Artery and 15 mg/kg Phenoxybenzamine Injection on Sodium Excretion in 15 min. (Fig. a) and Renal Blood Flow (Fig. b). The Control, Carotid Clamped and Phenoxybenzamine Periods represent 3 sequential 30 minute periods. Each bar represents the average value of the parameter in question over the entire 30 minute period.

Part 2) Protocol 3 - The Effects of Increasing Sodium Concentration in Blood Perfusing the Brain

a.) State of Hydration of the Animals

The state of hydration of the preparation in this protocol was determined through analysis of the hematocrit levels, plasma osmolality, plasma sodium concentration and plasma protein concentration of the animals throughout the protocol. With respect to hematocrit levels (Fig. 15a), as a result of both the control procedure and experimental procedure infusions, hematocrit levels were shown to significantly decrease ($p<0.05$). In the post-infusion periods of both the control and experimental procedures, the hematocrit levels were once again significantly decreased from the infusion period levels. There was no significant difference however between the baseline, infusion and post-infusion periods of the control and experimental procedures.

The plasma osmolality did not differ from baseline throughout both the control and experimental procedure infusion and post-infusion periods (Fig. 15b). The plasma osmolality also remained the same between the control and experimental procedures during each period within the protocol.

Plasma sodium concentrations increased significantly ($p<0.05$) from the baseline to the control procedure infusion period and remained significantly increased above baseline during the post-infusion period (Fig. 16a). Plasma sodium concentrations did

not change from baseline during the experimental procedure infusion or post-infusion periods. There was no difference at any time period in plasma sodium concentrations between the control and experimental procedures.

The plasma protein concentrations were significantly decreased ($p < 0.05$) from baseline during the control procedure post-infusion period (Fig. 16b). During the experimental procedure infusion period, plasma protein concentration was decreased from baseline and remained significantly decreased ($p < 0.05$) during the post-infusion period. There was no difference at any time period during the protocol between the control and experimental procedures.

In summary, although hematocrit levels, plasma sodium concentrations and plasma protein concentrations were shown to change at various points within control or experimental procedures, there was no difference between the control or experimental procedures at any point during the protocol for these three variables. Plasma osmolality was also constant between the control and experimental procedures at all periods. This indicates that animals infused with isotonic saline and animals infused with 677 mM sodium chloride, started off at the same baseline point and underwent similar changes during the infusion and post-infusion periods with respect to their state of hydration.

b.) Renal Effects

Urine flow was shown to increase significantly ($p < 0.05$) during the infusion period

of both the control and experimental procedures (Fig. 17). This increase in urine flow from baseline was maintained during both the control and experimental procedure post-infusion periods. Although the baseline periods were not statistically different, during the infusion period urine flow was significantly greater during the control procedure than during the experimental procedure. This difference was maintained in the post-infusion periods. This indicates that the control procedure had a greater effect on increasing absolute urine flow than the experimental procedure and that this effect was maintained in the post-infusion period.

The total amount of sodium excreted in 15 minutes was significantly increased above baseline in both the infusion and post-infusion periods of the control procedure experiments (Fig. 18). During the experimental procedure infusion period, the sodium excretion was not statistically different from baseline values although the increase in sodium excretion did reach statistical significance during the post-infusion period. There was no statistical difference at any point between the control and experimental procedures.

GFR normalized to weight was also measured as an indication of renal function (Fig. 19). GFR increased significantly ($p < 0.05$) from the baseline period during the control procedure infusion and this increase in GFR was maintained during the post-infusion period. During the experimental procedure infusion and post-infusion periods, GFR did not differ from the baseline. GFR did not differ between the experimental and

control procedures at any point during the protocol.

The filtered fractions of both sodium and potassium were shown to increase ($p < 0.05$) in response to the control procedure infusion; an increase that was maintained throughout the post-infusion period (Fig. 20a and 20b). During the experimental procedure infusion and post-infusion periods neither the filtered fraction of sodium nor potassium differed from their respective baseline values. There were no differences in the filtered fractions of sodium or potassium between the control and experimental procedures at any point during the protocol.

The actual urinary concentrations of sodium during the baseline, infusion and post-infusion periods were 60.41 ± 7.63, 81.37 ± 9.38 and 94.71 ± 11.66 mmol/l respectively during the control procedure and 51.09 ± 5.74, 85.68 ± 5.57 and 80.60 ± 6.98 mmol/l respectively during the experimental procedure. These changes represent significant increases in the urinary sodium concentration in the infusion period of both the experimental and control procedures that are maintained in the post-infusion periods of both procedures. There were no differences in urinary sodium concentration between the control or experimental procedures at any point during the protocol.

The actual urinary concentrations of potassium during the baseline, infusion and post-infusion periods were 23.5 ± 5.4, 7.5 ± 0.9, and 8.6 ± 1.0 mmol/l respectively during the control procedure and 38.8 ± 6.6, 17.4 ± 3.8, and 17.7 ± 3.0 mmol/l during the

experimental procedure. These values indicate that a significant decrease in the potassium concentration in the urine occurred during the infusion period in both the experimental and control procedures. This decrease in urinary potassium concentration was maintained during the post-infusion period in both procedures. The baseline and infusion periods were statistically identical in both the control and experimental procedures. However, during the post-infusion period, the urinary potassium concentration was significantly lower in the control procedure than in the experimental procedure.

The control and experimental procedures resulted in similar increases ($p < 0.05$) in osmolar clearance (Fig. 21). These increases were maintained during the post-infusion periods of both the control and experimental procedures. There were no differences in osmolar clearance between the control and experimental procedures at any point during the protocol.

Free water clearance increased significantly from baseline during both the control and experimental procedure infusion periods (Fig. 22). This increase was maintained during both the experimental and control post-infusion periods. The control procedure infusion resulted in a significantly larger ($p < 0.05$) increase in free water clearance than the increase in free water clearance seen during the experimental procedure infusion period. This difference between the control and experimental procedures was maintained during the post-infusion period.

In summary, the renal effects of the control and experimental procedures were examined using the parameters of urine flow, sodium excretion, GFR normalized to weight, the filtered fraction of sodium, the filtered fraction of potassium, osmolar clearance and free water clearance. In all of these parameters, the baseline values between the control and experimental procedures were not statistically different. Although the infusion of these two different solutions did result in differences between baseline, infusion and post-infusion periods within many of these procedures, only with respect to urine flow and free water clearance was any difference shown between the control and experimental procedures. With respect to both urine flow and free water clearance, the control procedure infusion caused a significantly ($p < 0.05$) greater absolute change from baseline than the experimental procedure infusion. This greater increase in the control procedure was maintained in both parameters during the post-infusion period.

c.) Cardiovascular Effects

The effects of carotid artery infusions of isotonic saline and 677 mM sodium chloride on arterial blood pressure are shown in Fig. 23. In both procedures, the infusions caused a small but significant increase from baseline in arterial pressure that was maintained during the post-infusion period. The absolute increase in arterial blood pressure observed during the experimental procedure infusion period was significantly greater than the increase observed during the control procedure infusion period. The absolute increase in arterial blood pressure observed during the experimental procedure

post-infusion period was also greater than the increase observed during the control procedure post-infusion period.

With respect to right atrial pressure, at no time during either the control or experimental procedures did right atrial pressure change from baseline (Fig. 24). Right atrial pressure was also not statistically different at all points between the control and experimental infusions.

Renal blood flow increased significantly and by the same amount in both the control and experimental procedure infusion periods from baselines that were not statistically different (Fig. 25). Renal blood flow started to decrease in the post-infusion period after both the control and experimental procedure infusions. This decrease was statistically significant only in the experimental procedure but in both the control and experimental procedures, the levels of renal blood flow remained significantly elevated from baseline. There was no difference between the control and experimental procedure post-infusion periods.

Vascular resistance in the kidney was also measured as an indication of cardiovascular function (Fig. 26). Vascular resistance decreased significantly during both the control and experimental procedure infusions. Although vascular resistance began to return to baseline values during the post-infusion periods of both the control and experimental procedures, vascular resistance remained significantly decreased from

baseline in both post-infusion periods and significantly increased from the infusion period only in the experimental procedure. At no time during the protocol was vascular resistance different between the control and experimental procedures.

In summary, the cardiovascular effects of carotid artery infusions of normal saline and 677 mM sodium chloride were examined using the parameters of arterial blood pressure, right atrial pressure, renal blood flow, and vascular resistance. The only difference between the two procedures with respect to these four parameters occurred in the arterial blood pressure which showed a significantly greater absolute increase in response to the 677 mM sodium chloride solution. This larger increase in arterial blood pressure in response to the 677 mM sodium chloride infusion was maintained in the post-infusion period.

d) Endocrine Parameters

The effect of carotid artery infusions of isotonic saline and 677 mM sodium chloride on the concentration of IR-ANP in the plasma is shown in Figure 27. Plasma IR-ANP concentrations did not change from baseline in response to either procedure. There was also no difference at any time point during the protocol between the control and experimental procedures.

The filtered fraction of IR-ANP appeared to increase during both the control and experimental procedure infusion periods but in neither one of the infusion or post-

infusion periods was this apparent increase statistically significant (Fig. 28a). There was also no difference between the control or experimental procedures at any point during the procedure. With respect to the amount of IR-ANP excreted in 15 minutes, both during and after the control procedure infusion there was no significant change from baseline (Fig. 28b). During the experimental procedure infusion, there was also no significant increase in the excreted IR-ANP from baseline although in the post-infusion period, the increase in excreted IR-ANP did achieve statistical significance. At no point during the protocol did the control and experimental values of excreted IR-ANP differ.

The baseline concentration of plasma arginine vasopressin (AVP) was not affected by either the control procedure or the experimental procedure infusion (Fig. 29). In the experimental procedure post-infusion period, the plasma AVP was significantly decreased from the slightly elevated infusion period plasma AVP concentration. At no point during the protocol did the control and experimental values of plasma AVP concentrations differ. The average plasma AVP concentrations of the baseline and post-infusion periods for the control and experimental procedures were 1.33 ± 0.31 and 1.60 ± 0.25 pg/ml respectively. When these values are compared with the infusion period plasma AVP concentrations from the control and experimental procedures (1.70 ± 0.25 and 2.31 ± 0.26 pg/ml respectively) the probability of the values being the same within the control procedure or the experimental procedure equals 0.11 for both procedures. Although these probabilities do not reach statistical significance, it should be noted that the differences in plasma AVP concentrations during the infusion

period are directionally appropriate with respect to the increase in plasma sodium concentration.

In summary, with respect to the four endocrine parameters measured, the plasma AVP and excreted IR-ANP did change during the experimental procedure post-infusion periods from the experimental procedure infusion periods. However, at no time during the protocol did the values of plasma ANP, excreted ANP, the filtered fraction of ANP or plasma AVP differ significantly between the experimental and control procedures.

e.) Validating the Hypertonic Saline Infusion

Figure 30 shows the average plasma sodium concentrations of the left and right ear or carotid arteries. Changes in plasma sodium concentration in response to the intracarotid infusions were not different between the ear and carotid artery samples. Therefore, ear and carotid artery sample results were combined and the sodium concentrations from the ear/carotid arteries were examined as being representative of the sodium concentration in the blood perfusing the brain. There was an increase in the plasma sodium concentration in these arteries during the carotid artery infusions in both the control and experimental procedures. These increases in sodium concentrations were significant ($p<0.05$) when compared with the average plasma sodium levels between the baseline and post-infusion periods. There was no difference in ear/carotid artery plasma sodium concentrations between the control and experimental procedures at any time during the protocol.

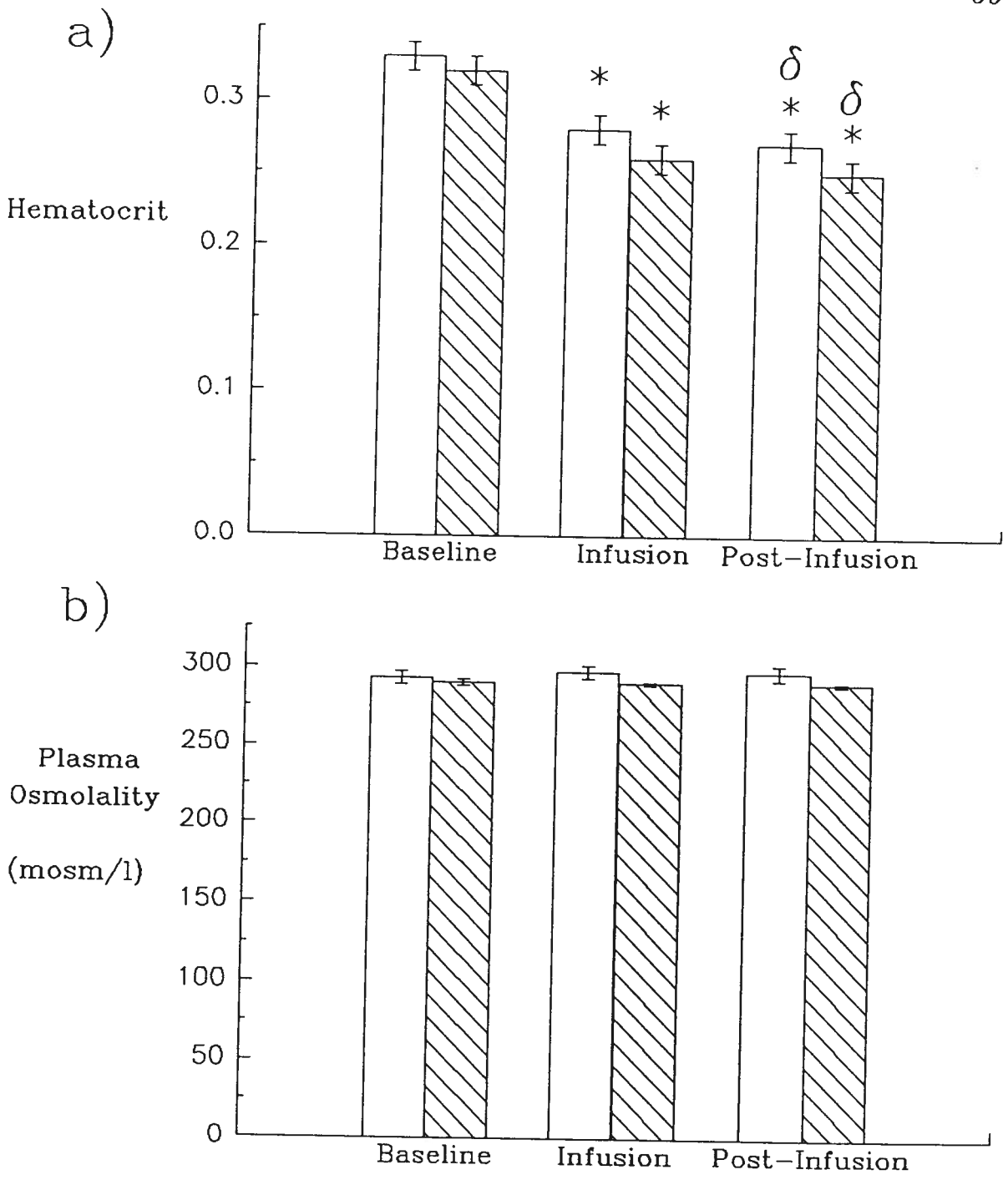

Fig. 15) Effects of Carotid Artery Infusions of Saline (open bars) and 677 mM Sodium Chloride (hatched bars) on Hematocrit Levels and Plasma Osmolality (Fig. a and Fig. b respectively). Each bar represents the average value of the parameter in question over the last 30 minutes of a 45 minute time period. The Baseline, Infusion and Post–Infusion periods represent 3 sequential 45 minute periods.

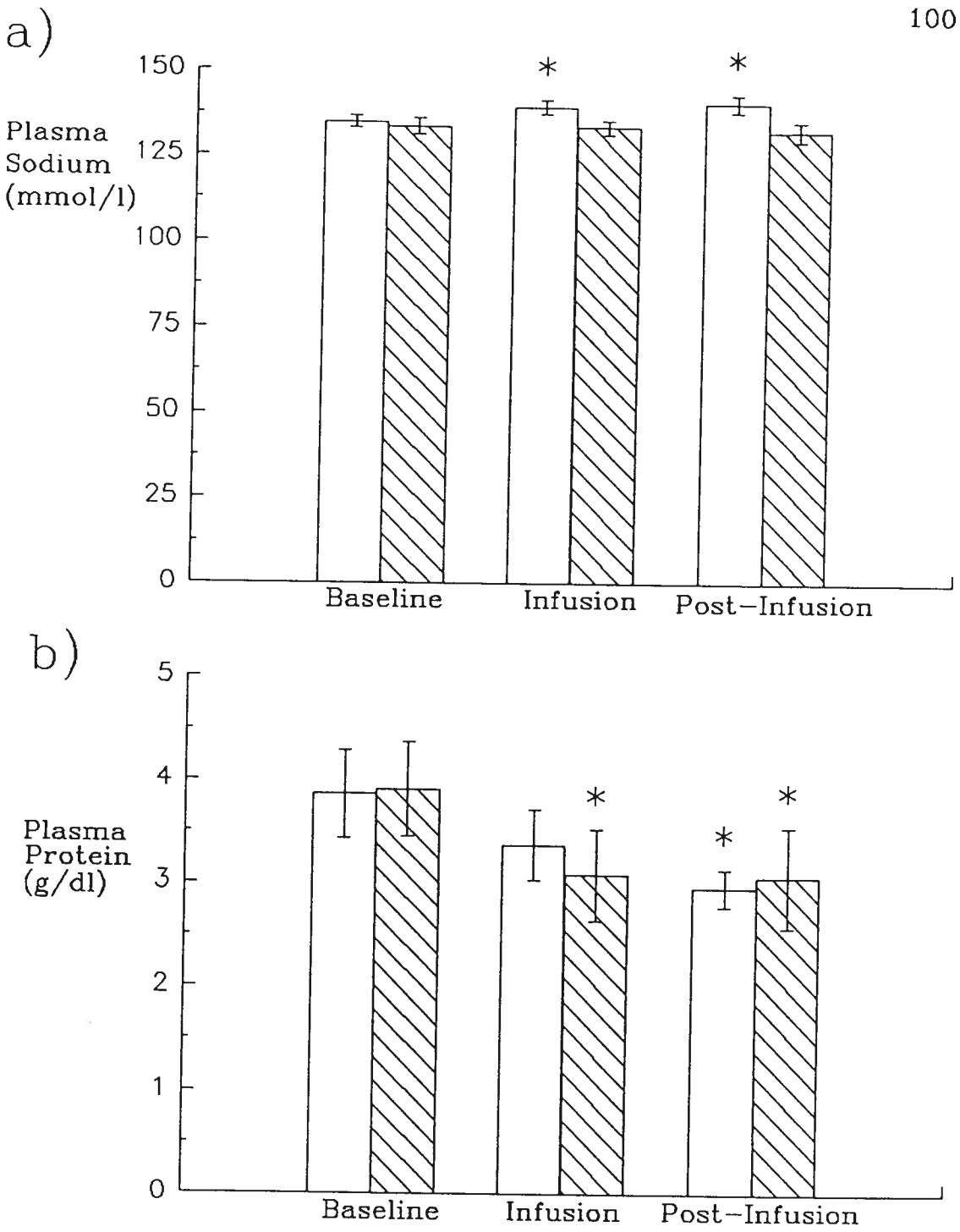

Fig. 16) Effects of Carotid Artery Infusions of Isotonic Saline (open bars) and 677 mM Sodium Chloride (hatched bars) on plasma sodium and plasma protein concentrations (Fig. a and Fig. b respectively). Each bar represents the average value of the parameter in question over the last 30 minutes of a 45 minute time period. Baseline, Infusion and Post-Infusion Periods represent sequential 45 minute periods.

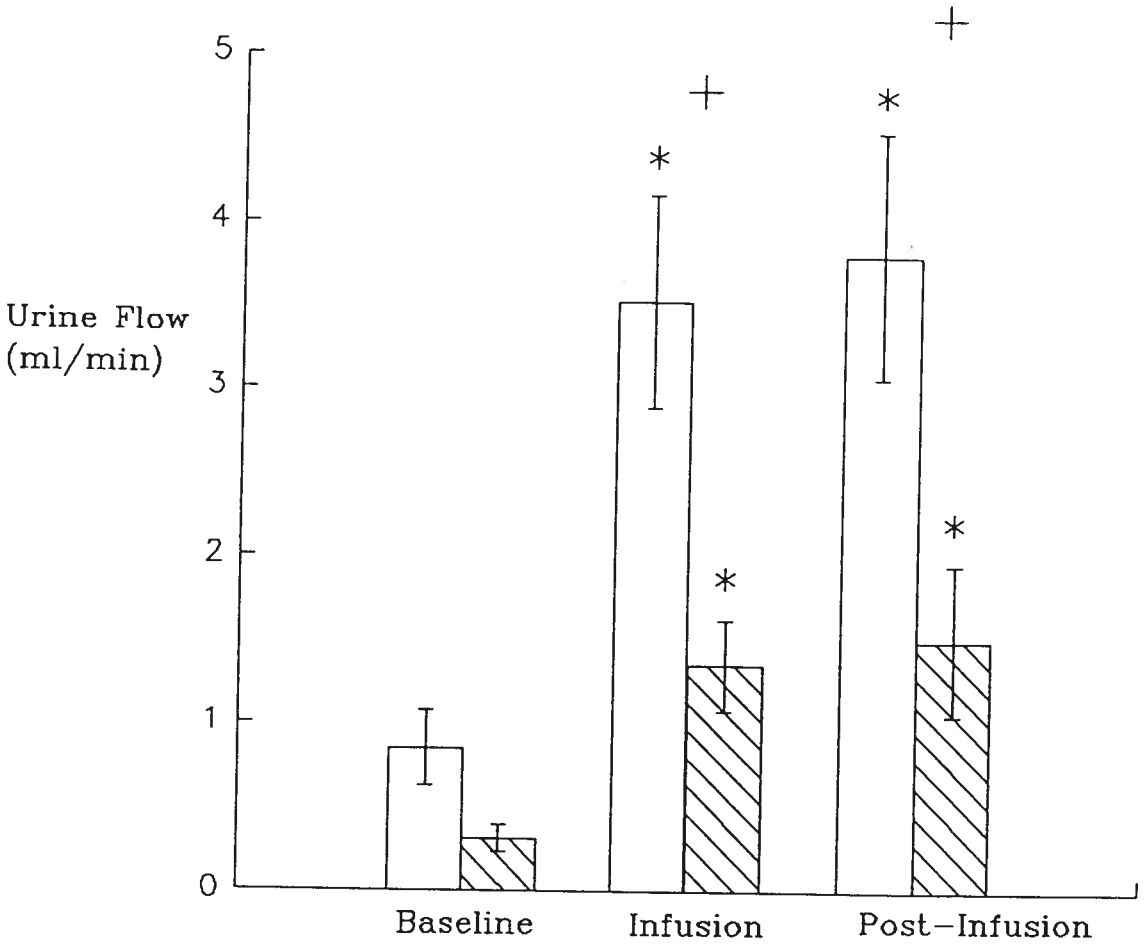

Fig. 17) Changes in Urine Flow resulting from Isotonic Saline Infusion (open bars) and 677 mM Sodium Chloride Infusion (hatched bars) into the carotid arteries. Each bar represents the average urine flow over the last 30 minutes of a 45 minute period. The Baseline, Infusion and Post–Infusion Periods represent 3 sequential 45 minute periods.

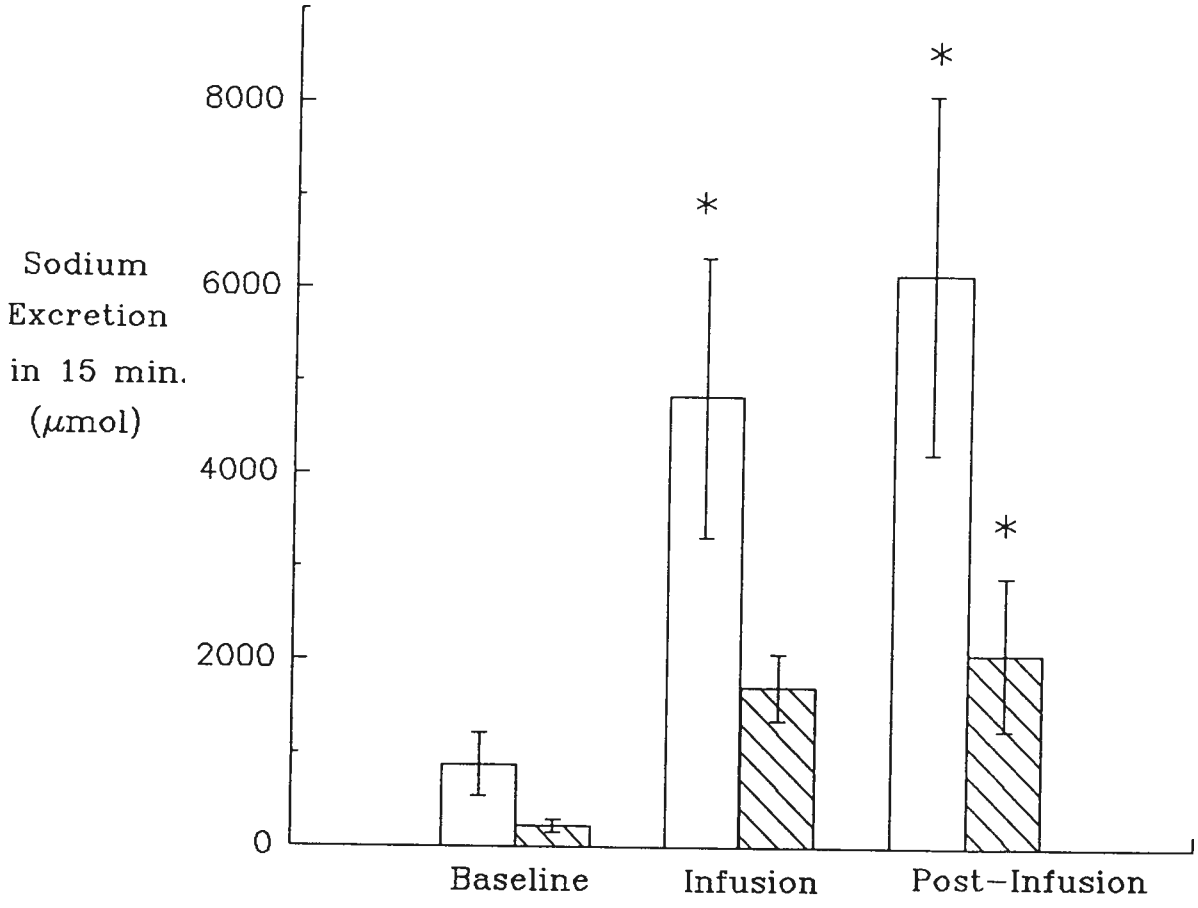

Fig. 18) Effect of Carotid Artery Infusions of Isotonic Saline (open bars) and 677 mM Sodium Chloride (hatched bars) on Sodium Excretion. Each bar represents the average sodium excretion in 15 minutes taken over the last 30 minutes of a 45 minute time period. The Baseline, Infusion and Post-Infusion periods represent 3 sequential 45 minute periods.
(N.B. The Post-Infusion period of 677 mM Sodium Chloride is statistically different from the Baseline according to Fisher's LSD Post Hoc Test.)

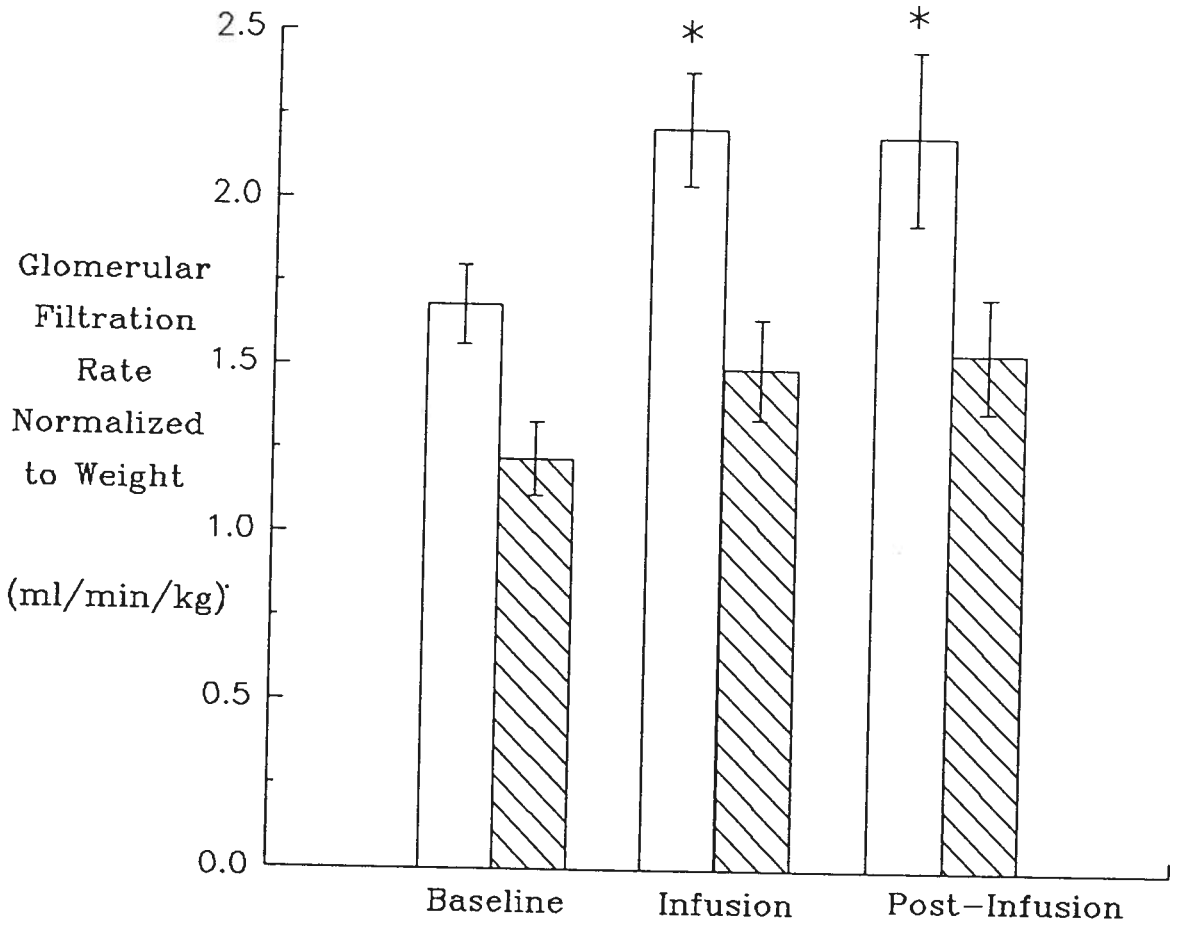

Fig. 19) The effect of Carotid Artery Infusions of Isotonic Saline (open bars) and 677 mM Sodium Chloride (hatched bars on Glomerular Filtration Rate (GFR) normalized to weight. Each bar represents the average GFR per kilogram body weight over the last 30 minutes of a 45 minute time period. The Baseline, Infusion and Post-Infusion periods represent 3 sequential 45 minute periods.

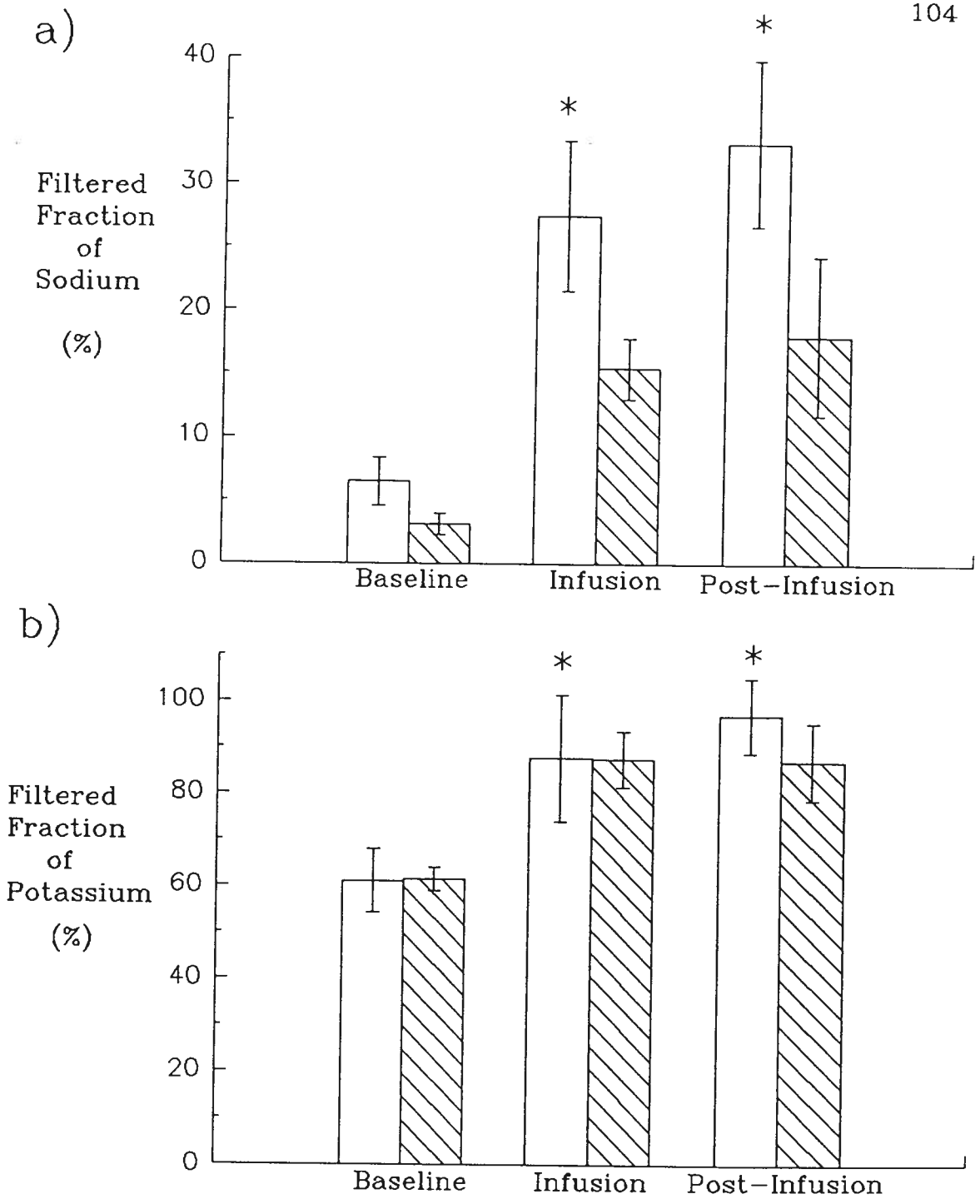

Fig. 20) The Effects of Carotid Artery Infusions of Isotonic Saline (open bars) and 677 mM Sodium Chloride (hatched bars) on the percentage of the filtered load of Sodium (Fig. a) and Potassium (Fig. b) excreted in the urine. Each bar represents the average filtered fraction of Sodium (Fig. a) or Potassium (Fig. b) over the last 30 minutes of a 45 minute time period. The Baseline, Infusion and Post-Infusion periods represent 3 sequential 45 minute periods.

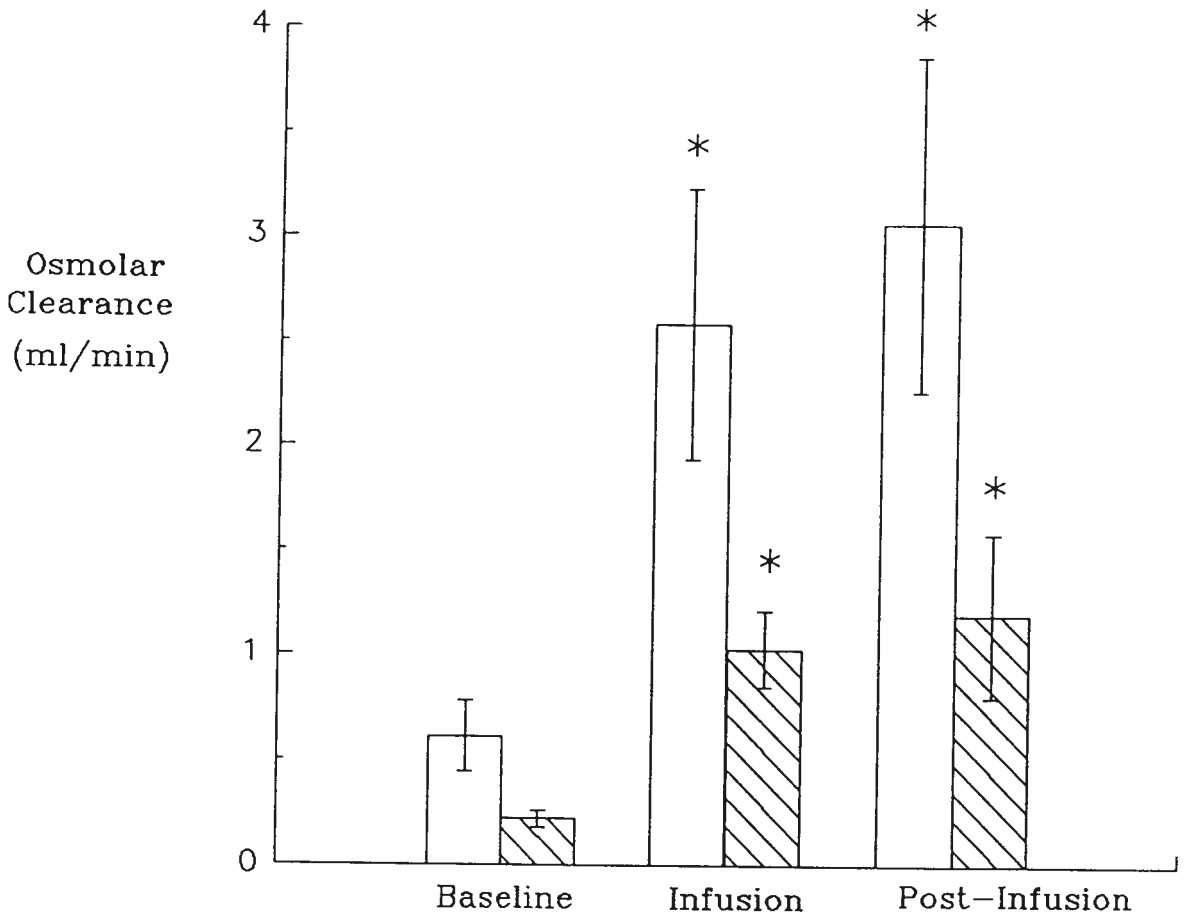

Fig. 21) The effect of Carotid Artery Infusions of Isotonic Saline (open bars) and 677 mM Sodium Chloride (hatched bars) on osmolar clearance. Each bar represents the average osmolar clearance over the last 30 minutes of a 45 minute time period. The Baseline, Infusion and Post-Infusion periods represent 3 sequential 45 minute periods.

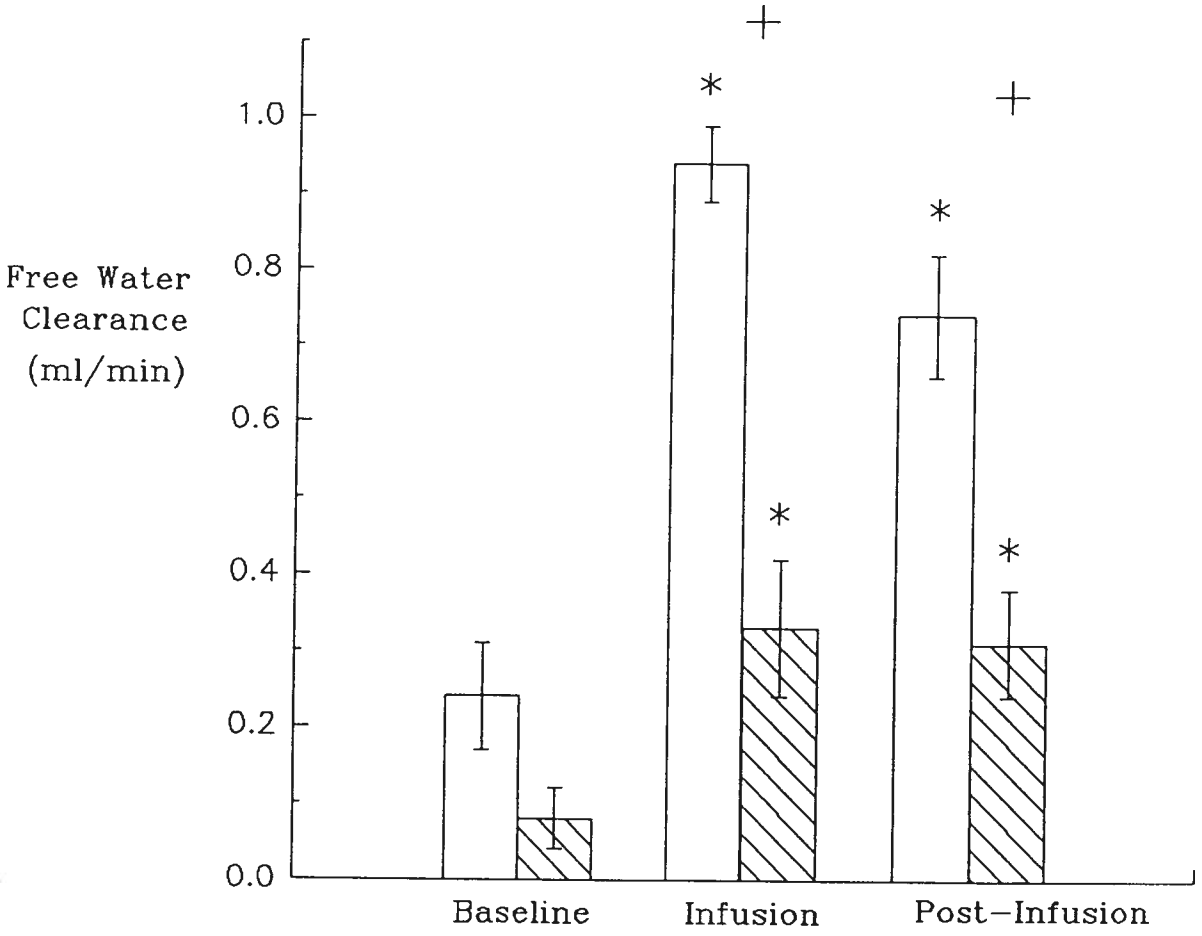

Fig. 22) The effect of Carotid Artery Infusions of Isotonic Saline (open bars) and 677 mM Sodium Chloride (hatched bars) on Free Water Clearance. Each bar represents the average Free Water Clearance over the last 30 minutes of a 45 minute time period. The Baseline, Infusion and Post-Infusion Periods represent 3 sequential 45 minute periods.

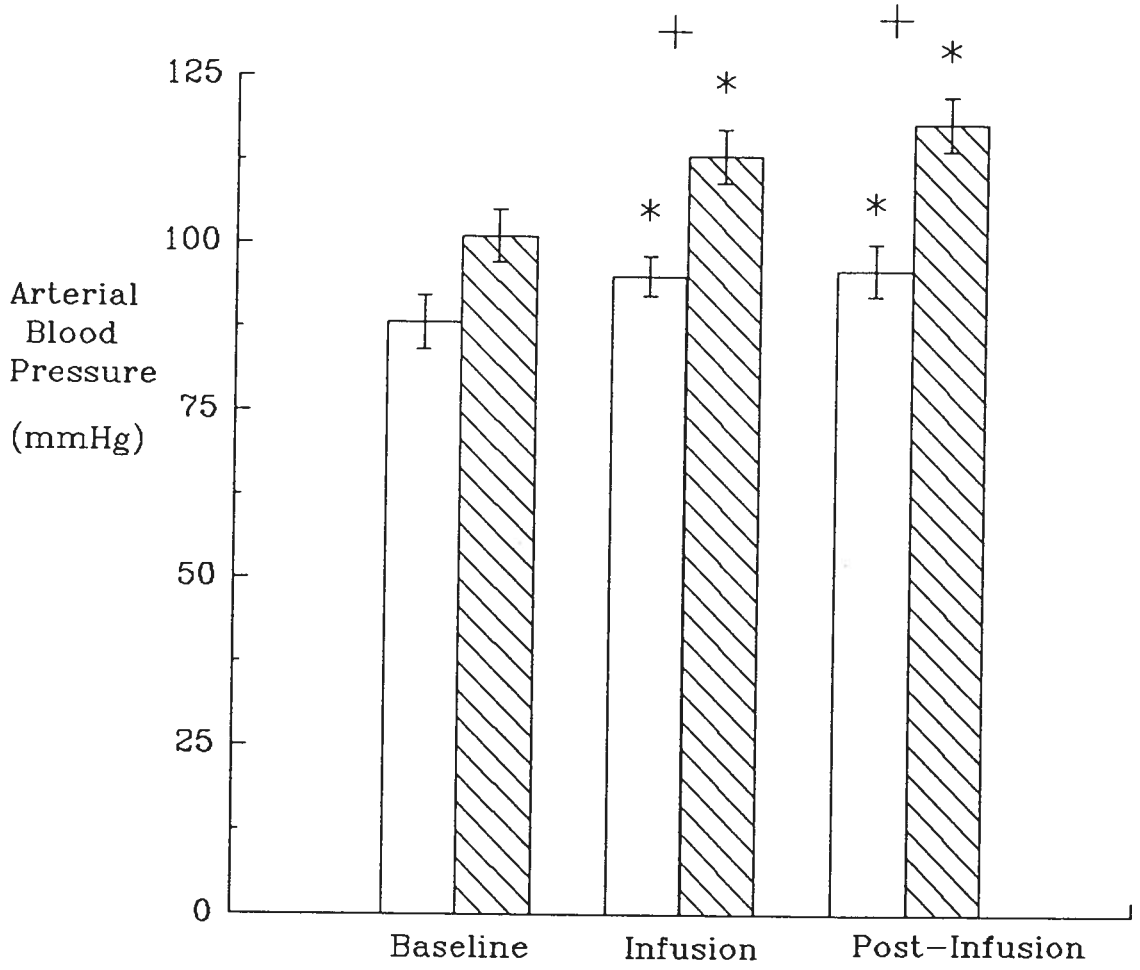

Fig. 23) The Effect of Carotid Artery Infusions of Isotonic Saline (open bars) and 677 mM Sodium Chloride (hatched bars) on Arterial Blood Pressure. Each bar represents the average Arterial Blood Pressure over the last 30 minutes of a 45 minute time period. The Baseline, Infusion and Post–Infusion Periods represent 3 sequential 45 minute periods.

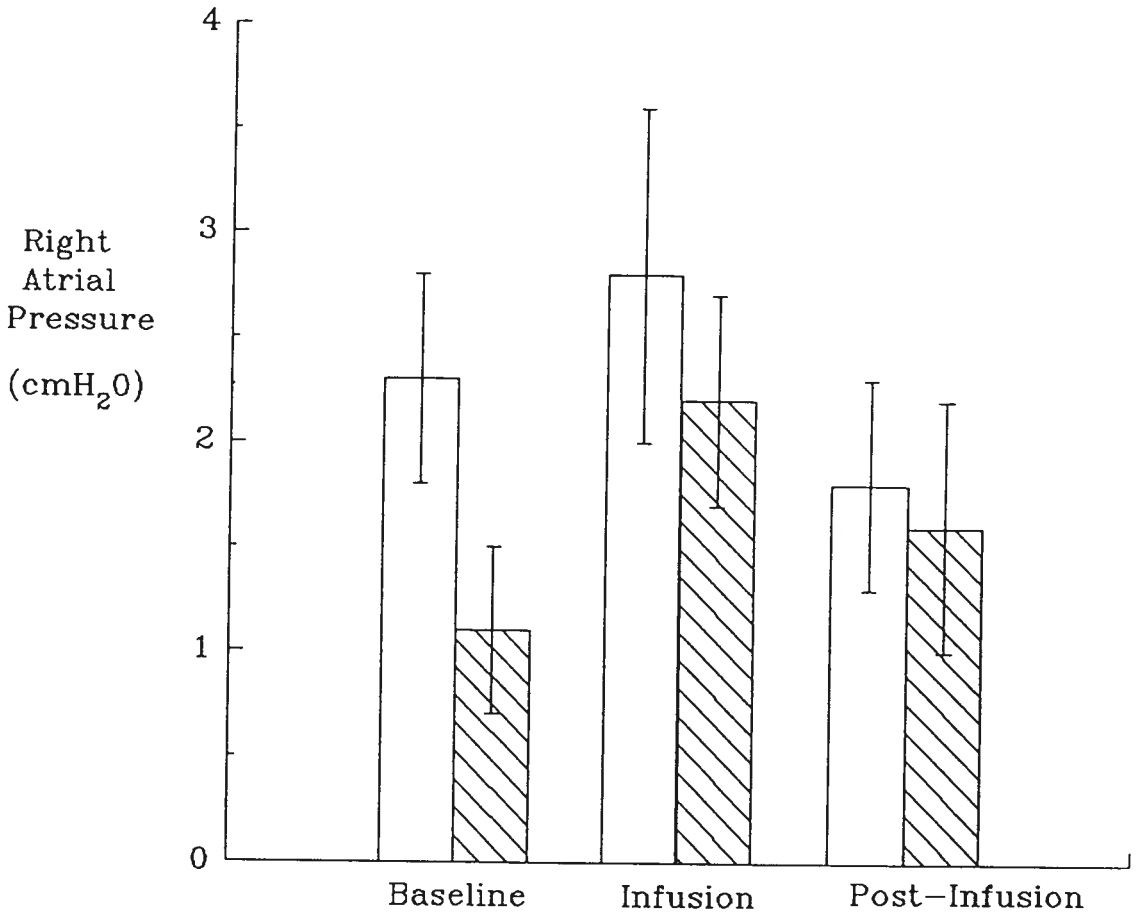

Fig. 24) The effect of Carotid Artery Infusions of Isotonic Saline (open bars) and 677 mM Sodium Chloride (hatched bars) on Right Atrial Pressure. Each bar represents the average Right Atrial Pressure over the last 30 minutes of a 45 minute time period. The Baseline, Infusion and Post-Infusion Periods represent 3 sequential 45 minute periods.

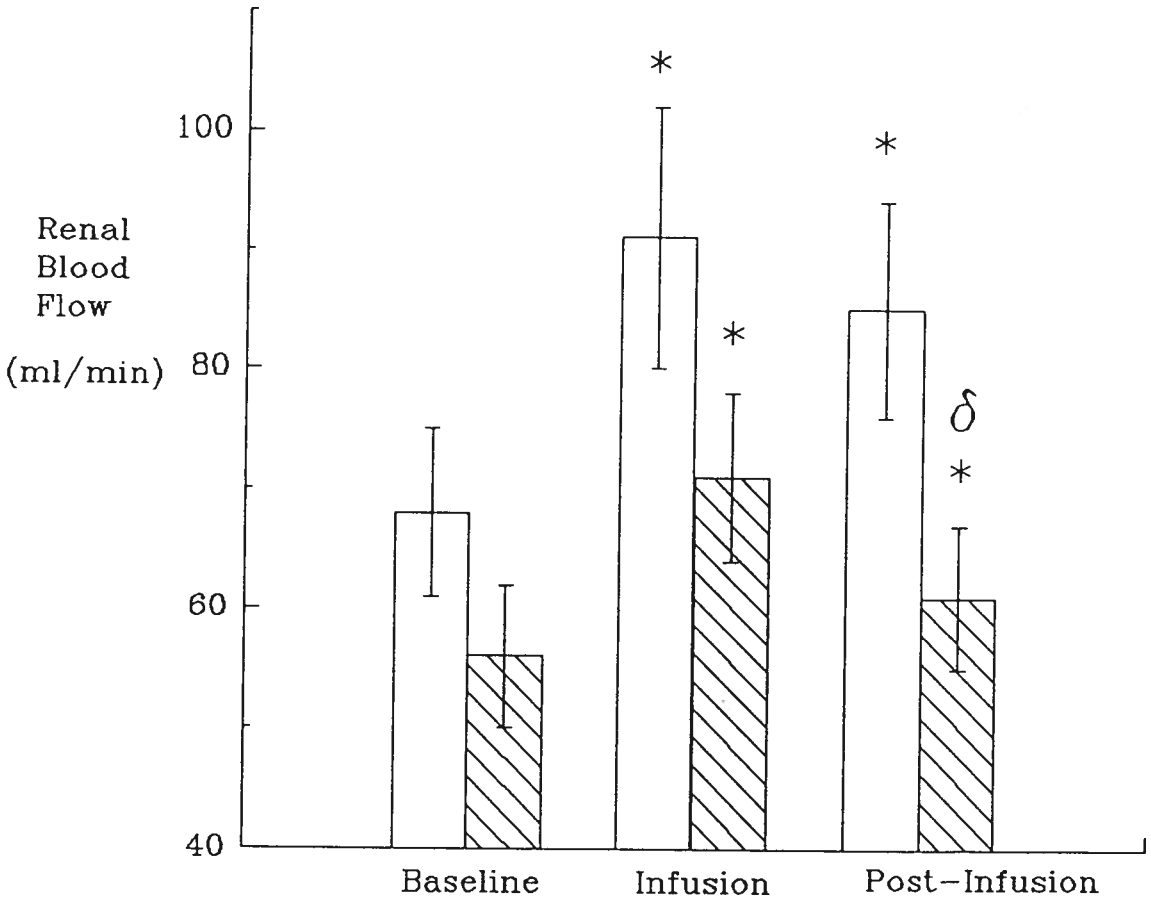

Fig. 25) The Effect of Carotid Artery Infusions of Isotonic Saline (open bars) and 677 mM Sodium Chloride (hatched bars) on Renal Blood Flow. Each bar represents the average Renal Blood Flow over the last 30 minutes of a 45 minute time period. The Baseline, Infusion and Post-Infusion Periods represent 3 sequential 45 minute periods.

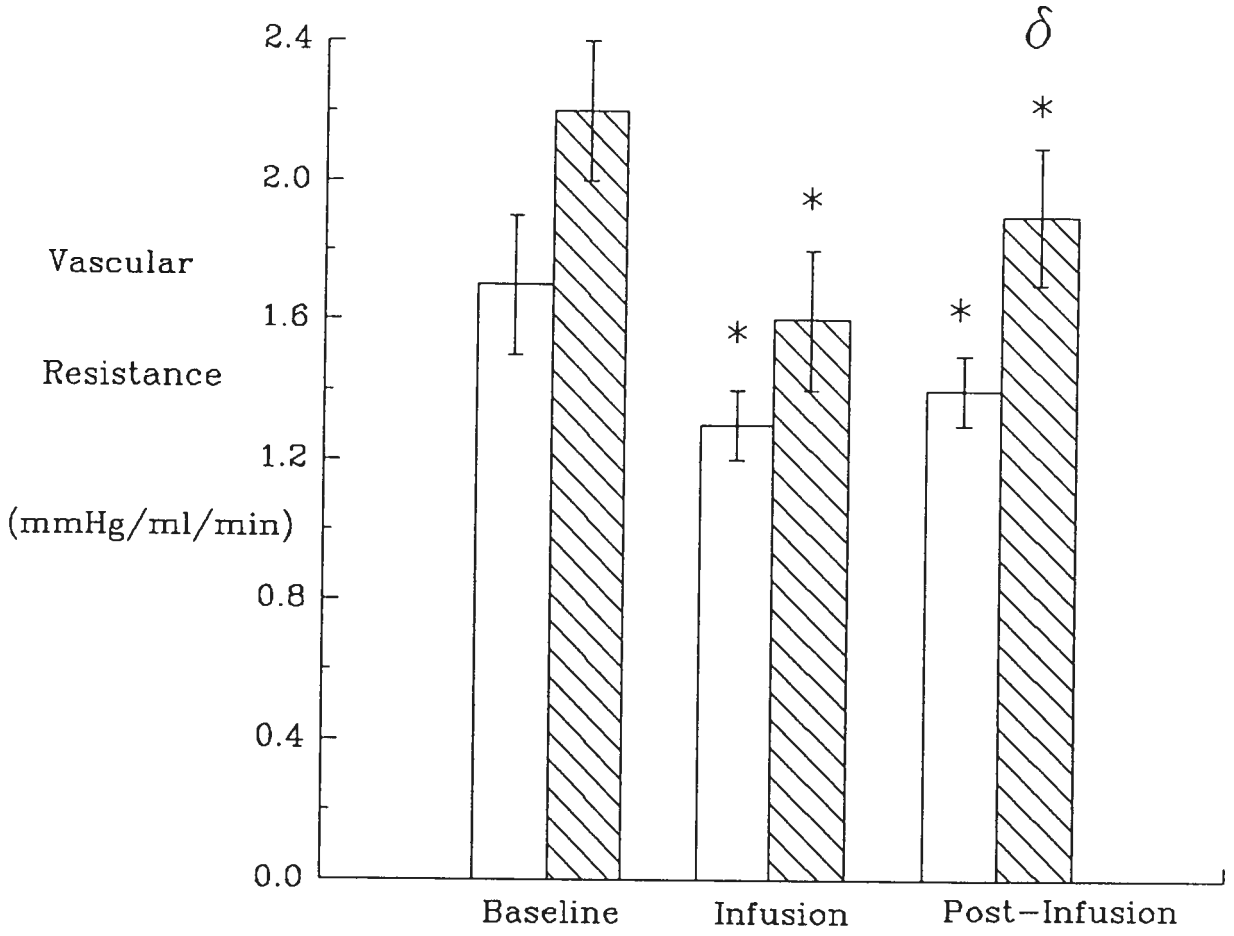

Fig. 26) The effect of Carotid Artery Infusions of Isotonic Saline (open bars) and 677 mM Sodium Chloride (hatched bars) on Vascular Resistance. Each bar represents the average Vascular Resistance over the last 30 minutes of a 45 minute time period. The Baseline, Infusion and Post-Infusion Periods represent 3 sequential 45 minute periods.

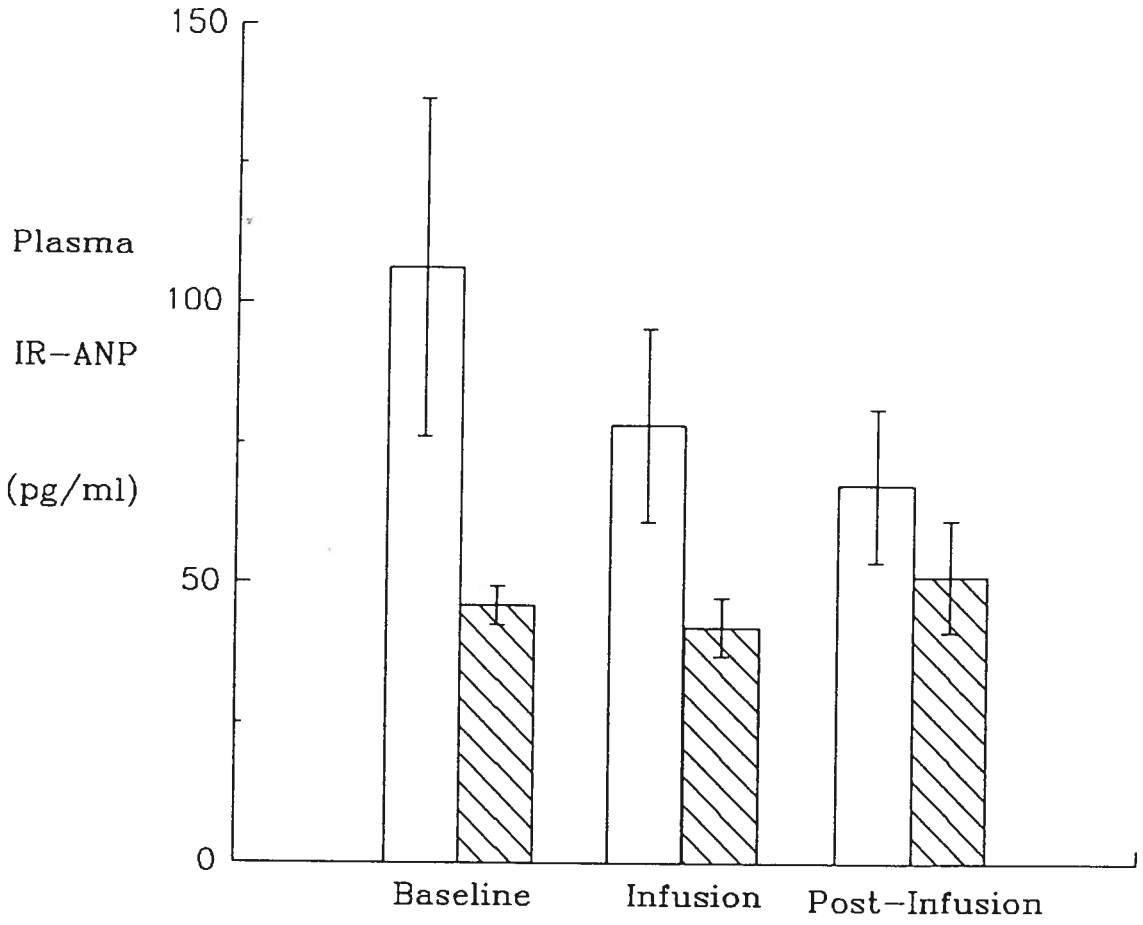

Fig. 27) The Effect of Carotid Artery Infusions of Isotonic Saline (open bars) and 677 mM Sodium Chloride (hatched bars) on the concentration of Immunoreactive ANP (IR-ANP) in the plasma. Each bar reresents the average Plasma IR-ANP concentration measured over the last 30 minutes of a 45 minute time period. The Baseline, Infusion and Post-Infusion Periods represent 3 sequential 45 minute time periods.

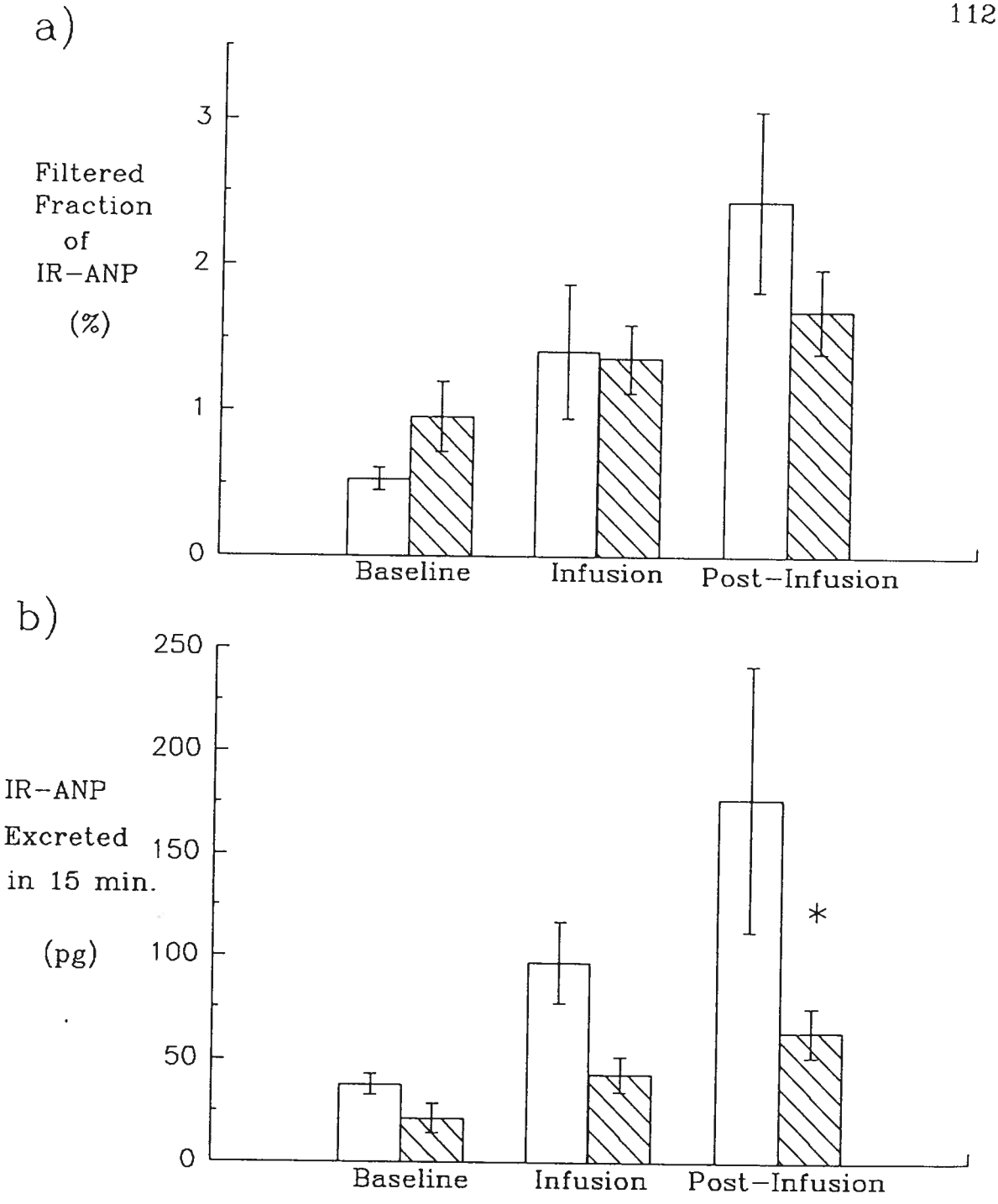

Fig. 28) The Effects of Carotid Artery Infusions of Isotonic Saline (open bars) and 677 mM Sodium Chloride (hatched bars) on the percentage of the filtered load of Immunoreactive-ANP (IR-ANP) excreted in the urine (Fig. a) and on the total amount of IR-ANP excreted in 15 minutes (Fig. b). Each bar represents the average value of the parameter in question over the last 30 minutes of a 45 minute time period. The Baseline, Infusion and Post-Infusion periods represent 3 sequential 45 minute periods.

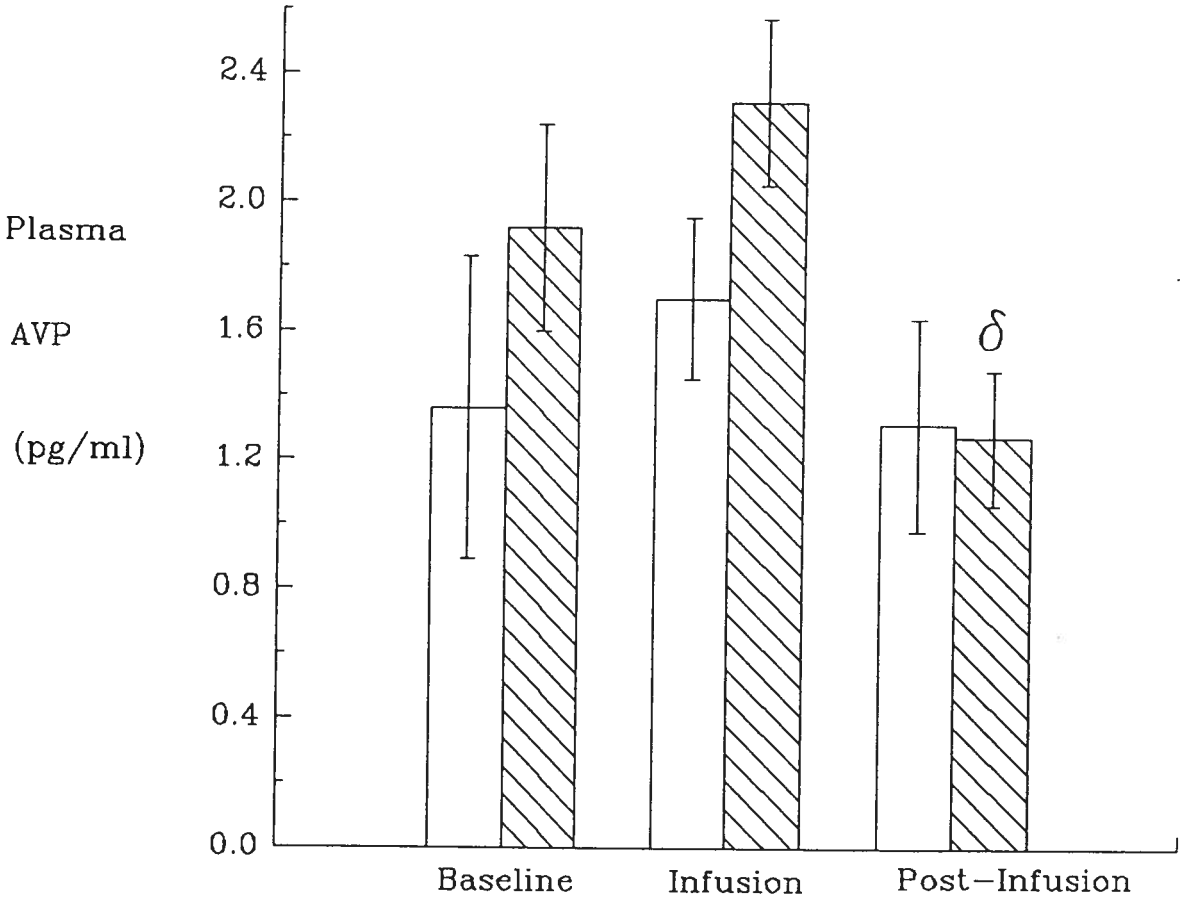

Fig. 29) The effect of Carotid Artery Infusions of Isotonic Saline (open bars) and 677 mM Sodium Chloride (hatched bars) on the concentration of AVP in the Plasma. Each bar represents the average Plasma AVP concentration over the last 30 minutes of a 45 minute time period. The Baseline, Infusion, and Post−Infusion Periods represent 3 sequential 45 minute periods.

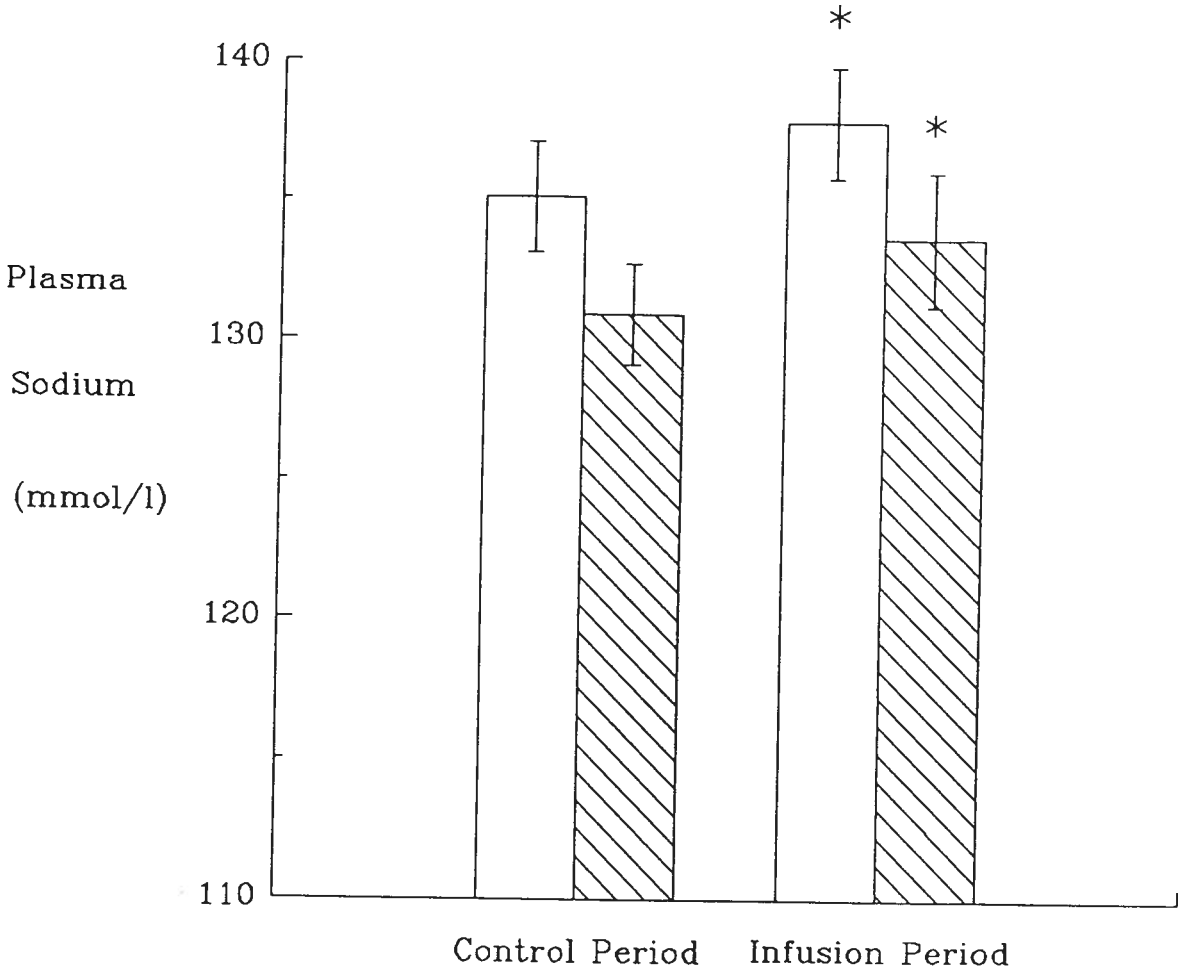

Fig. 30) The effect of Carotid Artery Infusions of Isotonic Saline (open bars) and 677 mM Sodium Chloride (hatched bars) on the concentration of Sodium in the Plasma taken from the Central Ear or Carotid Arteries. Each bar represents the average Plasma Sodium of the left and right arteries taken over the last 30 minutes of a 45 minute time period. The Control Period represents the average of the Baseline and Post-Infusion Periods. The Infusion Period represents the sodium concentrations in the Ear or Carotid Arteries during the carotid artery infusions.

DISCUSSION

Part 1) Protocols 1 & 2 - Validation of the Preparation

a.) State of Hydration of the Animals

In studies that examine the regulation of sodium excretion, it is very important that both the control and experimental preparations start at the same state of hydration. Otherwise, a number of complicating neural and endocrine reflexes affecting sodium excretion may occur in one group of animals and not in the other. It is also essential that the actual preparation used not affect the state of hydration (due to the effects of surgery, prolonged anaesthesia, etc.). As an index of the state of hydration of this preparation, four characteristics of the blood were measured: the hematocrit, plasma osmolality, plasma sodium concentration, and plasma protein concentration. In the conscious rabbit, the normal plasma osmolality is 290 mOsm/kg H_2O, hematocrit is 0.39 ± 0.007 (Dr. Courneya, personal communication), normal plasma sodium concentration is 140 mmol/l and the normal plasma protein concentration is 7.2 g/dl (Kaplan and Timmons, 1979; Kaplan, 1956). The levels of these four parameters were all slightly decreased from normal in this preparation (Fig. 6 and 7). This was expected because the plasma was deliberately diluted with hypotonic saline and hypotonic Kreb's Ringer infusions to increase urine flow. As RPP was changed between 80, 110 and 140 mmHg, none of the four parameters in question were altered (Fig. 6 and 7). This indicates that the rabbits undergoing this procedure, were maintaining consistent and well-hydrated states over the time course of the experiments.

b.) Renal Function

As an index of renal function in this preparation, the volume of urine produced in 15 minutes, the sodium excreted in 15 minutes, GFR/kg body weight, and the filtered fractions of sodium and potassium were measured. The volume of urine produced in 15 minutes increased significantly as RPP was increased from 80 to 110 mmHg and from 110 to 140 mmHg (Fig. 8). This phenomenon of pressure diuresis is expected in a normally functioning kidney (Brenner and Rector, 1991b; Schauf et al, 1990). Concurrent with the increase in urine production, the amount of sodium excreted in the urine increased as RPP was increased from 80 to 110 mmHg and from 110 to 140 mmHg (Fig. 9). This phenomenon of pressure natriuresis is also expected in the normally functioning kidney (Brenner and Rector, 1991b; Schauf et al, 1990) and was reflected in the significant increase in the filtered fraction of sodium seen at increased renal perfusion pressures of both 110 and 140 mmHg (Fig. 11a).

The filtered fraction of sodium seen at a RPP of 80 mmHg was 8.8% (Fig. 11a). In a normally functioning kidney, the filtered fraction of sodium would be expected to be about 1% (Schauf et al, 1990). The amount of sodium excreted in 15 minutes was increasing due to the large volume of urine being produced but the actual urinary concentration of sodium was also increasing as RPP increased (significant only when RPP increased to 140 mmHg). A decrease in plasma protein concentration of 1.0 g/dl has been shown to increase sodium excretion by 0.3 mequiv/min, primarily due to an increase in urinary sodium concentration (O'Connor, 1962). Therefore, this increase in

urinary sodium concentration was thought to be a result of the low levels of plasma protein seen throughout this procedure.

The filtered fraction of potassium did not change as RPP was changed and remained at high levels throughout the protocol (Fig. 11b). This occurred while the actual urinary potassium concentrations significantly decreased as RPP was increased from 80 to 110 mmHg and from 110 to 140 mmHg. GFR and plasma potassium concentrations remained unchanged while urinary potassium concentrations decreased. This indicates that the decrease in urinary potassium concentration was balanced by the increase in urine flow, resulting in a constant level of potassium excretion. A constant level of potassium excretion indicates that the acid-base state of the preparation was not changing. Acidosis leads to a decrease in potassium secretion as potassium is reabsorbed in exchange for excreted hydrogen ions (Schauf et al., 1991). During alkalosis, potassium secretion increases as hydrogen ions are reabsorbed in exchange for potassium (Schauf et al., 1991).

The glomerular filtration rates calculated during these experiments were normalized to body weight because GFR increases with size (Brenner and Rector, 1991b). GFR was not affected by changes in renal perfusion pressure at any point during the protocol. This autoregulation of GFR is expected in a normally functioning kidney and provides further evidence of the validity of this preparation with respect to renal function (Brenner and Rector, 1991b).

c.) Endocrine Parameters

Baseline plasma IR-ANP levels were not affected by changes in RPP (Fig. 12). This lack of change was expected as changes in RPP should not affect atrial stretch and subsequent release of ANP. This was confirmed by the fact that right atrial pressure did not change during this protocol.

The IR-ANP excreted in 15 minutes increased as RPP increased from 80 to 140 mmHg and from 80 to 110 mmHg (Fig. 13b). The filtered fraction of IR-ANP was also significantly increased when the RPP was increased to 140 mmHg from both 80 and 110 mmHg (Fig. 13a). These results indicate that the kidney was excreting increased amounts of IR-ANP as renal perfusion pressure was increased (although the urinary concentration of IR-ANP was not increasing). This IR-ANP could be comprised of immunoreactive degradation products of natriuretic peptides, urodilatin, and/or ANP. Plasma levels of IR-ANP did not change during these experiments, and the animals were well-hydrated to the point of diuresis. It was hypothesized that the increased amount of IR-ANP excreted in the urine was due to the increased excretion of natriuretic peptides, in turn caused by increased flow through the renal tubules. Unfortunately, radioimmunoassays specific for rabbit ANP and urodilatin were not available for these studies. Separation of these two peptides by high performance liquid chromatography is possible although this is a very time-consuming process. It would be interesting in future studies to determine the exact ANP and urodilatin concentrations of the urine during increased RPP to verify that they are not changing.

d.) Renal Nerve Function

In response to clamping the right carotid artery, renal blood flow was significantly decreased (Fig. 14b). This is a baroreceptor mediated neural reflex and demonstrates the presence of intact and functional renal nerves in this preparation. The baroreceptors detected a decrease in arterial pressure and mediated a decrease in renal blood flow to inhibit fluid excretion via the renal nerves. Clamping the carotid artery was also expected to cause a decrease in renal sodium excretion which was not shown (Fig. 14a). This is hypothesized to be due to compensation by other factors including renal autoregulation that are involved in the regulation of sodium excretion. Low plasma protein concentrations could also prevent a decrease in sodium excretion from being observed.

Phenoxybenzamine, an α_1-adrenergic receptor blocker, eliminates tonic sympathetic vasoconstriction of the renal vasculature. This vasodilation was expected to cause an increase in sodium excretion due to hemodynamic effects. This increase in sodium excretion in response to a phenoxybenzamine injection was apparent (Fig. 14a) and further indicated the presence of intact and functional renal nerves in this preparation. Phenoxybenzamine injection was also expected to cause an increase in renal blood flow which was not evident at the time period represented in this figure (Fig. 14b). Peak increases in renal blood flow in response to phenoxybenzamine injection were significant, however renal blood flow quickly returned to lower (not significantly increased) levels possibly due to renal autoregulation.

e.) Summary of Findings from Protocols 1 & 2 - Validation of the Preparation

In summary, a preparation of perfused rabbit kidneys was developed which will be useful in the study of factors that influence sodium excretion. This preparation was set-up without interrupting blood flow to the kidneys and was validated through the examination of a number of renal, endocrine and cardiovascular parameters. The rabbits were shown to maintain a relatively normal state of hydration in this preparation, as well as normal renal function. The renal nerves were also shown to remain intact and functional.

Part 2) Protocol 3 - The Effects of Increasing Sodium Concentration in Blood Perfusing the Brain

a.) State of Hydration of the Animals

In experiments performed using this protocol, differences in the regulation of sodium excretion between two groups of animals were studied. In one group, animals were given a control infusion of normal saline into the carotid arteries and left jugular vein. In the second group, animals were given the same salt and water load as the control group with an intracarotid hypertonic sodium chloride infusion and a left jugular vein infusion of water such that only the brain was exposed to increased plasma sodium levels. In order to ensure that the animals in both groups were receiving the same overall sodium chloride and water load, and that both groups were starting off at the same state of hydration, four characteristics of the blood were measured throughout the protocol.

The plasma osmolalities measured in experiments from this protocol were very close to normal levels of 290 mOsm/kg H_2O and did not change from baseline during the infusion or post-infusion periods of either procedure (Fig. 15b). It was expected that the infusions of hypotonic saline and Kreb's Ringer Solution occurring prior to and during the protocol would dilute the plasma and therefore decrease plasma osmolality. This dilution of the plasma was seen in the hematocrit levels (Fig. 15a), plasma sodium concentrations (Fig. 16a) and plasma protein concentrations (Fig. 16b) which were all at lower than normal levels during the baseline periods of both procedures. The hematocrit levels and plasma protein concentrations both decreased over the course of the protocol in both the experimental and control procedures. This indicates that the carotid artery and jugular vein infusions that occur during the course of the protocol were diluting the plasma as was expected.

The plasma sodium concentrations did not change during the experimental procedure. During the infusion and post-infusion periods of the control procedure, the plasma sodium concentration was significantly elevated from baseline (Fig. 16a). The sum of the infusions administered during the protocol equaled an isotonic saline solution. Plasma sodium levels were therefore not expected to change during the protocol. These animals were starting at low baseline plasma sodium levels and therefore isotonic saline infusions may have caused an increase in plasma sodium concentration due to the fact that isotonic saline had a higher sodium concentration than the plasma. It is hypothesized that this occurred during the control procedure.

Regardless of the changes in hematocrit, plasma osmolality, plasma sodium concentration, and plasma protein concentration that occurred within the different procedures, at no point in the protocol did any of these four parameters vary between the experimental and control procedures (Fig. 15 and Fig. 16). This indicates that the animals in the control and experimental procedures were both starting at the same level of hydration and maintained similar levels of hydration throughout the protocol. Any differences seen between the control and experimental procedures in endocrine, cardiovascular and renal parameters were therefore not the result of different levels of hydration in the two groups.

b.) Effects of the Control and Experimental Procedures on GFR, the Filtered Fractions of Sodium and Potassium and Osmolar Clearance

The GFR, filtered fraction of sodium, filtered fraction of potassium, and osmolar clearance did not differ between the control and experimental procedures in this protocol. Osmolar clearance increased in the infusion periods of both procedures as is expected during a diuresis (Fig.21). The GFR (Fig. 19) and the filtered fraction of sodium (Fig. 20a) both increased during the infusion period of the control procedure and remained significantly elevated from baseline in the post-infusion periods. This was surprising as the kidney is expected to maintain relatively constant levels of GFR (as occurred during the experimental procedure). This increase in GFR may have contributed to the increase in urine flow seen during the control procedure infusion period (Fig. 17).

The filtered fraction of potassium significantly increased from baseline during the infusion and post-infusion periods of the control procedure (Fig. 20b). This was most likely due to the large increase in urine flow that occured during the infusion and post-infusion periods of the control procedure (Fig. 17) which caused a high level of potassium excretion in a 15 minute period. This hypothesis is validated by the significant decrease in urinary potassium concentration that occurred in the control procedure. When compared to the experimental procedure, urinary potassium concentrations were much lower in the infusion and post-infusion periods of the control procedure (significant only during the post-infusion period). These results indicate that the kidneys were minimizing the potassium concentration in the urine to maintain constant levels of potassium excretion in spite of the diuresis that was occurring. This ability to maintain constant levels of potassium excretion became impaired during the control period infusion due to the large diuresis.

c.) Effects of the Control and Experimental Procedures on Renal Blood Flow, and Vascular Resistance

Renal blood flow increased during the infusion period by the same amount in both the control and experimental procedures (Fig. 25). This parameter was then decreased during the post-infusion period (significantly only in the experimental procedure). These changes in renal blood flow were expected in a normally functioning kidney subjected to a saline load where renal perfusion pressure is controlled. These changes in renal blood flow were confirmed by the changes in vascular resistance (Fig.

26). A decrease in the viscosity of the blood (caused by the decrease in hematocrit and dilution of plasma proteins) may have contributed to this increase in renal blood flow. Also, renal nerves likely contributed to this effect secondary to baroreceptor stimulation causing a vasodilation to maintain GFR during the increased saline load. There was no difference between the control and experimental procedures with respect to these two parameters.

d.) Effects of the Control and Experimental Procedures on Plasma IR-ANP, Right Atrial Pressure, IR-ANP Excretion and the Filtered Fraction of IR-ANP

Plasma IR-ANP levels did not change within the control and experimental procedures and did not differ at any period between the control and experimental procedures (Fig. 27). These results were confirmed by a lack of change in right atrial pressure either within or between the two procedures (Fig. 27). The filtered fraction of IR-ANP (Fig. 28a) and the amount of IR-ANP excreted in 15 minutes (Fig. 28b) also did not differ between the control and the experimental procedures. During the post-infusion period of the experimental procedure, the amount of IR-ANP excreted in 15 minutes was significantly increased above baseline. This may be due to increased degradation products of natriuretic peptides in the urine, an increase in ANP or an increase in urodilatin. Unfortunately, it was not possible to distinguish between these peptides due to the lack of availability of radioimmunoassays specific to rabbit ANP and urodilatin. It would be interesting to repeat these experiments and measure the exact concentrations of ANP and urodilatin excreted in the urine. If an increased

concentration of urodilatin was the cause of the increase in the amount of IR-ANP excreted, this would verify Emmeluth's conclusions that urodilatin is likely responsible for the larger natriuresis seen as a result of the intracarotid hypertonic sodium chloride infusion (Emmeluth et al., 1992). It should be noted that differences in urodilatin excretion did not reach statistical significance at any time between the intracarotid hypertonic sodium chloride infusion group and the intracarotid normal saline infusion group in Emmeluth's experiments.

e.) Effects of the Control and Experimental Procedures on Sodium Excretion

In contrast to results shown by Emmeluth et al. (1992), the infusion of hypertonic sodium chloride into the carotid arteries did not cause a significantly larger increase in sodium excretion than that seen due to an isotonic saline infusion. The sodium excretion in 15 minutes did increase significantly during the infusion and post-infusion periods of the control procedure, and during the post-infusion period of the experimental procedure. There are many possible reasons that might account for the differences seen between these studies and those done by Emmeluth. The infusion time in these experiments was 45 minutes compared to the 90 minute infusion time used by Emmeluth. One could argue that if left for a longer period, sodium excretion might increase significantly in the experimental procedure compared to the control procedure. However, even with the shorter infusion period much larger increases in sodium excretion were observed in these experiments than the largest increases seen during Emmeluth's experiments. Furthermore, in Emmeluth's experiments the difference

between the sodium excretion in the control and experimental procedures did not reach statistical significance until 15 minutes after the 90 minute infusion was ended. If the reflex release of urodilatin in response to increased cerebral plasma sodium is more sensitive than the reflex causing the release of AVP (as Emmeluth suggests), one could question why it takes 105 minutes for this reflex to take effect. In contrast, there is a ready releasable pool of AVP at axon terminals in the neurohypophysis and release of AVP from the SON and PVN can occur in less than 30 minutes (Dr. Courneya, personal communication).

The increases in the excretion of sodium were the result of both increases in the volume of urine produced and increases in the actual urinary sodium concentrations. Urinary sodium concentrations were significantly increased in the infusion and post-infusion periods of both the control and experimental procedures. It is hypothesized that the majority of this increase in urinary sodium concentration was caused by a decrease in plasma protein concentration. As was mentioned earlier, a decrease in plasma protein concentration of 1.0 g/dl has been shown to increase sodium excretion by 0.3 mequiv/min, primarily due to an increase in urinary sodium concentration (O'Connor, 1962). Plasma protein concentrations decreased by 0.85 and 0.91 g/dl over the course of the experimental and control procedures respectively. This equilibrates to calculated increases in the amount of sodium excreted in 15 minutes of 3825 and 4095 μmol respectively. The measured increases in sodium excretion were 1850 and 5283 μmol in 15 minutes during the experimental and control procedures. The calculated and

measured changes in sodium excretion are very close when experimental error is taken into account in the measurement of urine volume and plasma protein concentration. In the experiments done by Emmeluth et al. (1992) plasma protein concentration decreased by 0.7 g/dl and 0.6 g/dl in the experimental and control procedures respectively. The similar decreases in plasma protein concentrations would have similar effects on sodium excretion in both the experimental and control procedures of Emmeluth's experiments. Therefore, changes in plasma protein concentration cannot explain the increased sodium excretion seen during the experimental procedure in Emmeluth's experiments. In summary, the increase in sodium excretion seen in the present study can be primarily accounted for as the result of decreases in plasma protein concentration. In contrast to a similar study (Emmeluth et al., 1992) a difference in sodium excretion between the control and the experimental procedures was not observed.

f.) Different Effects of the Control and Experimental Procedures

Many renal, cardiovascular and endocrine parameters were measured in this study. However, only with respect to 4 parameters did significant differences occur between the control and experimental procedures. The control and experimental procedures caused differences in urinary potassium concentrations, arterial blood pressure, urine flow and free water clearance. The baseline periods were not statistically different between the control and experimental groups for all of these parameters. The differences in urinary potassium concentrations are discussed above and support the hypothesis that diuresis is attenuated during the experimental procedure.

Arterial blood pressure increased significantly more during the experimental procedure infusion than during the control procedure infusion (Fig. 23). This increased arterial pressure was maintained in the post-infusion period. The increase in arterial pressure that occurred in these experiments was primarily due to the large volume of fluid being infused. It is unlikely that this saline load would cause an increase in AVP sufficient to directly increase blood pressure through vasoconstriction. However, if AVP was released in response to the experimental procedure, water retention might result causing a further increase in blood pressure. The larger absolute increase in blood pressure that occurred during the experimental procedure therefore supports the hypothesis that AVP was being released during the experimental procedure although the small increases in plasma AVP concentrations that were shown were unlikely to be directly responsible for the increase in pressure.

Emmeluth et al. (1992) did not demonstrate a significant difference between the increase in arterial pressure seen in response to the control and experimental procedures. One of the fundamental differences between the experiments described here and those performed by Emmeluth is that in these experiments RPP was maintained at 110 mmHg. In contrast, RPP was permitted to fluctuate with arterial blood pressure in Emmeluth's experiments. It is possible that the larger increase in arterial blood pressure during the experimental procedure in these experiments was due to the fact that RPP was maintained constant. If these experiments were repeated without controlling RPP and sodium excretion was shown to be significantly increased

during the experimental procedure, this would indicate that an increase in RPP is responsible for the increased sodium excretion seen by Emmeluth et al. (1992) in response to the experimental procedure.

The control procedure infusions caused a larger absolute increase in urine flow (Fig. 17) and free water clearance (Fig. 22) than occurred in response to the infusions during the experimental procedure. The increased urine flow and free water clearance were maintained in the post-infusion period of the protocol. Both groups of animals were exposed to the same volume load as was verified by examining a number of parameters indicating the state of hydration of the animals. Therefore, an equal diuresis should have occurred in the two groups of animals. If AVP was released in response to the intracarotid hypertonic sodium chloride infusion (experimental procedure), AVP would be expected to attenuate the diuresis, resulting in a smaller increase in both urine flow and free water clearance. The differences in urine flow and free water clearance that occur between the control and experimental procedures therefore strongly support the theory that AVP was being released in response to the intracarotid hypertonic sodium chloride infusion (experimental procedure).

Emmeluth et al. (1992) did not demonstrate a difference in urine volume or free water clearance between similar control and experimental procedures. Due to the comparable increases in urine volume and free water clearance in both groups, Emmeluth concluded that AVP was not released in response to these infusions.

However, plasma levels of AVP were not measured in these experiments.

g.) Vasopressin Levels in the Control and Experimental Procedures

The hypotonic infusions of saline and Krebs Ringer Solution were administered in the present study to cause a diuresis and suppress plasma AVP levels. In response to increased plasma sodium levels in carotid blood, an increase in plasma AVP levels and a subsequent attenuation of the diuresis was expected. The change in plasma AVP concentration that occurred during the experimental procedure infusion period was directionally appropriate although not significantly different from the baseline value (Fig. 29). There was a significant drop in plasma AVP concentrations during the experimental procedure post-infusion period from the slightly elevated infusion period levels. This decrease in plasma AVP concentration would be expected after the intracarotid hypertonic sodium chloride infusion (which was supposedly causing AVP release) was terminated. During the isotonic infusions of the control procedure, a change in plasma AVP concentration was not expected as there was no osmotic (or sodium) stimulus for AVP release. There were no significant differences in plasma AVP concentrations within the control procedure (Fig. 29).

There were no differences in plasma AVP concentrations between the control and experimental procedures in this study at any time during the protocol. However, when the baseline and post-infusion values of the control and experimental procedures were averaged and compared to the infusion period plasma AVP levels, the p values were

both very small ($p<0.10$). This indicates that there was a 90% chance that these levels were different (i.e. that AVP is in fact increasing during the infusion period in both the control and experimental procedures). It is possible that if these infusions were maintained for a longer period of time and if more experiments were done that a significant increase in AVP excretion would occur. Furthermore, the fact that there is a 90% chance that AVP levels increased during both the control and experimental infusion periods was supported by the fact that carotid artery plasma sodium levels increased by the same amount in both procedures.

h.) Validating the Sodium Infusion

Emmeluth et al. (1992) calculated that a 677 mmol/l sodium chloride infusion bilaterally into the carotid arteries @ 0.2 ml/min/artery would increase carotid artery plasma sodium concentrations by 3 mmol/l in the dog. This concentration of sodium chloride was calculated assuming that carotid artery blood flow was 8.0 ml/min/kg/artery. These calculations were verified and this infusion rate and saline concentration were used in the present study. The carotid artery blood flow in the anaesthetized rabbit was later determined to be 10 ml/min/kg/artery. A larger volume of blood was therefore diluting the carotid artery infusions. Therefore, in these experiments, a smaller increase in carotid artery plasma sodium concentration was expected in response to the experimental procedure infusion than was calculated by Emmeluth et al. (1992).

In order to determine the exact increase in plasma sodium concentrations during the experimental procedure infusion, blood was sampled from the ear or carotid arteries as being representative of the plasma sodium concentration in the blood perfusing the brain. When compared to the average of baseline and post-infusion plasma sodium concentrations, in both the experimental and control infusions, the plasma sodium concentration in the carotid arteries was shown to significantly increase. The plasma sodium concentrations increased by 2.7 mmol/l in both the experimental and control infusion periods. The increase in cerebral plasma sodium concentrations in both the control and experimental procedure infusion periods was likely due to the fact that these animals have low levels of plasma sodium at the beginning of the protocol. Therefore, an infusion of 154 mmol/l (isotonic) sodium chloride is really hypernatremic when compared to the actual circulating plasma. In future studies, "isotonic" infusions should be verified to ensure that they are in fact isotonic with each rabbit's plasma during the protocol. It would also be interesting to repeat these experiments using a higher concentration of hypertonic saline in the experimental procedure infusion. When examining changes in plasma sodium concentration of only 3 mmol/l, the ability of the instruments used to accurately measure these small differences becomes questionable. Changes in plasma sodium concentrations of 5 mmol/l could be confirmed with greater confidence.

CONCLUDING COMMENTS

We have developed a preparation of perfused rabbit kidneys that was set-up without interrupting renal blood flow. The rabbits maintained a normal state of hydration in this procedure, and normal renal function. The renal nerves were also shown to remain intact and functional in this preparation. This preparation is therefore valid and can be used in future experiments to study the regulation of sodium excretion by the body as well as to separate neural and hormonal effectors of sodium excretion.

Some of the future experiments that might be done using this preparation include repeating the experiments described in this paper using radioimmunoassays specific to rabbit ANP and urodilatin to determine the exact concentrations of these natriuretic peptides in the excreted urine. This could provide further insight into the efferent mechanisms mediating the natriuresis seen in response to the control and experimental procedure infusions. The kidneys could also be denervated in this preparation to separate neural and endocrine mechanisms involved in the regulation of sodium excretion.

We have used this preparation to demonstrate the cardiovascular, renal and endocrine effects of administering an intracarotid hypertonic sodium chloride infusion concurrent with an intravenous infusion of water at rates such that the sum of the two infusions is isotonic and only the brain is subject to an increased plasma sodium

concentration (the experimental procedure). These effects were compared to those effects seen due to similar intracarotid and intravenous infusions of isotonic saline (the control procedure). The results of these experiments showed that both groups of animals started the protocol at similar states of hydration. Similar states of hydration were also maintained between the two groups throughout the protocol indicating that the animals in both groups were receiving the same volume and sodium load.

The isotonic and hypertonic infusions into the carotid arteries with the same total water and salt load differed with respect to urinary potassium concentration, arterial pressure, free water clearance and urine volume. All of these differences are compatible and were in accord with an increased secretion of AVP during the hypertonic infusion. Although changes in AVP levels were directionally appropriate when compared to the measured increases in carotid plasma sodium levels, they did not differ between the experimental and control infusions. However, it is expected that with a slightly longer infusion time or an increase in the sodium concentration of the hypertonic saline infusion, that the increase in AVP secretion would become significant. These changes combined with the use of infusions with the same sodium concentration as the plasma of the rabbit during the control procedure would be expected to demonstrate a significantly larger increase in AVP secretion during the experimental procedure infusion than during the control procedure infusion.

An increase in sodium excretion occurred in response to both isotonic and

hypertonic sodium chloride infusions into the carotid arteries with the same total water and salt load. The increase in sodium excretion was concluded to be primarily caused by the decrease in plasma protein concentration that occurred in these experiments. Changes in renal vascular resistance that accompanied the changes in sodium excretion indicate that increases in sodium excretion in both procedures may also have been due to changes in renal nerve activity. This could be verified by repeating these experiments with the renal nerves cut. The intracarotid infusion of hypertonic saline did not cause a greater increase in sodium excretion than the intracarotid infusion of isotonic saline. These results therefore do not support the presence of a mechanism in the rabbit that regulates sodium excretion in the distribution of the carotid arteries and that acts independently of RPP.

In a study done by Emmeluth et al. (1992), similar intracarotid infusions of hypertonic and isotonic sodium chloride with the same total water and salt load were made in conscious dogs. Emmeluth observed a significantly greater increase in sodium excretion in the dogs given the hypertonic infusion. This occurred concurrent with an increase in the urinary excretion of urodilatin and without any observed attenuation of free water clearance. In contrast, we did not demonstrate a larger increase in natriuresis in response to an increase in carotid artery plasma sodium concentration. Increases in carotid artery plasma sodium concentration in response to the intracarotid hypertonic and isotonic sodium chloride infusions in our experiments were similar. Emmeluth further concluded that the larger increase in urinary sodium excretion due to the

hypertonic sodium chloride infusion was likely caused by urodilatin and that this effector reflex was stimulated due to changes in plasma osmolality insufficient to cause AVP release. In contrast, in the perfused rabbit kidney preparation that we have designed, we did not show that sodium excretion is regulated in response to a smaller osmotic change than that which causes the excretion of AVP. Our results indicate that AVP and sodium excretion increase in response to the same degree of osmotic change.

In summary, our results do not support the concept of a specific sodium receptor in the distribution of the carotid arteries of the rabbit. An intracarotid infusion of hypertonic saline did not cause a greater increase in sodium excretion than an intracarotid infusion of isotonic saline. An intracarotid infusion of hypertonic saline also did not cause a greater decrease in renal vascular resistance than an intracarotid infusion of isotonic saline. Finally, sodium excretion did not increase in response to a smaller osmotic stimulus than that which causes the release of AVP.

REFERENCES

1.) Andersson, B., L. Eriksson, O. Fernandez, and C.G. Kolmodin and R. Oltner. Centrally Mediated Effects of Sodium and Angiotensin II on Arterial Blood Pressure and Fluid Balance. Acta Physiologica Scandinavica. 85, p. 398-407, 1972.

2.) Andersson, B. Regulation of Body Fluids. Annual Review of Physiology. 39, p. 185-200, 1977.

3.) Awazu, M., and I. Ichikawa. Biological Significance of Atrial Natriuretic Peptide in the Kidney. Nephron. 63. p. 1-14, 1993.

4.) Bie, P. Studies of Cerebral Osmoreceptors in Anaesthetized Dogs: The Effect of Intravenous and Intracarotid Infusion of Hyper-Osmolar Sodium Chloride Solutions during Sustained Water Diuresis. Acta Physiologica Scandinavica. 96, p. 306-318, 1976.

5.) Bie, P. Quantitative Aspects of Renal Actions of Vasopressin. Acta Physiologica Scandinavica. 139 (S. 591), p. 28-32, 1990.

6.) Brenner, B.M. and F.C. Rector. Control of Extracellular Fluid Volume. In: The Kidney (Fourth Edition), Vol. 1. W.B. Saunders Co., Toronto, p. 623-643, 1991a.

7.) Brenner, B.M. and F.C. Rector. The Kidney (Fourth edition), Vol. 1. W.B. Saunders Co., Toronto, p. 245-282, 424-444, 510-583, 1991b.

8.) Bricker, N.S., R.A. Straffon, E.P. Mahoney, and J.P. Merrill. The Functional Capacity of the Kidney Denervated by Autotransplantation in the Dog. 1957.

9.) Chen, C.J., and M.F. Lokhandwala. Role of Endogenous Dopamine in the Natriuretic Response to Various Degrees of Iso-Osmotic Volume Expansion in Rats. Clinical and Experimental Hypertension. - Theory and Practice. 13, p. 1117-1126, 1991.

10.) Chiu, P.J., G. Tetzloff, M.T. Romano, C.J. Foster, and E.J. Sybertz. Influence of C-ANF Receptor and Neutral Endopeptidase on Pharmacokinetics of ANF in Rats. American Journal of Physiology. 260, p. R208-R216, 1991.

11.) Chiu, P.J.S, and W.H. Sawyer. Third Ventricular Injection of Hypertonic Sodium Chloride and Natriuresis in Cats. American Journal of Physiology. 226, p. 463-469, 1974.

12.) Choi-Kwon, S., and A.J. Baertschi. Splanchnic Osmosensation and Vasopressin: Mechanisms and Neural Pathways. American Journal of Physiology. 261, p. E18-E25, 1991.

13.) Colindres, R.E., W.S. Spielman, N.G. Moss, W.W. Harrington, and C.W. Gottschalk. Functional Evidence for Renorenal Reflexes in the Rat. American Journal of Physiology. 239, p. F265-F270, 1980.

14.) Courneya, C.A., A.J. Rankin, N. Wilson, and J.R. Ledsome. Carotid Sinus Pressure and Plasma Vasopressin in Anaesthetized Rabbits. American Journal of Physiology. 255, p. H1199-H1205, 1988.

15.) Courneya, C.A., N. Wilson, and J.R. Ledsome. Carotid Sinus Pressure, Blood Volume and Vasopressin in the Anaesthetized Rabbit. Canadian Journal of Physiology and Pharmacology. 67, p. 1386-1390, 1989.

16.) Courneya, C.A., P.I. Korner, J.R. Oliver, and R.L. Woods. Afferent Control of Vasopressin and Renin Release During Haemorrhage in Normal and Autonomically Blocked Rabbits. Clinical and Experimental Physiology and Pharmacology. 19, p. 745-759, 1992.

17.) Cowley, A.W. Vasopressin and Cardiovascular Regulation. In: Cardiovascular Physiology IV, edited by Guyton, A.C. and Hall, J.E. p. 189-240, Baltimore, University Park Press, 1982.

18.) Cowley A.W. Jr., and M.M. Skelton. Dominance of Colloid Osmotic Pressure in Renal Excretion after Isotonic Volume Expansion. American Journal of Physiology. 261, p. H1214-H1225, 1991.

19.) DiBona, G.F. Role of Renal Nerves in Volume Homeostasis. Acta Physiologica Scandinavica. 139 (Suppl. 591), p. 18-27, 1990.

20.) Doumas, B.T., D.D. Bayse, and R.J. Carter. A Candidate Reference Method for Determination of Total Protein in Serum. Clinical Chemistry. 27, p. 1642, 1981.

21.) Drummer, C., F. Fiedler, A. Konig, and R. Gerzer. Urodilatin, a Kidney-Derived Natriuretic Factor, is Excreted with a Circadian Rhythm and is Stimulated by Saline Infusion in Man. Journal of the American Society of Nephrology. 1, p. 1109-1113, 1991.

22.) Drummer, C., R. Gerzer, M. Heer, B. Molz, P. Bie, M. Schlossberger, C. Stadaegher, L. Rocker, F. Strollo, B. Heryduck, K. Bauer, J. Warberg, F. Baisch, N.J. Christensen, A Konig, and P. Norsk. Effects of an Acute Saline Infusion on Fluid and Electrolyte Metabolism in Humans. American Journal of Physiology. 262, p. F744-F754, 1992.

23.) Emmeluth, C., C. Drummer, R. Gerzer, and Peter Bie. Roles of Cephalic Sodium Concentration and Urodilatin in Control of Renal Sodium Excretion. American Journal of Physiology. 262, p. F513-F516, 1992.

24.) Erdos, E.G., and R.A. Skidgel. Neutral Endopeptidase 24.11 (enkephalinase) and Related Regulators of Peptide Hormones. FASEB Journal. 3, p. 145-151, 1989.

25.) Eriksson, L., O. Fernandez, and K. Olsson. Differences in the Antidiuretic Response to Intracarotid Infusions of Various Hypertonic Solutions in the Conscious Goat. Acta Physiologica Scandinavica. 83, p. 554-562, 1971.

26.) Feller, S.M., M. Gagelman, and W.C. Forssmann. Urodilatin: A Newly Described Member of the ANP Family. Trends in Pharmaceutical Sciences. 10, p. 93-94, 1989.

27.) Gagelmann, M., D. Hock, and W. Forssmann. Urodilatin is not Biologically Inactivated by a Peptidase from Dog Kidney Cortex Membranes in Contrast to Atrial Natriuretic Peptides. FEBS Letters. 233, p. 249-254, 1988.

28.) Goetz, K.L., G.C. Bond, and D.D. Bloxham. Atrial Receptors and Renal Function. Physiology Reviews. 55, p. 157-205, 1975.

29.) Goetz, K.L. Physiology and Pathophysiology of Atrial Peptides. American Journal of Physiology. 254, p. E1-E14, 1988.

30.) Goetz, K.L. Evidence that Atriopeptin is Not a Physiological Regulator of Sodium Excretion. Hypertension. 15, p. 9-19, 1990a.

31.) Goetz, K.L. The Tenuous Relationship between Atriopeptin and Sodium Excretion. Acta Physiologica Scandinavica. 139 (S. 591), p. 88-96, 1990b.

32.) Goetz, K.L., C. Drummer, J.L. Zhu, R. Leadley, F. Fiedler, and R. Gerzer. Evidence that Urodilatin, Rather than ANP, Regulates Renal Sodium Excretion. Journal of the American Society of Nephology. 1, p. 867-874, 1990.

33.) Goetz, K.L. Renal Natriuretic Peptide (urodilatin?) and atriopeptin: Evolving Concepts. American Journal of Physiology. 261, p. F921-F932, 1991.

34.) Goto, A., K. Yamada, and T. Sugimoto. Minireview Endogenous Digitalis: Reality or Myth? Life Sciences. 48, p. 2109-2118, 1991.

35.) Granger, J.P. Pressure Natriuresis. Role of Renal Interstitial Hydrostatic Pressure. Hypertension. 19(S1), p. I9-I17, 1992.

36.) Greenwood, F.C., Hunter, W.M. and Glover, J.S. The Preparation of ^{131}I-Labelled Human Growth Hormone of a High Specific Radioactivity. Biochemistry Journal. 89, p. 114, 1963.

37.) Gross, P.M. and A. Weindl. Peering Through the Windows of the Brain. Journal of Cerebral Blood Flow and Metabolism. 7, p. 663-672, 1987.

38.) Gutman, M.B., J. Ciriello, and G.J. Mogenson. Effects of Plasma Angiotensin II and Hypernatremia on Subfornical Organ Neurons. American Journal of Physiology. 254, p. R746-R754, 1988.

39.) Guyton, A.C., J.H. Satterfield, and J.W. Harris. Dynamics of Central Venous Resistance with Observations on Static Blood Pressure. American Journal of Physiology. 169, p. 691-9, 1952.

40.) Guyton, A.C., T.G. Coleman, A.W. Cowley Jr., R.D. Manning Jr., R.A. Norman Jr., and J.D. Ferguson. A Systems Analysis Approach to Understanding Long-Range Arterial Blood Pressure Control and Hypertension. Circulation Research. 35, p. 159-176, 1974.

41.) Hall, J.E., H.L. Mizelle, and L.L. Woods. The Renin-Angiotensin System and Long-Term Regulation of Arterial Pressure. Journal of Hypertension. 4, p. 387-397, 1986a.

42.) Hall, J.E., A.C. Guyton, T.G. Coleman, H.L. Mizelle, and L.L. Woods. Regulation of Arterial Pressure: Role of Pressure Natriuresis and Diuresis. Federation Proceedings. 45, p. 2897-2903, 1986b.

43.) Hall, J.E., H.L. Mizelle, D.A. Hildebrandt, and M.W. Brands. Abnormal Pressure Natriuresis. A Cause or a Consequence of Hypertension? Hypertension. 15, p. 547-559, 1990.

44.) Hartter, E. and W. Woloszuk. Radioimmunological Determination of Arginine Vasopressin and Human Atrial Natriuretic Peptide After Simultaneous Extraction from Plasma. Journal of Clinical Chemistry and Clinical Biochemistry. 24, p. 559-563, 1986.

45.) Haupert Jr., G.T., C.T. Carilli, and L.C. Cantley. Hypothalamic Sodium-Transport Inhibitor is a High-Affinity Reversible Inhibitor of Sodium-Potassium-ATPase. American Journal of Physiology. 247, p. F919-F924, 1984.

46.) Henry, J.P., O.H. Gauer, and J.L. Reeves. Evidence of the Atrial Location of Receptors Influencing Urine Flow. Circulation Research. 4, p. 85-90, 1956.

47.) Henry, J.P., and J.W. Pearce. The Possible Role of Atrial Stretch Receptors in the Induction of Changes in Urine Flow. Journal of Physiology. 131, p. 572-585, 1956.

48.) Hildebrandt, D.A., H.L. Mizelle, M.W. Brands, and J.E. Hall. Comparison of Renal Actions of Urodilatin and Atrial Natriuretic Peptide. American Journal of Physiology. 262, p. R395-R399, 1992.

49.) Hubbard, J.I., R.G. Mills, and N.E. Sirett. Distribution of Osmosensitive Cells in the Preoptic and Adjacent Regions of Cat Brains. Brain Research. 345, p. 402-405, 1985.

50.) Jewell, P.A. and E.B. Verney. An Experimental Attempt to Determine the Site of the Neurohypophysial Osmoreceptors in the Dog. Proceedings of the Royal Society of London Series B. 240. p. 197-324, 1957.

51.) Kaplan, H.M. The Rabbit in Experimental Physiology. New York Scholar's Library, New York, 1956.

52.) Kaplan H.M., and E.H. Timmons, 1979. The Rabbit, A Model for the Principles of Mammalian Physiology and Surgery. Academic Press. New York, p. 6-7, 1979.

53.) Keeler, R. Natriuresis after Unilateral Stimulation of Carotid Receptors in Unanesthetized Rats. American Journal of Physiology. 226, p. 507-511, 1974.

54.) Ledsome, J.R. Atrial Receptors, Vasopressin and Blood Volume in the Dog. Life Sciences. 36, p. 1315-1330, 1985.

55.) Leng, G., W.T. Mason, and R.G. Dyer. The Supraoptic Nucleus as an Osmoreceptor. Neuroendocrinology. 34. p. 75-82, 1982.

56.) Leskell, L.G., M. Congiu, D.A. Denton, D.T.W. Fei, M.J. McKinley, E. Tarjan, and R.S. Weisinger. Influence of Mannitol-Induced Reduction in CSF Sodium on Nervous and Endocrine Mechanisms Involved in the Control of Fluid Balance. Acta Physiologica Scandinavica. 112, p. 33-40, 1981.

57.) Levy, M. and M.J. Wexler. Sodium Excretion in Dogs with Low-Grade Caval Constriction: Role of Hepatic Nerves. American Journal of Physiology. 253, p. F672-F678, 1987.

58.) Light, D.B., F.V. McCann, T.M. Keller, and B.A. Stanton. Amiloride-Sensitive Cation Channel in Rat Inner Medullary Collecting Duct. American Journal of Physiology. 255, p. F278-F286, 1988.

59.) Light, D.B., E.M. Schwiebert, K.H. Karlson, and B.A. Stanton. Atrial Natriuretic Peptide Inhibits a Cation Channel in Renal Inner Medullary Collecting Duct Cells. Science. 243, p. 383-385, 1989.

60.) Lokhandwala, M.F., S.J. Vyas, and S.S. Hegde. Renal Dopamine and Tubular DA-1 Receptors in the Regulation of Sodium Excretion. Journal of Autonomic Pharmacology. 10 (Suppl. 1), p. S31-S39, 1990.

61.) Mangiapane, M.L., T.N. Thrasher, L.C. Keil, J.B. Simpson, and W.F. Ganong. Role of the Subfornical Organ in Vasopressin Release. Brain Research Bulletin. 13, p. 43-47, 1984.

62.) McKinley, M.J., E.H. Blasine, and D.A. Denton. Brain Osmoreceptors, Cerebrospinal Fluid Electrolyte Composition and Thirst. Brain Research. 70, p. 532-537, 1974.

63.) McKinley, M.J., D.A. Denton, and R.S. Wiesinger. Sensors for Antidiuresis and Thirst - Osmoreceptors or CSF Sodium Detectors. Brain Research. 141. p. 89-103, 1978.

64.) McKinley, M.J., D.A. Denton, L.G. Leskell, D.R. Mouw, B.A. Scoggins, M.H. Smith, R.S. Weisinger, and R.D. Wright. Osmoregulatory Thirst in Sheep is Disrupted by Ablation of the Anterior Wall of the Optic Recess. Brain Research. 236, p. 210-215, 1982.

65.) McKinley, M.J., D.A. Denton, R.G. Park, and R.S. Weisinger. Ablation of Subfornical Organ Does Not Prevent Angiotensin-Induced Water Drinking in Sheep. American Journal of Physiology. 250, p. R1052-R1059, 1986.

66.) McKinley, M.J., Common Aspects of the Cerebral Regulation of Thirst and Renal Sodium Excretion. Kidney International, 41 S.37, p. S102-S106, 1992.

67.) Meister, B., and A. Aperia. Molecular Mechanisms Involved in Catecholamine Regulation of Sodium Transport. Seminars in Nephrology. 13, p. 41-49, 1993.

68.) Miller, R.D. Skeletal Muscle Relaxants. In: Basic and Clinical Pharmacology (4th Edition). B.E. Katzung Editor. Appleton and Lange, California. p. 323-324, 1989.

69.) Mizelle, H.L., J.E. Hall, and D.A. Hildebrandt. Atrial Natriuretic Peptide and Pressure Natriuresis: Interactions with the Renin-Angiotensin System. American Journal of Physiology. 257, p. R1169-R1174, 1989.

70.) Morris, S.J. Specific Radioactivity of Radioimmunoassay Tracer Determined by Self-Displacement; a Re-Evaluation. Clinicia Chimica Acta. 73, p. 213-216, 1976.

71.) Navar, L.G., and K.D. Mitchell. Contribution of the Tubuloglomerular Feedback Mechanism to Sodium, Homeostasis and Interaction with the Renin-Angiotensin System. Acta Physiologica Scandinavica. 139 (Suppl. 591), p. 66-73, 1990.

72.) O'Connor, W.J. Plasma Protein Concentration. In: Renal Function. Edward Arnold (Publishers) Ltd., London. p. 4-17, 1962.

73.) Oikawa, S., M. Imai, C. Inuzuka, Y. Tawaragi, H. Nakazato, and H. Matsuo. Structure of Dog and Rabbit Precursors of Atrial Natriuretic Polypeptides deduced from Nucleotide Sequence of Cloned cDNA. Biochemical and Biophysical Research Communications. 132(3), p. 892-899, 1985.

74.) Olsson, K. Dipsogenic Effects of Intracarotid Infusions of Various Hyperosmolal Solutions. Acta Physiologica Scandinavica. 85, p.517-522, 1972a

75.) Olsson, K. On the Importance of CSF Sodium Concentration in Central Control of Fluid Balance. Life Sciences. 11, p. 397-402, 1972b.

76.) Olsson, K. Further Evidence for the Importance of CSF Sodium Concentration in Central Control of Fluid Balance. Acta Physiologica Scandinavica. 88, p. 183-188, 1972c.

77.) Olsson, K. and R. Kolmodin. Dependence of Basic Secretion of Antidiuretic Hormone on Cerebrospinal Fluid Sodium Concentration. Acta Physiologica Scandinavica. 91. p. 286-288, 1974.

78.) Omvik, P., R.C. Tarazi, and E.L. Bravo. Regulation of Sodium Balance in Hypertension. Hypertension. 2, p. 515-523, 1980.

79.) Passo, S.S., J.R. Thornborough, and A.B. Rothballer. Hepatic Receptors in Control of Sodium Excretion of Anaesthetized Cats. American Journal of Physiology. 224, p. 373-375, 1972.

80.) Peterson, T.V., and B.A. Benjamin. The Heart and Control of Renal Excretion; Neural and Endocrine Mechanisms. FASEB Journal. 6, p. 2923-2932, 1992.

81.) Richards, A.M. Is Atrial Natriuretic Factor a Physiological Regulator of Sodium Excretion? A Review of the Evidence. Journal of Cardiovascular Pharmacology. 16 (Suppl. 7), p. S39-S42, 1990.

82.) Robertson, G.L., R.L. Shelton, and S. Athar. The Osmoregulation of Vasopressin. Kidney International. 10, p. 25-37, 1976.

83.) Romero, J.C., V. Lahera, M.G. Salom, and M.L. Biondi. Role of the Endothelium-Dependent Relaxing Factor Nitric Oxide on Renal Function. Journal of the American Society of Nephrology. 2, p. 1371-87, 1992.

84.) Rundgren, M., H. Jonasson, and H. Hjelmqvist. Water Intake and Changes in Plasma and CSF Composition in Response to Acute Administration of Hypertonic Sodium Chloride and Water Deprivation in Sheep. Acta Physiologica Scandinavica. 138, p. 85-92, 1990.

85.) Schad, H. and H. Seller. Influence of Intracranial Osmotic Stimuli on Renal Nerve Activity in Anaesthetized Cats. Pflugers Archives. 353, p. 107-121, 1975.

86.) Schauf, C.L., D.F. Moffett, and S.B. Moffett. Kidney and Body Fluid Homeostasis. In: Human Physiology. Foundations and Frontiers. Times Mirror/Mosby College Publishing. Toronto, p. 473-530, 1990.

87.) Schulz-Knappe, P., K Forssmann, F. Herbst, D. Hock, R. Pipkorn, and W.G. Forssmann. Isolation and Structural Analysis of "Urodilatin"k, a New Peptide of the Cardiodilatin-(ANP)-Family, Extracted from Human Urine. Klinische Wochenschrift. 66, p. 752-759, 1988.

88.) Scott, C.S., J. Sharp-Kehl, C.A. Redekopp, and J.R. Ledsome. Regulation of Plasma Vasopressin by Plasma Osmolality and Carotid Sinus Pressure in Anaesthetized Rabbits. American Journal of Physiology. 266, p. R796-R801, 1994.

89.) Seldin, D.W., and G. Giebisch. The Regulation of Sodium and Chloride Balance. Raven Press, New York, p. 59-104, 1990.

90.) Slot, C. Plasma Creatinine Determination. A New and Specific Jaffe Reaction Method. Scandinavian Journal of Clinical Laboratory Investigation. 17, p. 381, 1965.

91.) Skowsky, W.R., and D.A. Fisher. The Use of Thyroglobulin to Induce Antigenicity to Small Molecules. Journal of Laboratory and Clinical Medicine. 80, p. 134-144, 1972.

92.) Sonnenberg, H., U. Honrath, C.K. Chong, and D.R. Wilson. Atrial Natriuretic Factor Inhibits Sodium Transport in Medullary Collecting Duct. American Journal of Physiology. 250, p. F963-F966, 1986.

93.) Stanton, B. Role of Adrenal Hormones in Regulating Distal Nephron Structure and Ion Transport. 44, p. 2717-2722, 1985.

94.) Swaminathan, S. Osmoreceptors or Sodium Receptors: An Investigation into AVP Release in the Rhesus Monkey. Journal of Physiology. 307, p. 71-83, 1980.

95.) Takemoto, F., H.T. Cohen, T. Satoh, and A.I. Katz. Dopamine Inhibits Na/K-ATPase in Single Tubules and Cultured Cells from Distal Nephron. Pflugers Archives. European Journal of Physiology. 421, p. 302-306, 1992.

96.) Thompson, C.J., J. Bland, J. Burd, and P.H. Baylis. The Osmotic Thresholds for Thirst and Vasopressin Release are Similar in Healthy Man. Clinical Science. 71, p. 651-656, 1986.

97.) Thornton, S.N., B.A. Baldwin, and T. Purdew. Osmotically Induced Drinking in the Goat: An Osmoreceptor or a Sodium Receptor Mechanism? Quarterly Journal of Experimental Physiology. 70, p. 549-556, 1985.

98.) Thrasher, T.N., C.J. Brown, L.C. Keil, and D.J. Ramsay. Thirst and Vasopressin Release in the Dog: An Osmoreceptor or Sodium Receptor Mechanism? American Journal of Physiology. 238, p. R333-R339, 1980a.

99.) Thrasher, T.N., R.G. Jones, L.C. Keil, C.J. Brown, and D.J. Ramsay. Drinking and Vasopressin Release During Ventricular Infusions of Hypertonic Solutions. American Journal of Physiology. 238. p. R340-R345, 1980b.

100) Thrasher, T.N., L.C. Keil, and D.J. Ramsay. Lesions of the Organum Vasculosum of the Lamina Terminalis (OVLT) Attenuate Osmotically-Induced Drinking and Vasopressin Secretion in the Dog. Endocrinology. 110, p. 1837-1839, 1982a.

101) Thrasher, T.N., J.B. Simpson, D.J. Ramsay. Lesions of the Subfornical Organ Block Angiotensin-induced Drinking in the Dog. Neuroendocrinology. p. 68-72, 1982b.

102) Wang, B.C., G. Flora-Ginter, R.J. Leadley Jr., and K.L. Goetz. Ventricular Receptors Stimulate Vasopressin Release During Hemorrhage. American Journal of Physiology. 254, p. R204-211, 1988.

103) Weaver, L.C. Cardiopulmonary Sympathetic Afferent Influences on Renal Nerve Activity. American Journal of Physiology. 233, p. H592-H599, 1977.

104) Wu, A.P. and J.P. Gilmore. Lack of Evidence for a Cerebral Sodium Modulating Mechanism in the Monkey. Basic Research in Cardiology. 82, p. 319-325, 1987.

105) Verney, E.B. The Antidiuretic Hormone and Factors which Determine its Release. Proceedings of the Royal Society of London Series B. 135, p. 25-106, 1949.

106) Zambraski, E.J. Renal Nerves in Renal Sodium-Retaining States: Cirrhotic Ascites, Congestive Heart Failure, Nephrotic Syndrome. Mineral and Electrolyte Metabolism. 15, p. 88-96, 1989.

107) Zeidonis, D.M., W.B. Severs, R.W. Brennan, and R.B. Page. Blood Flow and Functional Responses Correlate in the Ovine Neural Lobe. Brain Research. 373, p. 27-34, 1985.