EFFECT OF GENDER ON THE ANTIDIURETIC ACTIVITY OF VASOPRESSIN IN

THE SPONTANEOUSLY HYPERTENSIVE RAT

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAI'I IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

BIOMEDICAL SCIENCES (PHARMACOLOGY)

August 2003

By
Dailin Ye

Dissertation Committee:

Catherine F.T. Uyehara, Chairperson
Bert K.B. Lum
Eiichi Furusawa
S. Ramanathan
John R. Claybaugh

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to thank everyone who has provided assistance to me during my graduate training and made my graduation a possibility.

My deepest appreciation goes to Dr. Catherine F.T. Uyehara, my mentor, without whose support this dissertation would not have been possible.

I would like to express my grateful appreciation to Dr. Lum, Dr. Ramanathan, Dr. Furusawa, and Dr. Claybaugh for serving on my committee and providing me their generous advice and encouragement throughout this research.

I am indebted to the Department of Clinical Investigation at Tripler Army Medical Center, Hawaii, in which the work of this dissertation was carried out from 1996 to 2000. I want to thank all the personnel in the department who has worked together with me. Without their patience and support, this research could never have been completed.

I am especially grateful to Glenn Hashiro, Claudia Burghardt, and Aileen Sato for their invaluable technical assistance. I owe a lot to Dorena Cheng, who patiently assisted me during the long hours of experimentation throughout the years.

I would like to thank my fellow graduate students: Wenqun Lee, Baozhi Yuan, Kevin Urada, and Jenny Lim, who always allowed me to discuss my problems and share excitement in my research.

Special thanks must be given to Dr. Scott Lozanoff for not only his financial support for part of my graduate training but also his kindness and understanding.

ABSTRACT

Recently, studies have found that circulating vasopressin levels, as well as the cardiovascular and antidiuretic actions of vasopressin are greater in males than in females, but the consequences of these gender differences are unclear. Further, animal and human studies have revealed that hypertension develops more rapidly and is more prevalent in males than in females. Because vasopressin is involved with blood pressure regulation, whether vasopressin gender differences are involved in the gender difference of hypertension development should be examined.

Thus, this dissertation tested the hypothesis that a gender effect on vasopressin renal action contributes to gender differences in hypertension. Renal function of male and female adult spontaneously hypertensive rats (SHR) were compared to that of normotensive (WKY) rats. Vasopressin V2 receptors were pharmacologically stimulated with a selective V2 receptor agonist, which revealed that maximal urine concentrating abilities of SHR were higher than that of WKY. While there were significant differences in concentrating abilities with hypertension, there were no significant gender differences in responses to maximal V2 stimulation in normotensive or hypertensive rats.

The contribution of endogenous vasopressin on renal fluid handling to a gender difference in hypertension was also examined. Vasopressin inhibition with a selective V2 antagonist resulted in higher free water clearance in females than in males of both strains. Also, the renal response to endogenous vasopressin blockade was lower in SHR than WKY in both sexes. Expression of V2 receptors in renal inner medulla of male SHR and WKY showed that levels of V2 receptor mRNA in SHR were significantly

lower than those in WKY. Thus, differences in renal fluid handling abilities between strains and gender, may be due to the differences in endogenous vasopressin levels, renal concentrating abilities, or expression of renal vasopressin receptors.

In conclusion, vasopressin influence on renal fluid handling is altered in established hypertension. While there are differences in vasopressin renal action between males and females in both strains, there were no significant differences in the gender effect between normotensive and hypertensive adult rats. Thus, whether gender differences in fluid handling contribute to gender differences in hypertension development remains to be determined.

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

μl	microliter
μU	microunit
1K1C	one-kidney, one-clip
2K1C	two-kidney, one-clip
A	adenosine
ADH	antidiuretic hormone
ANOVA	analysis of variance
AQP-2	aquaporin-2
ATP	adenosine triphosphate
AVP	arginine vasopressin
BW	body weight
C	cytidine
Ca	calcium
cAMP	cyclic adenosine monophosphate
cDNA	complementary deoxyribonucleic acid
C_{H2O}	free water clearance
cm	centimeter
C_{osm}	osmolar clearance
CSPD	disodium 3-(4-methoxyspiro{1,2-dioxetane3,2'-(5'-chloro) tricyclo[3.3.1.1^{3,7}]decan}-4-yl)phenyl phosphate
d(CH$_2$)$_5$VDAVP	vasopressin V1 receptor antagonist
dATP	deoxyadenosine triphosphate
DBP	diastolic blood pressure
dCTP	deoxycytidine triphosphate
dDAVP	[deamino-Cys^1,D-Arg^8]-vasopressin
DEPC	diethyl pyrocarbonate
dGTP	deoxyguanosine triphosphate
DI	diabetes insipidus
DIG	digoxin
DNA	deoxyribonucleic acid
DNase I	deoxyribonuclease I
dNTP	deoxyribonucleoside triphosphate
DOC	deoxycorticosterone
DOCA	deoxycorticosterone acetate
dPVDAVP	vasopressin V1 receptor antagonist
dTTP	deoxythymidine triphosphate
ED_{50}	effective dose for fifty percent of maximum effect
ERBF	effective renal blood flow
ERPF	effective renal plasma flow
G	guanosine
g	gram
GAPDH	glyceraldehyde-3-phosphate dehydrogenase

GFR	glomerular filtration rate
G-protein	guanine-nucleotide-binding-proteins
Gs-protein	stimulatory guanine-nucleotide-binding-protein
h	hour
H_2O	water
H_2SO_4	sulfuric acid
HCl	hydrochloric acid
Hct	hematocrit
i.g.	intragastric
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IP3	inositol triphosphate
KCl	potassium chloride
kg	kilogram
L-NAME	NG-nitro-L-arginine methyl ester
M	molality
MABP	mean arterial blood pressure
mg	milligram
$MgCl_2$	magnesium chloride
min	minute
ml	milliliter
mM	millimolality
mm Hg	millimeter mercury column
mOsm	milliosmolarity
mRNA	messenger ribonucleic acid
mV	millivolt
n	number of samples
NaCl	Sodium chloride
N-ED	N-(1-naphthyl)-ethylenediamine dihydrochloride
ng	nanogram
nm	nanometer
O.D.	outer diameter
PAH	para-aminohippurate
PCR	polymerase chain reaction
pg	picogram
pH	hydrogen ion exponent
pM	picomolality
PVN	paraventricular nuclei
RNA	ribonucleic acid
RT	reverse transcription
RVR	renal vascular resistance
SBP	systolic blood pressure
SDS	sodium dodecyl sulfate
SEM	standard error mean

SHR	spontaneously hypertensive rat
SHRSP	spontaneously hypertensive rat (stroke-prone strain)
SIADH	syndrome of inappropriate antidiuretic hormone secretion
SON	supraoptic nuclei
SSC buffer	sodium chloride, sodium citrate buffer
T	thymidine
TCA	trichloroacetic acid
U	urine sample
UT1	urea transporter-1
UV	ultraviolet
V1	vasopressin subtype 1 receptor
V1a	vasopressin subtype 1a receptor
V1b	vasopressin subtype 1b receptor
V2	vasopressin subtype 2 receptor
WKY	Wistar-Kyoto rat

PREFACE

Vasopressin is a neurohypophysial hormone, which has two major biological actions: vasoconstriction and antidiuretic action. Vasopressin acts via V1a receptors on vascular smooth muscle cells to cause vasoconstriction and acts via V2 receptors on the distal tubules and collecting ducts of the kidney to promote water reabsorption. Since both actions of vasopressin may contribute to the regulation of blood pressure, the possible role of vasopressin in the pathogenesis of hypertension has been studied but the importance of this role remains unclear. In recent years, studies demonstrated that the release and excretion of vasopressin as well as the cardiovascular action and antidiuretic action of vasopressin, are greater in males than in females, but the physiological and pathological consequences of these gender differences are not clear. Gender difference has also been found in the development of hypertension in several animal models that high blood pressure develops more rapidly and to a higher level in males than in females. In clinical studies, the prevalence of hypertension and the mortality due to cardiovascular disease is greater in men than in women. Therefore, the potential effects of gender differences in vasopressin action may contribute to the gender difference of hypertension development.

CHAPTER I

INTRODUCTION

1.   Vasopressin: An Overview

1.1   Vasopressin: Synthesis and Secretion

Arginine Vasopressin (AVP), also known as the antidiuretic hormone (ADH), is a

peptide hormone synthesized in the magnocellular neurons in the supraoptic nuclei

(SON) and paraventricular nuclei (PVN) of the hypothalamus. The magnocellular

neurons have long axons, which traverse the supraopticohypophyseal tract and terminate

in the posterior lobe of the pituitary (North, 1987).

In the cell body of magnocellular neurons, vasopressin is first translated as a

preprohormone and then converted to a prohormone. This prohormone is then packaged

into neurosecretory vesicles and transported to the axon terminals in the posterior

pituitary. During transport, the prohormone undergoes enzymatic modification to form a

vasopressin nonapeptide, a carrier protein called neurophysin, and a glycoprotein (North,

1987). Vasopressin and its by-products are stored in the posterior pituitary where

vasopressin is released into the systemic circulation by exocytosis (Dreifuss, 1975) in

response to various stimuli.

The normal range of plasma vasopressin concentration varies between species.

In normal humans and animals, daily plasma vasopressin concentrations range from 0.3

to 30 pg/ml (Cowley et al., 1983). The secretion of AVP is mainly regulated by changes

in body fluid osmolality, blood volume and/or blood pressure. Pain, nausea, and hypoxia

as well as endogenous hormones may also modulate the secretion of vasopressin.

Plasma osmolality is the major physiological determinant of vasopressin secretion, which is regulated by a group of specific cells, called osmoreceptors (Verney, 1946). Osmoreceptors were originally proposed to be located in the anterior hypothalamus (Verney, 1947), but the precise site has not yet been identified. There is evidence that the magnocellular neurons synthesizing vasopressin may also be osmotically sensitive (Mason, 1980; Leng et al., 1982). Vasopressin secretion occurs when plasma osmolality exceeds a critical level of approximately 280 mOsm/kg (Robertson et al., 1976). Above this level, there is a linear relationship between plasma vasopressin concentration and plasma osmolality, and below this level, vasopressin is low or undetectable in plasma. However, there is no direct evidence that the secretion of vasopressin is completely suppressed at plasma osmolality less than 280 mOsm/kg.

Vasopressin secretion is also regulated by changes in blood volume and/or blood pressure through two classes of baroreceptors: low- and high-pressure receptors. Low-pressure receptors in the left atrium, left ventricle, and pulmonary veins sense the changes of blood volume, whereas high-pressure receptors in the carotid sinus and aorta monitor arterial blood pressure (Gauer and Henry, 1963). Johnson et al. (1970) suggested that at least a 10% reduction in blood volume was necessary to increase plasma vasopressin concentration. While small decreases (5-10%) in blood volume and/or pressure have little effect on vasopressin secretion, large decreases (20-30%) in blood volume and/or pressure lead to exponential rises in plasma vasopressin concentration to 20-30 times the normal level, which far exceeds the concentration of vasopressin required to induce maximal antidiuresis.

1.2 Vasopressin: Biological Actions

Vasopressin has various biological actions. Two major biological actions of vasopressin are vasocontriction and antidiuretic action. Specific vasopressin receptor subtypes mediate the different biological actions of vasopressin.

1.2.1 Vasopressin: Receptors

There are at least three types of vasopressin receptors which have been identified as mediating the biological actions of vasopressin: V1a, V1b, and V2 receptors (Michel et al., 1979; Jard et al., 1986). V1a receptors have been found abundantly in vascular vessels and liver. V1a receptors are also found in the kidney and many other peripheral tissues. V1b receptors are predominantly located in the anterior pituitary, whereas V2 receptors are expressed predominantly in the renal collecting ducts.

Vasopressin V1 and V2 receptors are cell surface receptors coupled to different types of guanine-nucleotide-binding-proteins (G-proteins) that trigger a series of specific intracellular events in various target tissues, and in turn, evoke various biological responses (Jard, 1983). Activation of V1 receptors results in hydrolysis of phosphotidylinositol-4,5-bisphosphate and formation of inositol-1,4,5-triphosphate (IP3), which causes mobilization of intracellular calcium (Ca^{2+}). Activation of V2 receptors results in the conversion of intracellular adenosine triphosphate (ATP) to adenosine-3',5'-cyclic monophosphate (cAMP). Vasopressin acts on V1a receptors on vascular smooth muscle cells to mediate vasoconstriction and acts on V2 receptors on the principal cells of the renal collecting ducts to mediate antidiuretic action.

It has been demonstrated that expressions of V1a and V2 receptor mRNA were

tissue specific and altered throughout development (Hirasawa et al., 1993; Ostrowski et

al., 1993). Both the V1a and V2 receptor genes are expressed in adult rat kidney. In the

nephron segments of the kidney, Terada et al. (1993) reported that expression of V1a

receptor mRNA was mainly detected in the glomerulus, initial cortical collecting duct,

and inner medullary collecting duct. V1a receptor mRNA was also expressed in

proximal convoluted and straight tubules, inner medullary thin limbs, and medullary

thick ascending limbs. Expression of V2 receptor mRNA was mainly found in the

cortical collecting duct, outer medullary collecting duct, and inner medullary collecting

duct. V2 receptor mRNA was also expressed in the inner medullary thick limbs,

medullary thick ascending limbs, and initial cortical collecting duct. In addition, V2

receptors were found not only in the basolateral membrane but also in the luminal

membrane of terminal inner medullary collecting ducts (Nonoguchi et al., 1995). In the

renal vasculature, Park et al. (1997) demonstrated that the V1a receptor mRNA was

present in renal artery, interlobar artery, arcuate artery, interlobular artery, afferent

arteriole, glomerulus, efferent arteriole, and both cortical and mudullary vasa recta

microvasculature. There was no evidence for the presence of V2 mRNA in renal

vasculature and extrarenal blood vessels.

Alterations of vasopressin receptor expression and their mediated functions have

been reported in both physiological and pathological conditions. Landgraf et al. (1991)

reported a downregulation of vasopressin receptors in rat kidneys in response to osmotic

stimuli. Downregulation of vasopressin V2 receptors was also reported in rats after 72-

hour water restriction (Terada et al., 1993) and in a rat model of chronic renal failure

4

(Teitelbaum and McGuinness, 1995). Park et al. (1998) demonstrated that V2 receptor mRNA was regionally regulated where cortical and outer medullary V2 receptor mRNA and protein decreased after 48 hours of water restriction, but remained unchanged in the inner medulla. Klingler et al. (1997) observed an age-related impaired response to vasopressin of the V2 receptor in female rats but not in male rats.

The importance of vasopressin in the regulation of cardiovascular function and body fluid homeostasis has long been recognized. However, as Ostrowski and colleagues (1993) wrote: 'The relationship among the appearance of vasopressin receptors, their linkage to second message systems, circulating AVP levels, and the ability to concentrate urine in the kidney is not clear.'

1.2.2 Vasopressin: Cardiovascular Actions

The vasopressor activity of vasopressin was first discovered by Oliver and Schafer (1895), who observed a marked increase in blood pressure with intravenous injection of pituitary extract in dog. Three years later, Howell (1898) confirmed this vasopressor activity, and associated this activity with the posterior lobe of the pituitary. Vasopressin derived its name from its vasopressor activity. However, the amounts of vasopressin required to elicit vasoconstriction were far in excess of those required for maximal antidiuretic activity as well as systemic circulating concentrations of vasopressin under normal physiological conditions (Saameli, 1968; Sawyer, 1971; Nakano, 1974). Therefore, the vasopressor activity of vasopressin was believed to be only a pharmacological event and the importance of vasopressin in the daily regulation of cardiovascular functions was not appreciated for many years.

It was not until the 1950s that the vasopressor activity of vasopressin was re-evaluated and extensive studies of the role of vasopressin in cardiovascular function were undertaken. Several important findings arose at this time. Frieden and Keller (1954) found that dogs with diabetes insipidus were more susceptible to hemorrhage than normal dogs and giving pitressin, a synthetic vasopressin, reversed this. Goodman and Gilman (1955) reported a significant increase in vasopressin circulating levels during severe hemorrhage. Furthermore, Wagner and Braunwald (1956) found that administration of vasopressin to human subjects with autonomic insufficiency and orthostatic hypotension had a greater effect on blood pressure than seen in normal subjects. These findings suggested that endogenous vasopressin plays a role in the daily regulation of cardiovascular function and contributes to blood pressure control by maintaining normal vascular tone.

Studies performed in vivo and in vitro have demonstrated that vasopressin is one of the most powerful vasoconstrictors that has been found (Altura and Altura, 1977). In animals with an intact baroreflex system, elevation of plasma vasopressin concentration within the normal daily range of levels can produce a significant vasoconstriction effect and result in increased total peripheral resistance (Cowley et al., 1983), however, the vasopressin vasoconstrictor effect on blood pressure is minimal. Cowley et al. (1974) reported that intravenous infusion of exogenous vasopressin, which produced plasma vasopressin concentrations well within the daily physiological range, resulted in an increase of arterial blood pressure of only 5 mmHg in intact dogs while an increase of 33 mmHg was seen in baroreceptor-denervated dogs. Similar results were also reported by Montani et al. (1980). Studies demonstrated that vasopressin could enhance the ability of

6

the baroreceptor reflex to buffer blood pressure elevations caused by a rise in total peripheral resistance by reducing cardiac output and heart rate (Cowley et al., 1974; Montani et al., 1980).

It is also evident that vasopressin has varying vasoconstrictor effects in different vascular beds. While some organs showed reduced blood flow in response to vasopressin infusion, other organs do not, in particular, the kidney. This is probably due to a V2 receptor mediated vasodilator effect that offsets the V1a receptor mediated vasoconstrictor effect of vasopressin. V2 receptor-mediated vasodilatation has been shown in the kidney of dogs, rats, and rabbits (Naitoh et al., 1993; Aki et al., 1994; Tamaki et al., 1996), and in the forearm (Hirsch et al., 1989; van Lieburg et al., 1995) and renal arteries (Medina et al., 1999) of humans. However, the mechanism by which vasodilation is produced is not clear. As there is no evidence for V2 receptors within vascular smooth muscle, this vasodilating effect does not appear to be due to direct action of the vascular smooth muscle. It is believed that the release of nitric oxide (Aki et al., 1994; Lieburg et al., 1995) or vasodilatory prostaglandins (Medina et al., 1999) induced by V2 receptor stimulation of endothelial receptors contributes to the vasodilation effect of vasopressin.

The role of vasopressin in the regulation of renal hemodynamics is of particular interest due to the importance of the kidney on body fluid homeostasis and long-term blood pressure control.

Studies have demonstrated that the vascular effects of vasopressin are different in the renal cortex and medulla in rats, where the renal medulla seems more sensitive to the vasoconstrictor effect of vasopressin. Intravenous infusion of vasopressin causing an

increase in plasma vasopressin concentration still within the physiological range which had no effect on mean arterial blood pressure, total renal blood flow, and blood flow in the renal cortex, lowered blood flow in the renal medulla (Franchini and Cowley, 1996; Franchini et al., 1997). These results indicate that vasopressin may regulate regional blood flow shifts within the kidney.

Vasopressin appears to have a dual effect on the renal medullary circulation in rats, causing vasoconstriction via V1 receptors and vasodilatation via V2 receptors (Nakanishi et al., 1995). While infusion of a selective V1 receptor agonist into the medullary interstitium reduced medullary blood flow, infusion of a selective V2 agonist increased medullary blood flow. Furthermore, equimolar doses of vasopressin also reduced medullary blood flow but to a lesser degree than the effect of a V1 agonist. This study indicated that the vasoconstrictor effect of vasopressin was the predominant effect in the renal medulla circulation, but stimulation of the V2 receptor could partially attenuate V1 receptor-mediated vasoconstriction. The net effect of vasopressin was to decrease renal medullary blood flow.

While chronic intravenous or renal medullary infusion of vasopressin failed to increase blood pressure (Cowley et al., 1994; Cowley et al., 1998), infusion of a V1 agonist produced sustained hypertension in rats (Szczepanska-Sadowska et al., 1994; Cowley et al., 1998). Cowley et al. (1998) demonstrated that chronic renal medullary infusion of vasopressin decreased medullary blood flow transiently and insignificantly, whereas infusion of V1 agonist resulted in a significant reduction of renal medullary blood flow. These results indicated that the V1 vasoconstrictor effect of vasopressin played an important role in the development of hypertension due to a chronic reduction of

8

medullary blood pressure. Chronic stimulation of V2 receptors might offset the vasoconstrictor and hypertensive effects of V1 receptors by vasopressin.

V1a receptor mRNA was found in all regions of the renal vasculature, while V2 receptor mRNA was only present in the renal tubule (Park et al., 1997). This indicated that V2 receptor mediated renal medullary vasodilation was not a direct effect of vasopressin in the renal vasculature, but likely an indirect effect of nitric oxide release through tubular V2 receptor stimulation (Park et al, 1998). While vasopressin or vasopressin V2 receptor agonist increased renal medullary nitric oxide concentrations, vasopressin V1 receptor agonist had no effect. On one hand, there was evidence that stimulation of V2 receptors increased concentration of nitric oxide in the renal medulla, buffering the hypertensive effects of vasopressin (Szentivanyi et al., 2000). On the other hand, administration of the nitric oxide synthase inhibitor, NG-nitro-L-arginine methyl ester (L-NAME) increased blood pressure by lowering medullary blood flow and inducing sodium and water retention (Mattson et al., 1992, 1994, 1997).

Therefore, Cowley (1997) suggested that the renal medullary circulation could play an important role in determining the level of blood pressure required to achieve long-term fluid and electrolyte homeostasis by establishing the slope and set point of the pressure-natriuresis relationship.

1.2.3   Vasopressin: Renal Actions

The antidiuretic action of vasopressin was not known until 1913 when Von den Velden (1913) reported successful treatment of patients with diabetes insipidus by injections of posterior pituitary extracts. In 1924, Starling and Verney (1924) localized

the antidiuretic effect of posterior pituitary extracts to the kidney. Under normal

circumstances, the antidiuretic action is the most prominent action of vasopressin, which

regulates renal collecting duct water reabsorption and plays an important role in body

fluid homeostasis.

The antidiuretic action of vasopressin is mediated by V2 receptors coupled to

stimulatory G proteins (Gs-protein). Without the presence of vasopressin, the entire

collecting duct is impermeable to water. Binding of vasopressin to V2 receptors

stimulates Gs-proteins, which then activate adenylate cyclase to convert ATP to cAMP.

As a second messager, cAMP activates a cAMP-dependent protein kinase, followed by

protein phosphorylation-dephosphorylation. Finally, aquaporin-2 (AQP-2) water

channels are inserted in the luminal membrane of the collecting duct principal cells,

thereby increasing permeability and reabsorption of water. Vasopressin is involved in

both short and long term regulation of collecting duct AQP-2 water channels (Knepper,

1997). In normal rats subjected to water restriction or treated with a vasopressin V2

receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP), the expression of AQP-2

was increased (Hayashi et al., 1994; Terris et al., 1996).

The antidiuretic action of vasopressin depends upon the generation of the osmotic

gradient across the collecting duct cells (Rector, 1977). Activation of V2 receptors

increases urea permeability in the terminal portions of the medullary collecting duct.

Accumulation of urea in the medullary interstitium contributes to the establishment of the

osmotic gradient, which provides the osmotic driving force for water reabsorption, which

in turn, enhances the ability to form concentrated urine in the collecting ducts. Similar to

regulation of water reabsorption, vasopressin regulates urea reabsorption via a cAMP-

dependent pathway and activation of a specialized urea transporter UT1 located in the apical membrane (Shayakul, 1996).

It has been shown that V2 receptors were located not only in the basolateral membrane but also in the luminal membrane of terminal inner medullary collecting ducts (Nonoguchi et al., 1995). Luminal vasopressin inhibited basolateral vasopressin-stimulated water and urea permeability in terminal inner medullary collecting ducts. This suggests that vasopressin acts on both basolateral and luminal sides to regulate water and urea transport in terminal inner medullary collecting ducts. Luminal action of vasopressin serves as a negative feedback system upon basolateral action of vasopressin.

Vasopressin may also enhance NaCl reabsorption in the medullary thick ascending limbs of Henle's loop (Sasaki and Imi, 1980; Hebert and Andreoli, 1984) via $Na+/K+/2Cl-$ cotransport (Hebert and Andreoli, 1984) driven by $Na+/K+-ATPase$ (Jorgensen, 1976). Vasopressin or V2 agonist dDAVP have been shown to stimulate $Na+/K+-ATPase$ (Charlton and Bayliss, 1990) and increased expression of the $Na+/K+/2Cl-$ cotransporter in the medullary thick ascending limbs of Henle's loop (Kim et al., 1999).

Apart from its direct tubular actions, vasopressin vascular actions on the renal hemodynamics discussed in the previous section may also influence sodium and water excretion. Franchini et al. (1997) reported that intravenous infusion of vasopressin, which increased plasma concentration of vasopressin within the physiological range, had no effect on mean arterial blood pressure, total renal blood flow, and blood flow in the renal cortex. However, blood flow in the renal medulla was reduced and was accompanied by decrease in urinary sodium excretion. Renal medullary interstitial

11

infusion of a nitric oxide synthase inhibitor, NG-nitro-L-arginine methyl ester (L-NAME), which inhibited nitric oxide production by stimulation of V2 receptors lowered medullary blood flow and decreased sodium and water excretion (Mattson et al., 1992, 1994, 1997). Thus vasopressin-mediated shifts in blood flow within the kidney appear to alter renal sodium handling.

One of the most important mechanisms by which the kidneys regulate blood pressure is by altering renal excretion of salt and water. Vasopressin actions on regulation of renal sodium and water excretion indicate that vasopressin may play an important role in blood pressure control through its influence on body fluid homeostasis.

2.   Kidney and Vasopressin in Hypertension

2.1   Kidney in Hypertension

Guyton et al. (1974) first proposed that the kidneys are central to the establishment and maintenance of hypertension. Since then numerous studies conducted regarding the role of the kidney in blood pressure control and hypertension support their view. As Abe and Ito (1997) stated 'The kidney is important not only as a target organ of hypertension but also as an organ that may cause hypertension.'

Renal transplantation studies in rats and humans have provided strong evidence for a pivotal role of the kidney in the pathogenesis of hypertension. Renal cross-transplantation has been successfully performed in several rat models of genetic hypertension and their normotensive controls, including spontaneously hypertensive rats (Bianchi et al., 1974; Kawabe et al., 1978; Patchan et al., 1997), stroke-prone spontaneously hypertensive rats (Kopf et al., 1993), Dahl salt-sensitive rats (Dahl and

Heine, 1975; Morgan et al., 1990; Churchill et al., 1992), Milan hypertensive rats

(Bianchi et al., 1977), and Prague hypertensive rats (Heller et al., 1993). It appears that

hypertension "travels with the kidney". Generally, transplantation of a kidney from a

genetically hypertensive rat to a genetically normotensive rat causes the normotensive

recipient to become hypertensive. Conversely, transplantation of a kidney from a

genetically normotensive rat to a genetically hypertensive rat, results in the hypertensive

recipient becoming normotensive. It is particularly interesting that transplantation of a

kidney from an adult SHR which received lifetime antihypertensive treatment with

normalized blood pressure or from a young SHR which has not yet developed

hypertension to an age-matched normotensive rat might still produce hypertension (Rettig

et al., 1990; Kopf et al., 1993). These findings indicated that the genetic background of

the kidney determines the development of hypertension.

Similarly, genetic predisposition to hypertension was also transmitted with the

kidney in humans. Curtis et al. (1983) reported that the blood pressure was completely

normalized in patients with severe hypertension and end-stage hypertensive renal disease

by bilateral nephrectomy and seqsequent transplantation of a kidney from normotensive

donor. Guidi et al. (1985) demonstrated that mean blood pressure in patients receiving a

kidney from donors with a family history of hypertension was significantly higher than

the mean blood pressure in patients receiving a kidney from donors with a normotensive

family history and required more antihypertensive treatment.

Taken together, renal transplantation studies both in rats and in humans supported

a view that the kidney plays a primary role in the pathogenesis of genetic hypertension

and this is probably due to a genetic defect influencing renal function. However, the

underlying mechanisms have not yet been identified. Studies have demonstrated that transplanted SHR kidneys had a decreased capacity to excrete dietary sodium and the development of post transplantation hypertension in normotensive recipients of a SHR kidney was shown to be associated with excess renal sodium retention (Graf et al., 1993; Frey et al., 2000).

The most important mechanism by which the kidney contributes to long-term blood pressure regulation is its ability to excrete sodium and water so that a balance between sodium and water intake and sodium and water excretion is maintained. This in turn maintains body fluid volume and blood pressure. According to the pressure-natriuresis-diuresis theory proposed by Guyton and associates (1974), renal perfusion pressure is a major determinant of sodium and water excretion. An elevation of renal perfusion pressure results in increased excretion of sodium and water. In normotensive subjects, elevation of arterial blood pressure leads to an increase in renal perfusion pressure, which promotes sodium and water excretion, and consequently reduces blood volume and returns arterial blood pressure to normal. In hypertensive subjects, however, the inability of the kidney to excrete sodium and water requires an increased arterial blood pressure to reestablish and maintain sodium and water balance. Any factor that impairs the ability of the kidney to excrete sodium and water and shifts the relationship between sodium excretion and arterial blood pressure toward higher pressures may induce hypertension.

In fact, all forms of experimental models of hypertension studied to date use some maneuver to reduce the ability of the kidney to excrete sodium and water. For example, in DOCA (deoxycorticosterone acetate) salt hypertension, animals were treated with

14

deoxycorticosterone acetate at the same time fed on a high sodium diet. Obviously, this type of experimental hypertension was induced by excess sodium and water retention due to a high sodium intake and an enhanced renal tubular sodium reabsorption. Impaired renal excretory function was also found in genetic models of hypertension. Uyehara and Gellai (1993) demonstrated a reduced renal solute excretion in SHR at 4 weeks of age preceding the development of hypertension, which appeared to be due to an increased renal vascular resistance and a decreased glomerular filtration rate. This supports the theory that an increased blood pressure developed as it was needed to compensate the impaired renal excretory function in SHR. Results reported by Roman and Kaldunski (1988) showing that, medullary vascular resistance was elevated even in very young SHR, also supported Guyton's theory.

Studies have demonstrated that many factors, including neural, hormonal, and humoral factors, may influence the ability of kidney to excrete sodium and water hence arterial blood pressure. Furthermore, many of these factors have been shown to play a role in various forms of hypertension, such as arterial baroreflexes, renin-angiotensin-aldosterone, vasopressin, adrenal catecholamines, atrial natriuretic peptide, endothelial and nitric oxide, kallikrein-kinin system, and prostaglandins (Navar, 1997).

It has long been recognized that vasopressin, with its antidiuretic action on renal tubular system via V2 receptors and its vasoconstrictor effect on renal vasculature via V1 receptors, may influence renal excretion of sodium and water, and participate in body fluid homeostasis and blood pressure control. In addition, elevated concentrations of vasopressin have been found in several forms of hypertension. Therefore, it is reasonable

to believe that vasopressin may be involved in the pathogenesis of hypertension through its renal actions.

2.2   Vasopressin in Hypertension

A possible role of vasopressin in hypertension was first suggested by Page and Sweet (1937) who observed a decrease in blood pressure in dogs with Goldblatt hypertension following hypophysectomy, and thus, presumably removal of vasopressin. Elevated levels of vasopressin have been found in several animal models of hypertension as well as in human essential hypertension. The contribution of vasopressin to the hypertensive process has been examined in animals and in humans to provide evidence that vasopressin is likely to be involved in the development and/or maintenance of hypertension, through its pressor effect, or its antidiuretic effect, or a combination of both effects.

2.2.1   Vasopressin in Animal Models of Hypertension

In DOC-salt hypertension, animals are subjected to unilateral nephrectomy and treated with deoxycorticosterone (DOC) and salt. Mohring et al. (1977) found plasma vasopressin concentrations in DOC-salt hypertensive rats increased three-fold in the benign phase of elevated blood pressure, and ten-fold in the malignant phase of hypertension compared to vasopressin levels in the control animals. Intravenous injection of a specific vasopressin antiserum resulted in a transient reduction of blood pressure to normal or subnormal levels in both phases of hypertension, but especially in the malignant phase. Based on these findings, the authors proposed that vasopressin

16

plays a role as a vasopressor hormone in the pathogenesis of malignant DOC-salt hypertension. Elevated plasma levels of vasopressin in DOC-salt hypertension were also reported subsequently by other investigators (Crofton et al., 1979, 1980; Berecek et al., 1981, 1982; Morton et al., 1982).

In order to determine the role of vasopressin in DOC-salt hypertension, rats with hereditary diabetes insipidus (DI rats) which were unable to synthesize vasopressin were studied. While DI rats treated with DOC and salt or with vasopressin alone failed to develop hypertension (Crofton et al., 1979; Berecek et al., 1982; Saito and Yajima, 1982), those treated with DOC and salt together with vasopressin did develop hypertension (Berecek et al., 1982; Saito and Yajima, 1982). These findings support a view that vasopressin is essential in the onset and/or maintenance of DOC-salt hypertension.

Despite the fact that vasopressin plays a role in the DOC-salt hypertension and it is a potent vasoconstrictor, the involvement of vasopressin as a pressor hormone in this form of hypertension is still uncertain. Elevated plasma vasopressin concentrations found in the animals with DOC-salt hypertension were far below those required to increase blood pressure. This led to the postulation that there is an increased sensitivity to the pressor effect of vasopressin in DOC-salt hypertension (Crofton et al., 1979). Berecek et al. (1982) reported that renal vascular reactivity to vasopressin was increased in DOC-salt hypertensive rats and that the changes occurred as early as 3 days post DOCA treatment and preceded the rise in blood pressure. However, Morton et al. (1982) failed to find enhanced pressor sensitivity to vasopressin.

Specific vasopressin antagonists which block the pressor effect and/or antidiuretic activity of vasopressin were also employed to examine the role of vasopressin in DOC-salt hypertension. Crofton et al. (1979) observed a substantial reduction in blood pressure in rats with DOC-salt hypertension after intravenous injection of vascular vasopressin antagonists dPVDAVP or $d(CH_2)_5VDAVP$. However, using the same vasopressin antagonists, Rabito et al. (1981) failed to observe a fall in blood pressure in DOC-salt hypertensive rats. Okada et al. (1995) administered two orally effective vasopressin analogs, V1 antagonist (OPC-21268) and V2 antagonist (OPC-31260), separately or combined, to rats treated with DOCA and salt for 10 weeks. The V1 antagonist slightly attenuated the increase in blood pressure, but this attenuation was not statistically significant. In contrast, the V2 antagonist could significantly attenuate the increase in blood pressure. Combined administration of V1 and V2 antagonist prevented the increase in blood pressure. In rats treated with DOCA and salt for 3 weeks, V1 antagonist alone did not significantly reduce blood pressure, whereas V2 antagonist alone or a combined V1 and V2 antagonist treatment significantly reduced blood pressure. Okada et al. (1995) concluded that both V1 vascular contraction and V2 volume expansion effects contributed to the development of DOC-salt hypertension, while the V2 effect was also important in the maintenance of high blood pressure at the established stage. These results were inconsistent with the findings by Saito and Yajima (1982) who found that administration of dDAVP, which is a potent antidiuretic agonist of vasopressin with markedly reduced pressor activity, caused an increase in blood pressure. Although the height of blood pressure was lower than that in rats infused with vasopressin with comparable antidiuretic activity, this finding suggested that not only the vasopressor

18

action but also the antidiuretic action of vasopressin is involved in the development of DOCA-salt hypertension.

Pettinger et al. (1986) has demonstrated an increased cAMP response to vasopressin in cortical collecting tubules in DOCA-salt hypertensive rats. This increased response to vasopressin was DOCA dependent and appears to be exaggerated by dietary NaCl.

The Okamoto-Aoki spontaneously hypertensive rat (SHR) is a genetic model of hypertension resembling human essential hypertension.

Elevated levels of plasma vasopressin concentration, urinary excretion of vasopressin, and pituitary vasopressin content have been found in the young SHR during the early developmental phase of hypertension at 33-75 days of age, compared to the Wistar-Kyoto (WKY) normotensive control rats (Crofton et al., 1978). Morhing et al. (1979) also reported that plasma vasopressin concentrations were elevated in a SHR stroke-prone strain (SHRSP) with established hypertension (22-28 weeks of age). They found that the elevation of arterial pressure was correlated with elevated plasma vasopressin concentrations. Plasma vasopressin concentrations in SHR with benign hypertension were elevated twofold, and in SHR with severe or malignant hypertension fourfold, compared to normotensive WKY rats. In addition, intravenous injection of a specific vasopressin antiserum lowered or normalized blood pressure transiently in SHR. The authors concluded that in SHR with established hypertension, plasma vasopressin plays an important role in the maintenance of high blood pressure. However, using the same model of SHRSP as by Mohring, Rascher et al. (1981) reported that vasopressin concentrations were higher than normal only if the rats were more than 24 weeks of age.

In 6-, 9-, 12-week-old rats, plasma vasopressin levels were lower than that of age-match

normotensive WKY rats. Due to the contradictory results, altered vasopressin levels in

blood pressure regulation and fluid homeostasis during hypertension in SHR is unclear.

Specific vasopressin V1 and V2 receptor antagonists were employed to examine

the role of endogenous vasopressin in spontaneous hypertension. Based on the finding

that injection of vascular vasopressin antagonists in SHR failed to produce a significant

fall in blood pressure, Crofton et al (1978) questioned the role of vasopressin in the

pathogenesis of genetic hypertension. Similar result was also reported by Rascher et al

(1982) who did not observe a fall in blood pressure or peripheral resistance in 40-week-

old SHRSP after administration of a vascular vasopressin antagonist $d(CH_2)_5AVP$. In

addition, chronic administration of a V1 antagonist, alone or combined with V2

antagonist for 10 weeks failed to significantly reduce the increases in blood pressure of

SHR rats (Okada et al., 1994). In contrast, Sladek et al. (1988) reported that while

administration of a vasopressin antagonist selective for the V1 receptor alone did not alter

the course of hypertension, administration of a vasopressin antagonist with both V1 and

V2 antagonist properties could attenuate the development of hypertension in SHR.

Results from this experiment also indicated a difference in water balance between SHR

and WKY. SHR exhibited a larger water intake and urine output than WKY at 6 weeks

of age; baseline plasma osmolality was greater in SHR than WKY at 12 weeks of age;

and administration of V1/V2 antagonist resulted in a greater increase in plasma

osmolality in SHR than in WKY. Further study by Hosoya et al. (1996) showed that the

binding capacity for V2 receptors was significantly increased in 12-week-old SHR rats

but not significantly different in 3- and 7- week-old compared with age-matched control

WKY rats. This finding suggested that V2 receptors in the kidney changed with development and played a role in maintaining fluid homeostasis in SHR with established hypertension.

To determine whether the vasopressor and antidiuretic actions of vasopressin may participate in the development of salt-induced hypertension, Hashimoto et al. (1995) examined the long-term effcts of vasopressin V1 and V2 receptor antagonists on blood pressure in Dahl-salt sensitive and salt-resistant rats. Neither the V1 nor V2 receptor antagonists (OPC-21268 or OPC-31260) altered the course of hypertension in DS rats.

Mohring et al. (1978) found that plasma vasopressin concentrations in rats with malignant two-kidney, one-clip Goldblatt hypertension were 4- to 5- fold higher than vasopressin levels in normotensive control rats. Intravenous injection of a special vasopressin antiserum lowered blood pressure transiently in some, but not all of the rats. In contrast, injection of a specific angiotensin II antiserum always induced a transient fall in blood pressure. The authors concluded that the increased release of vasopressin in the hypertensive rats was simply a consequence of sodium and water depletion and activation of the renin-angiotensin system, and they proposed that vasopressin may contribute to the development of malignant renal hypertension via enhanced systemic vasoconstriction. However, administration of antagonists (dDVDAVP or cyclo dDVDAVP) of the vasopressor effect of vasopressin failed to reduce blood pressure in malignant two-kidney, one clip Goldblatt hypertension, and provided evidence against a role of vasopressin-mediated vasoconstriction in the maintenance of high blood pressure in renovascular hypertension (Rabito et al., 1981). Woods and Johnston (1982) also reported elevated levels of plasma vasopressin concentrations in two-kidney, one-clip and

21

one-kidney, one-clip hypertension rats. It was interesting that two-kidney, one clip hypertension and one-kidney, one clip hypertension may be induced in homozygous Brattleboro rats with hereditary hypothalamic diabetes insipidus (Woods and Johnston, 1982). While DI rats developed two-kidney, one-clip hypertension at the same rate and to the same degree as control Long-Evans rats, DI rats with one-kidney, one-clip hypertension had lower blood pressure than that of control Long-Evans rats. However, administration of dDAVP, a V2 selective agonist with high antidiuretic but low pressor activity, elevated the blood pressure in the DI rats to the same level as that seen in the control rats. These findings indicate that the vasopressor effect of vasopressin is not essential for the development of renal hypertension, but that the antidiuretic action of vasopressin, via its effects on body fluid volume, may be important in determining the long-term hypertensive level of blood pressure regulation.

2.2.2   Vasopressin in Human Essential Hypertension

The role of vasopressin in human essential hypertension is not yet clear.

Ellis and Grollman in 1949 first reported that patients with hypertension had increased antidiuretic activity in their urine. Khokhar et al. (1976) found increased urinary excretion of vasopressin in patients with mild essential hypertension. Padfield et al. (1976) found plasma concentration of vasopressin was significantly lower in patients with benign hypertension than in normal subjects, whereas, it was significantly higher in those with malignant hypertension. However, the increased levels of plasma vasopressin in malignant hypertension had no correlation with the blood pressure. Since intravenous infusion of vasopressin in normal subjects which increased plasma vasopressin

22

concentration to a level greater than those seen in malignant hypertension had no effect on blood pressure, it appeared unlikely that vasopressin was involved in the pathogenesis of human essential hypertension by a direct vasoconstrictor effect. Therefore, an increased sensitivity to the vasoconstrictor effects of vasopressin in patients with essential hypertension was proposed and examined by Padfield et al. (1981). Intravenous infusion of vasopressin in patients with mild-moderate essential hypertension to a level in excess of those seen in malignant hypertension produced only minor changes in blood pressure. Based on their findings, and the fact that patients with the syndrome of inappropriate antidiuretic hormone secretion (SIADH) have extremely high plasma vasopressin levels without hypertension, Padfield et al. (1981) concluded that the direct vasoconstrictor action of vasopressin was an unlikely cause of malignant hypertension. It appeared that the raised plasma vasopressin concentrations found in patients with malignant hypertension were a result of volume depletion following salt and water loss, rather than a cause. In contrast to the findings of Padfield and colleagues, Cowley et al. (1981) found that the plasma vasopressin concentration was significantly higher in human subjects with moderate essential hypertension than vasopressin levels of normotensive subjects, and that there was a significant correlation between diastolic pressure and changes in plasma vasopressin, as well as urine sodium concentration. Despite elevated plasma vasopressin concentration, renal concentrating ability appeared to be diminished in hypertensive subjects.

3.	Gender and Vasopressin

3.1	Sexual Dimorphism in Vasopressin Secretion and Excretion

Dai et al. (1996) demonstrated a gender difference in the basal hypothalamic

AVP-mRNA level in Sprague-Dawley rats which was 45% higher in intact males than in

intact females. While the hypothalamic AVP-mRNA level tended to be lower (but not

statistically significant) in castrated males than in intact males, the hypothalamic AVP-

mRNA level in ovariectomized rats was 30% higher than that in intact females.

Gender-related differences in the osmotic control of vasopressin secretion have

been reported. Infusion of hypertonic saline (2.5%) in normal human subjects resulted in

a greater increase in the urinary excretion of vasopressin in women than in men

(Merkelbach et al. 1975). Pretreating men with estrogen for 4 days reduced this

difference (Vallotton et al., 1983). Baylis et al. (1985) found a reduction of basal plasma

osmolality in the luteal phase of the human menstrual cycle, which was caused by a

lowering of the osmotic threshold for the release of vasopressin and for thirst. The

sensitivity of the osmoreceptor-vasopressin-releasing unit was also found to be lowered

in the luteal phase. However, Crofton et al. (1989) did not find a difference in the

sensitivity of the osmotic control of vasopressin release between male and female rats.

Instead, they observed a higher osmotic threshold for vasopressin release in males than in

females in each of the four phases of the estrous cycle.

Skowsky et al. (1979) found serum vasopressin levels varied during the estrous

cycle in rats. Serum vasopressin levels in adult female Wistar rats were 0.6 μU/ml during

diestrous, 4.6 μU/ml on the morning of proestrous, 1.3 μU/ml on the afternoon of

proestrous, and 1.5 μU/ml on the day of estrous, whereas, serum vasopressin levels in

24

adult male Wistar rats were only 0.4 μU/ml, which were similar or lower than those in age-matched females depending upon the phase of the estrous cycle. The serum vasopressin concentration in adult male Wistar rats increased significantly, from 0.4 μU/ml to 2.6 μU/ml after 2 weeks of castration, which was prevented by daily injections of testosterone. In adult female Wistar rats, however, the serum vasopressin concentration was 1.4 μU/ml after 2 weeks of ovariectomy. Daily injections of estradiol resulted in a significant rise in serum vasopressin concentration to 5.0 μU/ml. These authors concluded that the testosterone inhibits vasopressin release in males and estrogen stimulates vasopressin release in females. In contrast, Crofton et al. (1985) reported that the plasma vasopressin concentrations were unchanged during the estrous cycle, which were 0.60 μU/ml at diestrus, 0.68 μU/ml at proestrus, 0.60 μU/ml at estrus, and 0.66 μU/ml at metestrus. Also, the plasma vasopressin concentrations were higher in male rats (1.1 μU/ml) than in female Wistar rats (0.41 μU/ml). Gonadectomy performed at 3 weeks of age reduced plasma vasopressin concentration in males and increased plasma vasopressin concentration in females at 10-11 weeks of age. Treatment of castrated males with testosterone increased vasopressin release, whereas treatment of ovariectomized female rats with estrogen had little effect on the plasma concentration of vasopressin. Moreover, Crofton et al. (1986) reported that the higher plasma vasopressin concentration in male than in female rats was due to a higher rate of secretion of the hormone since the metabolic clearance rate of vasopressin did not differ between male and female rats.

Variations in plasma concentrations of vasopressin were also reported in women with normal menstrual cycles. Vasopressin levels were highest at the time of ovulation and lowest at the onset of menstruation (Forsling et al., 1981). Administration of estradiol augmented vasopressin release in postmenopausal women (Forsling et al., 1982).

In rats, the 24-hour urinary excretion of vasopressin was higher in males than in females (Crofton et al., 1985). This gender-related difference in urinary excretion of vasopressin in rats was abolished by gonadectomy as evidenced by a reduction in urinary vasopressin excretion in castrated males and an increase in that of ovariectomized females. Treatment of castrated male rats with testosterone and ovariectomized female rats with progesterone alone or in combination with estradiol restored the gender-related differences.

In normal human subjects, Merkelbach et al. (1975) found that the 24-hour urinary excretion of vasopressin was approximately two times higher in men (70 ng) than in women (34 ng). Similarly, Crofton et al. (1986) reported the 24-hour urinary excretion of vasopressin was higher in black and white men than in black and white women on both normal and low sodium diets.

Taken together, findings from both animals and humans suggest that gonadal steroid hormones can modulate the secretion and excretion of vasopressin.

3.2 Sexual Dimorphism in Vasopressin Biological Actions

3.2.1 Sexual Dimorphism in Vasopressin Cardiovascular Actions

In 1938, Byrom observed an enhanced sensitivity of the rat to vasopressin with preliminary treatment with estrogen. Later, Lloyd (1959) found that intravenous administration of vasopressin caused a greater pressor response at the time of estrus than at other times in female rats. Their studies suggested that the gonadal steroid hormones could modulate the vascular actions of vasopressin.

In conscious, chronically instrumented hydrated rats, graded intravenous infusion of vasopressin resulted in a greater pressor response in males and estrous females than in females in diestrus, proestrus, and metestrus (Crofton et al., 1986, 1988). This greater pressor response to vasopressin was due to greater increases in total peripheral resistance in males and estrous females than in females in the other phases of the estrous cycle, since there were no gender- or cycle-related differences in the vasopressin-induced reductions in cardiac output (Toba et al., 1991). Gonadectomy was without effect on the pressor response to vasopressin in males, but in gonadectomized females the pressor response was increased to a level similar to that observed in males. Chronic treatment of ovariectomized rats with estradiol restored the pressor response to vasopressin to levels similar to those in nonestrous intact females, whereas, treatment of ovariectomized rats with progesterone was without effect. Further studies showed that vasopressin has a greater vasoconstrictor action in the mesenteric vascular beds in male than in female rats (Wang et al., 1997).

3.2.2. Sexual Dimorphism in Vasopressin Renal Actions

A number of studies have indicated that the gonadal steroid hormones modulate the antidiuretic action of vasopressin. Crofton et al. (1985) found a higher urine osmolality and a lower urine volume in intact male rats than in intact female rats with free access to food and water. In conscious, chronically instrumented hydrated rats, intravenous infusion of vasopressin at the rates of 3-100 pg/kg/min resulted in a dose-dependent antidiuresis, which was significantly greater in intact male and estrous female rats than in intact female rats in the other phases of the estrous cycle (Wang et al., 1993; 1994). Urine volume and free water clearance decreased and urinary osmolality increased in both male and female rats without significant changes in mean arterial blood pressure, heart rate, osmolar clearance, and urinary sodium and potassium excretion. Similar results were also found in rats after 24 h water deprivation, where urine flow was significantly higher and urine osmolality lower in females than in males. Gonadectomy was without effect on the antidiuretic response of vasopressin in males, but in gonadectomized females, the antidiuretic response to vasopressin was enhanced to a level similar to that observed in intact males (Wang et al., 1994). Estradiol attenuated and restored the antidiuretic response of vasopressin in ovariectomized female rats, whereas progesterone, alone or in combination with estradiol, had no effect (Wang et al., 1995).

Wang et al. (1993) reported that the greater antidiuretic action of vasopressin in male than in female rats was associated with an increased number of vasopressin V2 receptors and a greater ability of vasopressin to stimulate adenylate cyclase in papillary collecting duct cells in male than in female rats.

28

Estrogen receptors have been found in the kidney (Christy et al., 1974). While Hatano et al. (1988) reported that physiological concentrations of estradiol and progesterone significantly reduced the cAMP response to vasopressin in both rat and human renal cells, Wang et al. (1995) reported that it is estrogen, not progesterone that reduces the antidiuretic response to vasopressin in the female rat. Regardless of whether progesterone may be involved, both studies suggested that estrogen contributed to the sex differences of antidiuretic activity of vasopressin. There is evidence that estradiol stimulates the renal production of prostaglandin E2 that inhibits the antidiuretic activity of vasopressin by attenuation of vasopressin-stimulated cAMP accumulation. Wang et al. (1997) demonstrated that inhibition of prostaglandin synthesis by the cyclooxygenase inhibitor indomethacin abolished the sexually dimorphic antidiuretic action of vasopressin by enhancing the antidiuretic action of vasopressin to a greater degree in nonestrous female rats than in male rats.

4.   Sexual Dimorphism in Hypertension

A sexually dimorphic pattern in the development of hypertension is evident in both laboratory animals and human subjects: high blood pressure develops more rapidly and/or severely in males than in females. Gonadectomy and/or treatment of gonadal steroids that could alter the course of hypertension development in both males and females suggest that the gonadal steroids might play a role.

4.1   Sexual Dimorphism in Animal Models of Hypertension

Sexual dimorphism in hypertension has been manifested in a number of experimental animal models of hypertension, including deoxycorticosterone (DOC)-salt hypertensive rats, Dahl salt hypertensive rats, spontaneously hypertensive rats, and rats with induced renal hypertension.

In the deoxycorticosterone acetate (DOCA)-salt hypertension model where animals are subjected to unilateral nephrectomy and treatment with DOCA and salt, both male and female rats developed hypertension in response to treatment with DOC and salt. Hypertension development was more rapid in male than in female rats, and the systolic blood pressure was 20-30 mmHg higher in male than in female rats (Ouchi et al., 1987, 1988). This sexual dimorphism was abolished by gonadectomy, which attenuated the development of hypertension in males and exacerbated it in females (Crofton et al., 1989). Estrogen attenuated the course of the hypertension in male and in gonadectomized female rats, whereas, testosterone exacerbated the development of the hypertension in gonadectomized male rats but had no effect in females (Crofton and Share, 1997).

In spontaneously hypertensive rats, blood pressure increases progressively with age. Gonadectomy performed at 30 days of age, when the animals were still normotensive retarded the development of high blood pressure when they matured (Iams and Wexler, 1977). In both intact and gonadectomized rats, the blood pressure levels were higher in males than in females. Similar results were reported by Masubuchi et al. (1982) where the systolic blood pressure levels in intact SHR were 194-208 in males versus 163-173 mmHg in females when they were 29-30 weeks old. Gonadectomy

30

performed at 17 weeks of age resulted in a significant reduction in blood pressure, to 168-175 in males versus 158-163 mmHg in females. Treatment with estrodial inhibited the rise in blood pressure in intact or gonadectomized male and female rats (Iams and Wexler, 1979). Cambotti et al. (1984) reported that neonatally castrated males showed a similar blood pressure increase to that of intact females, while neonatally androgenized females exhibit a pattern of blood pressure similar to intact males. Similar results were reported by Ganten et al. (1989). Surgical castration and treatment with testosterone receptor antagonists reduced blood pressure in young (9 weeks of age) hypertensive rats. However, these treatments had no effect on high blood pressure in 25-week-old hypertensive rats.

Androgen receptor antagonists attenuated high blood pressure development when given for the first 10 days after birth. In spontaneously hypertensive rats on a high salt diet, males developed high blood pressure more rapidly than females (Blizard et al., 1991). In addition, increases in blood pressure were accompanied by increased urinary protein excretion and increased renal vascular lesions, which were more severe in males than in females. Chen and Meng (1991) found the systolic blood pressure levels of intact SHR were 244 ± 6 mmHg in males and 205 ± 3 mmHg in females in 22 weeks of age. Orchidectomy at age 4 weeks significantly attenuated the systolic blood pressure elevation in the males (195 ± 4 mmHg at age 22 weeks), but ovariectomy at age 4 weeks had no effect on the development of hypertension in the females. Administration of testosterone to gonadectomized rats of both sexes conferred a male pattern of high blood pressure development. These results indicated that the sexually dimorphic pattern of hypertension in the SHR is androgen dependent, rather than estrogen dependent. Plasma

norepinephrine levels did not differ between the sexes, nor were they altered by gonadectomy or testosterone replacement, suggesting that the higher blood pressures in the intact male and androgen treated male and female SHR are not dependent on increased sympathetic outflow in the established phase of hypertension. Reckelhoff et al. (1998) reported that systolic blood pressure in SHR at 12 weeks of age was significantly higher in males (195 ± 3 mmHg) than in females (168 ± 3 mmHg) and males castrated at 4 weeks of age (173 ± 4 mmHg). Treatment with testosterone to overiectomized females caused arterial pressure to increase (175 ± 2 mmHg), which was significantly higher than in intact females, castrated male, or untreated ovariectomized females (158 ± 2 mmHg).

In Dahl salt-sensitive rats fed a high salt diet at 6 weeks of age, the male rats developed hypertension more rapidly than the age-matched female rats (Dahl et al., 1975). The mean systolic blood pressure of the males was 194 ± 4.20 mmHg at 10 weeks of age, while that of the females was only 162.8 ± 8.70 mmHg. It was not until 20 weeks of age that the mean systolic blood pressure of the females reached the same level (200.9 ± 7.66 mmHg) as that of male rats. Similarly, Crofton et al. (1993) reported that the systolic blood pressure was approximated 20 mm Hg higher in male than in female Dahl salt-sensitive rats after 3 weeks of high-salt diet treatment, which was started at 7 weeks of age. Dahl et al. (1975) found that gonadectomy performed at 6 week of age had no influence in the development of hypertension in male rats but enhanced the rate of hypertension development in female rats to be equivalent to that of male rats. Rowland et al. (1992) reported that castration of neonatal male Dahl salt-sensitive rats attenuated the development of hypertension (in contrast to findings of no influence on hypertension development reported by Dahl et al (1975)), whereas, neonatal female rats treated with

testosterone exacerbated the development of hypertension. Administration of estradiol had no effect on hypertension in either ovariectomized females or intact males. Two weeks of 8% sodium diet in Dahl salt-sensitive rats caused blood pressure to increase significantly higher in intact male rats and ovariectomized female rats than in intact females: 152 ± 4, 154 ± 5, and 141 ± 3 mmHg, respectively (Hinojosa-Labode et al., 2000).

Induction of renal hypertension in male and female Sprague-Dawley rats using the Goldblatt two-kidney, one-clip (2K1C) model, Okuniewski et al. (1998) found that the success rate of induction of hypertension was higher in males (83%) and overiectomized females (78%) than in intact females (53%). Blood pressure increased more rapidly in male and ovariectomized female hypertensive rats, although no difference was found in the severity of hypertension in male and female rats.

4.2   Sexual Dimorphism in Human Essential Hypertension

Longitudinal and epidemiological studies have shown that blood pressure increases with age and a sexual dimorphism exits in the development of hypertension. Generally, men have higher blood pressures and a higher prevalence of hypertension.

According to the Community Hypertension Evaluation Clinic Program, which screened more than 1 million Americans between 1973 and 1975, found mean diastolic blood pressure (DBP) was higher in men than in women at all ages, whereas mean systolic blood pressure (SBP) was higher in men than in women until age 50 for blacks and until age 65 for whites, and was higher in women thereafter (Stamler et al., 1976). The National Health and Nutrition Examination Survey II examined the blood pressure of

33

16,204 Americans aged 6-74 years between 1976 and 1980, and found mean DBP was higher in adult men than in women at all ages, whereas mean SBP was higher in men until the age of 54 years, but was higher in women in the oldest (post-menopausal) age group (Rowland and Roberts, 1982). Similar results were also reported by Kotchen and his colleagues (1982) who found that SBP of males was higher than that of females between ages 16 and 40 years, whereas above age 60, females had higher SBP. The Hypertension Detection and Follow-up Program Cooperative Group (1977) screened 158,906 persons, aged 30 to 69 years, in 14 communities between 1973 and 1974 to identify those with a DBP of 95 mmHg or higher, and found that hypertension was more prevalent in men than in women both black and white.

Hypertension, defined as SBP $\geq$ 160 mmHg, DBP $\geq$ 95 mmHg, was more common in men in younger age groups, but tended to be more common in women over the age of 64 than in age-matched men (Cornoni-Hhuntley et al., 1989).

The Dormont High School Follow-up Study examined the changes of blood pressure over a 30-year period in the Dormont High School cohort of 86 men and 116 women with mean ages of 17 years during high school (1957-1963), 34 years at follow-up I (1977-1978), and 47 years at follow-up II (1989-1990) (Yong et al., 1993). There was relatively little change in mean SBP during the 30-year period, while the increase in mean DBP was significantly higher in men than in women. Data from phase I of the Third National Health and Nutrition Examination Survey, collected from 9901 participates 18 years of age and older from 1988 through 1991, indicate that 24%, or an estimated 43 million of American adults had hypertension (SBP $\geq$ 140 mmHg, DBP $\geq$ 90 mmHg. In all age and racial (non-hispanic blank, non-hispanic white, and Mexican

34

American) groups surveyed, men had average SBP and DBP that were 6-7 and 3-5 mmHg higher, respectively, than the average values in women. In each of the gender-race groups, age was associated not only with an increasing prevalence, but also with an increasing severity of hypertension. Mean values for SBP were lower in women than men during early adulthood (18-49 years of age), but the subsequent rate of rise of blood pressure with age was steeper for women than for men. As a result, average SBP for women in each of the three racial/ethnic groups was as high as or higher than the corresponding value for men during and after the seventh decade (Burt et al., 1995). These results indicate that any protective effect of gonadal hormone in females before menopause is gone after menopause.

4.3   Vasopressin Sexual Dimorphism in Hypertension

Despite the fact that vasopressin may contribute to some forms of hypertension and there are gender differences in the secretion and actions of vasopressin, as well as in the development of hypertension, the possible role of vasopressin in the gender difference of hypertension or the influence of gender on vasopressin actions in hypertension has not yet been fully investigated.

Vasopressin is essential for the development and maintenance of DOC-salt hypertension contributing via both its antidiuretic action and its vasopressor action (Morhing et al., 1977; Saito et al., 1979; Crofton et al., 1979, 1980). A gender difference in the development of DOC-salt hypertension has been demonstrated where male rats develop hypertension rapidly and to a higher level than female rats (Ouchi et al., 1987, 1988; Crofton et al., 1989, 1997; Stallone, 1995). Plasma vasopressin concentration and

35

24-hour urinary excretion of vasopressin were significantly higher in DOC-salt

hypertensive rats than in control rats, however, there was no difference between male and

female rats (Ouchi et al., 1987). Since the metabolic clearance rate of vasopressin did not

differ from male and female DOC-salt hypertensive rats (Ouchi et al., 1987), it was

unlikely that the gender difference in the development of DOC-salt hypertension was due

to the difference in vasopressin secretion. Ouchi et al. (1988) reported that there was a

greater pressor responsiveness to graded intravenous infusion of vasopressin in male than

in female DOC-salt hypertensive rats. In addition, a depression of baroreflex sensitivity

was observed in male and female DOC-salt hypertensive rats, which was greater in males

that in females. These authors concluded that the diminished pressor responsiveness to

vasopressin and a smaller impairment of baroreflex sensitivity might contribute to the

reduced development of DOC-salt hypertension in female rats. Although the antidiuretic

action of vasopressin is also a major contributing factor to the DOC-salt hypertension, the

gender effect on the vasopressin antidiuretic action in DOC-salt hypertension has not yet

been reported.

Similar to DOC-salt hypertensive rats, plasma vasopressin levels have been

shown to be increased in Dahl-salt sensitive rats fed on a high salt diet. In this model,

male rats developed hypertension more rapidly than female rats. Vasopressin V1

receptor antagonist in males prevented further increase in systolic blood pressure during

the first week of treatment, but systolic blood pressure rose thereafter towards the levels

found in the untreated males. In females, V1 receptor antagonist had no effect (Crofton

et al., 1993).

Elevated levels of vasopressin have been reported in SHR, with male rats developing hypertension more rapidly than female rats. Burrell et al. (1995) demonstrated a gender difference in the efficacy of a nonpeptide vasopressin V1a receptor antagonist OPC-21268 to lower blood pressure, which is greater in male than in female SHR.

In human patients with essential hypertension, plasma vasopressin levels were significantly higher in hypertensive men than in normal men (Cowley et al., 1985). In addition, there was a significantly positive correlation between plasma vasopressin levels and both systolic and diastolic blood pressure in men. However, in women with comparable age and similar levels of hypertension as men (men, 146/100; women, 153/102 mmHg), elevated levels of plasma vasopressin were not found. This suggested that there is a gender difference in the development of hypertension and vasopressin may contribute to this gender difference.

5.   Summary

Vasopressin is important in the regulation of body fluid volume and the maintenance of blood pressure. Studies have shown that vasopressin may play an important role in the development and maintenance of hypertension in several animal models and further, that its antidiuretic action may be involved. Recent studies found that the release and excretion of vasopressin, as well as the cardiovascular action and antidiuretic action of vasopressin, are greater in males than in females, but the physiological and pathological consequences of these gender differences are not clear. Gender difference has also been found in the development of hypertension in several

animal models where high blood pressure develops more rapidly and to a higher level in males than in females. In clinical studies, the prevalence of hypertension and the mortality due to cardiovascular disease is greater in men than in women. However, whether vasopressin gender differences are involved in the gender difference of hypertension has not yet been determined.

This dissertation will test the hypothesis that a gender effect on vasopressin renal action contributes to gender differences in hypertension. If antidiuretic action of vasopressin indeed is involved in hypertension, altered fluid handling by the kidney in hypertensive rats compared with normotensive control rats should be evident. Further, if the gender effect on vasopressin action contributes to a difference in the degree of hypertension development, a difference in the magnitude of a gender effect in normotensive vs hypertensive rats may by evident.

In this study, the effect of gender on the antidiuretic activity of vasopressin in the spontaneously hypertensive rat (SHR) was examined. Specifically, the followings were compared in male SHR and WKY and female SHR and WKY during the estrous and nonestrous phases of the estrous cycle:

1) Renal function as measured by renal clearance studies.
2) The antidiuretic activity of exogenous vasopressin to determine possible hypertension-induced changes in renal sensitivity.
3) The contribution of endogenous vasopressin to hypertension-induced differences in renal fluid handling.

In addition, vasopressin V2 receptors play an important role in mediating the development of hypertension via altered antidiuretic action of vasopressin on the kidneys of hypertensive animals. If antidiuretic action of vasopressin indeed is involved in

38

hypertension, altered renal fluid handling due to an alteration of V2 receptor expression in the kidney as well as their intracellular responses may also be possible. Thus, expression of V2 receptors in the kidney of male SHR and WKY were also compared.

CHAPTER II

MATERIALS AND METHODS

1.   Animals

Age-matched adult male and female Spontaneously hypertensive rats (SHR) and

their normotensive counterpart, Wistar-Kyoto (WKY) rats were used in this study.

SHR is a genetic model of hypertension resembling human essential hypertension.

High blood pressure develops spontaneously in SHR with age. According to the study by

Uyehara and Gellai (1993), SHR has normal mean arterial blood pressure at 4 weeks of

age. Mean arterial blood pressure then increases progressively and hypertension is fully

developed approximately at 12 weeks of age. In this study, WKY rats served as

normotensive controls.

The reason for choosing this animal model is that the effect of gender on the renal

actions of vasopressin could be studied in a model where hypertension develops in intact

animals without artificial manipulation of the renal arteries and the kidneys. In addition,

there was no additional salt required for the development of hypertension. This avoided

complications of varied contributions of degree of salt loading or other effects on renal

function that might occur in other animal models of hypertension, in which the animals

are subjected to nephrectomy and/or constriction of the renal artery, as well as being fed a

high salt diet, such as in DOCA-salt hypertension, Goldblatt hypertension, and Dahl salt-

sensitive hypertension.

Adult female SHR (Okamoto strain, Taconic Farms) and WKY rats (Okamoto

strain, Taconic Farms) were bred in-house for timed pregnancies to ensure precise birth

40

dates. Rat pups were weaned 18-21 days after delivery and segregated in groups by sex. Both male and female rats were used for the subsequent in vivo studies. Surgical implantation of vascular and stomach catheters as well as bladder cannula in rats for in vivo studies was performed when the rats were 11 to 15 weeks of age. In vivo experiments were conducted one week after surgery, when the rats were 12 to 16 weeks of age, and when they were fully recovered from surgery. By this age, hypertension was also fully established. Only male rats at 4 weeks and 12 to 16 weeks of age were used for the in vitro study examining renal V2 receptor expression.

The stage of the estrous cycle in female rats was determined by daily microscopic examination of vaginal smears previously described by Hafez (1970). At lease one complete estrous cycle (4-6 days in length) was followed before a rat was used in an experiment. In addition, vaginal smears were always obtained immediately before and after each experiment.

Animals were housed in a room with controlled temperature and lighting (lights on from 0600 to 1800) and allowed free access to food and tap water.

All surgical and experimental procedures performed in this study were reviewed and approved by the Tripler Army Medical Center Animal Care and Use Committee and procedures were done in accordance with National Institutes of Health Guidelines for the care and use of animals.

2.   Surgical Preparation

Eleven to 15-week-old age-matched male and female SHR and WKY rats were

surgically prepared as described previously by Uyehara and Gellai (1993), and Gellai and

Valtin (1979).

With the use of sterile surgical procedures, rats were anesthetized with ketamine

hydrochloride (60 mg/kg body weight, i.m.) and pentobarbital sodium (21 mg/kg body

weight, i.p.) for chronic implantation of vascular and stomach catheters as well as bladder

cannula.

Vascular catheters were made of 0.030 inch O.D. medical grade Tygon Microbore

tubing (S-54-HL, Norton Performance Plastics, Akron, Ohio). One vascular catheter was

implanted in the abdominal aorta via left femoral artery for blood pressure recording and

blood sampling. Another vascular catheter was implanted into the abdominal vena cava

via the femoral vein for solution infusion and drug administration.

The stomach catheter was made of 0.040 inch O.D. medical grade Tygon

Microbore tubing (S-54-HL, Norton Performance Plastics, Akron, Ohio) and implanted

for water loading or replacement of water lost during the experiment.

All of the catheters were tunneled subcutaneously and externalized at the back of

the neck. Vascular catheters were filled with a 25% heparin-25% dextrose solution, while

the stomach catheter was filled with sterile water. Then, all of the catheters were plugged

with stainless steel pins.

The bladder cannula was constructed of a stainless steel tube (14-16 G, 1-1.5 cm

long) covered with a silastic tubing (0.125 inch O.D., Dow Corning, Midland, MI) and

implanted into the bladder for urine collection through a suprapubic midline incision and brought directly through the adjacent abdominal wall.

After surgery, rats were housed in individual cages with free assess to food and water. Every other day after surgery, vascular catheters were maintained by flushing with sterile saline and filling with a 25% heparin-25% dextrose solution, stomach catheter was maintained by flushing and filling with sterile water, and bladder cannula was maintained by flushing with 1% neomycin (Monarch Pharmaceuticals, Bristol, TN) in sterile water.

The animals were allowed at least 4 to 7 days for recovery before any experiment procedures were conducted. Before surgery and during recovery, each rat was trained 2 to 3 times to rest quietly in a plastic experiment cage designed specifically for renal clearance studies (Braintree Scientific, Inc., Braintree, MA) for the duration of an experiment.

3.   Experimental Procedures: In vivo Studies

3.1   Experimental Preparation

On the day of the experiment, a rat with indwelling vascular catheters and bladder cannula was weighed and placed in a plastic experiment cage designed specifically for renal clearance studies. The arterial catheter was connected to a pressure transducer (model 5900, Gould Inc., Valley View, Ohio) and recorder (model RS 3800, Gould Inc., Valley View, Ohio) for measurement of mean arterial pressure and collection of blood samples. The venous catheter was connected to a syringe pump (model 355, Sage Instruments, Cambridge, MA) for infusion of fluid and administration of drugs. The stomach catheter was attached to a syringe filled with sterile water for water loading or

43

replacement. The bladder cannula was extended with a silastic tube to facilitate urine collection into pre-weighed tubes. Blood pressure was monitored and urine was collected throughout the experiment. Urine volume was measured gravimetrically. Body weight was recorded before and after the experiment to assess maintenance of fluid balance throughout the experiment.

3.2   Experimental Protocol 1: In vivo Assessment of Maximum Urine Concentrating Ability of SHR.

Antidiuretic actions of the vasopressin V2 receptor agonist, [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) in adult SHR males (n=7) and SHR females (n=9) were compared to the antidiuretic actions in age-matched WKY males (n=6) and WKY females (n=8). Antidiuretic actions of dDAVP were also compared in female rats during estrous (SHR n=7, WKY n=5) and nonestrous (SHR n=9, WKY n=8) phases of the estrous cycle.

The experimental protocol was modified from that previously described by Wang et al. (1993) and is shown in figure 1. At the beginning of the experiment, sterile water in the amount of 2% body weight was slowly administered intragastrically over 5 min. This was followed by a constant intragastric infusion of sterile water at a rate of 80 μl/100 g body weight/min for an equilibration period of 90-120 minutes. This hydration procedure might suppress endogenous vasopressin secretion and created a high but constant urine flow rate that exogenous vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) could act on, which was approximately equivalent to the fluid infusion rate. Simultaneously, para-aminohippurate sodium (PAH, 1%) and polyfructosan (Inutest, 10%) in 0.45% sodium chloride solution was infused intravenously at a rate of 10

44

μl/100 g body weight/min during the experiment for assessment of renal blood flow and glomerular filtration rate. After the hydration procedure, two 15-min control urine samples were collected. A blood sample (0.2-0.4 ml) was taken between the two control urine collections. At the same time, a small amount of blood was also taken into microcapillary tubes for measurement of hemotocrit. Then, the blood sample was centrifuged and plasma was separated for analysis of osmolality and concentrations of sodium, potassium, PAH, and polyfructosan. The red blood cells were returned in an equivalent volume of 0.9% sodium chloride within 5 min of blood sampling to maintain the constant composition of the blood. Vasopressin V2 receptor agonist dDAVP (1 μg/ml) diluted with 0.45% sodium chloride solution containing 1% PAH and 10% polyfructosan were then infused intravenously at increasing doses of 0.3, 1, 3, 10, 30, 100, and 300 pg/100 g body weight/min over a 30 min period for each dose. Urine was collected into pre-weighed tubes for 2 x 15 min and the tubes were kept in ice during the experiment. According to the preliminary data from Dr. Uyehara's lab, a steady-state response to dDAVP could be achieved within15 min after intravenous infusion of dDAVP. Therefore, the second 15 min urine collection in each infusion period was used for measurement of urinary osmolality and concentrations of sodium, potassium, urea nitrogen, PAH, and polyfructosan after the experiment. Due to the antidiuretic action of dDAVP, urine flow rate decreased in response to dDAVP infusions. Therefore, the infusion rate of water into the stomach was adjusted to equal to urine flow rate after each urine collection, in order to maintain a constant level of hydration in the rat. At the end of the experiment, a second blood sample was obtained. A small amount of blood was taken in microcapillary tubes for measurement

Figure 1. Experimental protocol 1: in vivo assessment of maximum urine concentrating ability of SHR using vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP).

H₂O (2% BW, i.g.)

↓

H₂O i.g. infusion (80 µl/100 g BW/min)	H₂O i.g. infusion, rate adjusted to equal urine flow rate						
1% *p*-aminohippurate and 10% polyfructosan in 0.45% NaCl solution i.v. infusion (10 µl/100 g BW/min)							
	dDAVP i.v. infusion (pg/100 g BW/min)						
Equilibration	0	0.3	1	3	10	30	100	300
90-120 min	U1,2 15 min x 2	U3,4 15 min x 2	U5,6 15 min x 2	U7,8 15 min x 2	U9,10 15 min x 2	U11,12 15 min x 2	U13,14 15 min x 2	U15,16 15 min x 2

of hemotocrit. Then, the blood sample (0.2 to 0.4 ml) was centrifuged and plasma was separated analysis of osmolality and concentrations of sodium, potassium, PAH, and polyfructosan. The red blood cells were returned in an equivalent volume of 0.9% sodium chloride within 5 min of blood sampling to maintain the constant composition of the blood.

Vasopressin V2 receptor agonist [deamino-Cys[1],D-Arg[8]]-vasopressin (dDAVP) was purchased from Sigma Chemical Co. (St. Louis, MO). dDAVP was dissolved in a solution containing 0.9% sodium chloride and 0.03% Acetic Acid at the concentration of 1 μg/ml and stored in 350-μl aliquots at $-20^{\circ}C$ for the duration of the study. Prior to use, an aliquot was thawed and further diluted with 0.45% sodium chloride solution containing 1% para-aminohippurate and 10% polyfructosan.

Para-aminohippurate sodium (PAH) was purchased from Merck Sharp & Dohme (West Point, PA). Polyfructosan (Inutest) was purchased from Laevosan Gesellshaft (Linz-Donau, Austria).

3.3   Experimental Protocol 2: In vivo Assessment of Endogenous Vasopressin on Renal Water Handling Ability.

Responses to vasopressin V2 receptor antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin in adult SHR males (n=8) and SHR females (n=8) were compared to the responses in age-match WKY males (n=10) and females (n=10). Responses to vasopressin V2 receptor antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin were also compared in female rats during estrous (SHR n=5, WKY n=8) and nonestrous (SHR n=8, WKY n=10) phases of the estrous cycle.

48

The experimental protocol is shown in figure 2. 0.9% sodium chloride solution was infused intravenously at a rate of 10 μl/100 g body weight/min during the experiment to keep the animal euhydrated. The animal was allowed to equilibrate for 90 to 120 min. Then, two 15-min control urine samples were collected. A blood sample (0.2-0.4 ml) was obtained between the two control urine collections. At the same time, a small amount of blood was also taken into microcapillary tubes for measurement of hemotocrit. Then, the blood sample was centrifuged and the plasma was separated for analysis of osmolality and sodium and potassium concentration. The red blood cells were returned in an equivalent volume of 0.9% sodium chloride within 5 min of blood sampling. V_2 receptor antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-Vasopressin in 5% dextrose in water at doses of 0.3, 1, 3, 10, 30, and 100 μg/kg body weight was injected intravenously in a bolus volume of 50 μl/100 g body weight. Each bolus dose of V2 receptor antagonist injected intravenously was followed by several 5 min urine collections until the maximum urine flow in response to the dose was reached. Then, the next higher dose was administered. Urine loss was replaced with an equivalent volume of water via the stomach catheter to maintain adequate hydration. After the last urine collection, a blood sample was obtained. A small amount of blood was also taken into microcapillary tubes for measurement of hemotocrit. Then, the blood sample (0.2-0.4 ml) was centrifuged and the plasma was separated for analysis of osmolality and sodium and potassium concentration. The red blood cells were returned in an equivalent volume of 0.9% sodium chloride within 5 min of blood sampling.

49

Figure 2. Experimental protocol 2: in vivo assessment of endogenous vasopressin renal action of SHR using vasopressin V_2 receptor antagonist [1-Adamantane acetyl^1, D-Tyr(Et)^2, Val^4, Abu^6, Arg^9]-arg^8-vasopressin

0.9% NaCl solution i.v. infusion (10 μl/100 g BW/min)							
	H$_2$O i.g. infusion, rate adjusted to equal urine flow rate						
	V2 antagonist i.v. injection (μg/kg BW in 50 μl/100 g BW) in 5% dextrose						
Equilibration	0	0.3	1	3	10	30	100
90 min	U1,2 15 min x 2	U 5 min x	U 5 min x	U 5 min x	U 5 min x	U 5 min x	U 5 min x

Vasopressin V_2 receptor antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin was purchased from Sigma Chemical Co. (St. Louis, MO). It was dissolved in a solution containing 0.9% sodium chloride and 0.03% Acetic Acid at the concentration of 1 mg/ml and stored in 200-µl aliquots at $-20^{\circ}C$ for the duration of the study. Prior to use, an aliquot was thawed and further diluted with 5% dextrose in sterile water.

3.4   Sample Analysis

3.4.1   Hematocrit, Osmolality and Electrolytes

Blood samples collected into microcapillary tube were analyzed for hematocrit immediately after experiment. Blood hematocrit was measured with a microhematocrit centrifuge.

Plasma and urine samples for osmolality and electrolytes were kept in ice or $4^{\circ}C$ refrigerator and analyzed within 24 to 48 hours after experiment. Plasma samples were analyzed for osmolality, and concentrations of sodium and potassium. Urine samples were analyzed for osmolality, concentrations of sodium, potassium, and urea nitrogen. Plasma and urine osmolalities were determined by the method of depression of freezing point (The Advanced Micro-Osmometer, Model 3MO, Advanced Instruments, Inc., Needham Heights, MA). Plasma concentrations of sodium and potassium were measured using 614 ISE NA+/K+ Analyzer (Giba Corning Diagnostics Corp., Medfield, MA). Urine concentrations of sodium, potassium and urea nitrogen were measured using SYNCHRON CX3 Delta Clinical System (Beckman Instruments, Inc., Fullerton, CA).

3.4.2 Para-aminohipurate (PAH) and Polyfructosan

Plasma and urine samples from experimental protocol 1 were also analyzed for concentrations of para-aminohipurate (PAH) and polyfructosan. Samples were diluted and deproteinized on the day of the experiment. Plasma samples for PAH assay were diluted and deproteinized by adding 1 ml of 3.2% Trichloroacetic acid (TCA) to 30 µl of each plasma sample. Plasma samples for polyfructosan assay were diluted and deproteinized by adding 200 µl of 3.2% TCA to 20 µl of each plasma sample. Urine samples were diluted by adding 1 ml of 3.2% TCA to 10 µl of each urine sample for both PAH and polyfructosan assays. Diluted plasma and urine samples were stored in 4 °C refrigerator and analyzed within one week of sampling.

Plasma and urine concentrations of PAH were measured using the method of Smith et al. (1945). On the day of assay, a second dilution of urine sample was made according to urine flow rate during the period in which the urine was collected, so that the estimated PAH concentration would fall within the range of the standard curve.

PAH at concentrations of 0, 1, 2, 4, 6, and 10 mg% were prepared for the standard curve. Two aliquots of 0.5 ml from each standard or sample were dispensed into 12x75 borosilicate tubes. 100 µl of 1.2 N HCl and 50 µl of 0.1% sodium nitrite were added to each tube. The tubes were vortexed and stood at room temperature for 3 to 5 min. 0.5% ammonium sulfamate was then added to each tube and the tubes were vortexed. After 3 to 5 min, 50 µl of 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride (N-ED) was added to each tube and the tubes were vortexed. The tubes were let stand at room temperature for at least 1 hour. The absorbency of each sample was then measured at

540 nm on a spectrophotometer (spectronic 21, Milton Roy, Rochester, NY). Plasma and

urine concentrations of PAH were used to calculate effective renal blood flow rates.

Plasma and urine concentrations of polyfructosan were measured using the

anthrone method (Führ et al., 1955). On the day of assay, a second dilution of urine

sample was made according to urine flow rate during the period in which the urine was

collected, so that the estimated polyfructosan concentration would fall within the range of

the standard curve.

Polyfructosan at concentrations of 0, 25, 50, 100, 200, and 300 mg% were

prepared for the standard curve. Two aliquots of 40 μl from each standard or sample

were dispensed into 12 x 75 borosilicate tubes. 2.0 ml of anthrone reagent (0.2%

anthrone in 70% H_2SO_4) was added to each tube. After well vortex, all sample tubes

were tightly covered with parafilm. The samples were then incubated at 56°C for 10 min,

and cool in room temperature. The absorbency of each sample was then measured at 620

nm on a spectrophotometer (spectronic 21, Milton Roy, Rochester, NY). Plasma and

urine concentrations of polyfructosan were used to calculate glomerular filtration rates.

3.5   Calculations

3.5.1   Renal Function Parameters

Osmolar clearance (Cosm), free water clearance (C_{H2O}), excretion of sodium,

excretion of potassium, excretion of urea, glomerular filtration rate (GFR), effective renal

plasma flow (ERPF), effective renal blood flow (ERBF), and renal vascular resistance

(RVR) were calculated by standard renal clearance equations as follows:

Cosm = (Urine flow x Urine osmolality)/Plasma osmolality

C_{H2O} = Urine flow – Cosm

Excretion of solute = (Urine flow) x (Urine solute concentration)

GFR = (Urine flow x Urine polyfructosan)/Plasma polyfructosan

ERPF = (Urine flow x Urine PAH)/Plasma PAH

ERBF = (ERPF)/(1-Hct)

RVR = Mean arterial pressure/ERBF

3.5.2 Dose-response Curves

Dose response curves for the effects of V2 receptor agonist and antagonist were

generated by curve fitting the data with the following equation using GraphPad Prism

package (GraphPad Software, Inc., San Diego, CA):

$$RESPONSE = \{(A-D)/[1+(DOSE/C)^B]\}+D$$

where A represents the minimum response, B is the slope, C represents the ED50

response, and D represents the maximum response.

4.   Experimental Procedures: In vitro Study

Expression of vasopressin V2 receptor mRNA in renal medulla collecting ducts in

male SHR at 4 weeks and 12 to 16 weeks of age was measured and compared to that of

age-matched male WKY. Impairment of renal function has been demonstrated in young

SHR preceding the establishment of hypertension where 4-week-old SHR was still

normotensive, hypertension had been fully established in 12- to 16-week-old SHR

(Uyehara and Gellai, 1993). Determination of V2 receptor expression in the kidney in

different stages of hypertension development in SHR might provide useful information to

55

unmask whether alteration of V2 receptor expression in the kidney was related to the altered renal function during the development of hypertension.

The relative quantitation of V2 receptor mRNA level of renal inner medulla was measured using a combination of reverse transcription and polymerase chain reaction method. This method is one of the most sensitive and specific methods that have been developed to date to investigate the gene expression at the transcription level. It may be used to identify specific mRNA in a small amount of sample source such as V2 receptor mRNA in renal medullary collecting ducts.

4.1   Tissue Preparation

Male WKY and SHR at 4 weeks (WKY n=3, SHR n=5) and 12 to 16 weeks of age (WKY n=9, SHR n=9) were killed by intraperitoneal administration of overdose of pentobarbital sodium. The kidneys were rapidly removed and placed on ice. Inner medulla was dissected and stored in $-70^{\circ}C$ for later total RNA extraction and quantitation of V2 receptor mRNA level.

4.2   Total RNA Extraction

The total RNA from renal papilla was isolated using a TRIzol Reagent (Life Technologies, Inc., Grand Island, NY) following the protocol provide by the manufacturer.

In polypropylene tubes, tissue samples were homogenized in 1 ml of TRIzol Reagent per 50-100 mg of tissue using a power homogenizer (Omni International, Inc., Warrenton, VA). Homogenized samples then were incubated for 5 min at room

temperature, and 0.2 ml of chloroform per 1 ml of TRIzol Reagent was added. Sample

tubes were capped securely and shaken vigorously by hand for 15 second. Tubes were

incubated at room temperature for 3 min, and then centrifuged at no more than 12,000 x g

for 15 min at 4 °C. The colorless upper aqueous phase was transferred to a fresh tube.

0.5 ml of isopropyl alcohol per 1 ml of TRIzol Reagent was added to precipitate the RNA

from the aqueous phase. Samples were incubated at room temperature for 10 min and

centrifuged at no more than 12,000 x g for 10 min at 4 °C. The supernatant was removed

and the RNA pellet was washed once with 75% ethanol, then centrifuged at no more than

7,500 x g for 5 min at 4 °C. After briefly dried the RNA pellet (air-dry), the RNA was

dissolved in 50 μl RNase-free water (DEPC (diethylpyrocarbonate)-treated, autoclaved

water). The quality of the extracted RNA were confirmed by the A_{260}/A_{280} ratio using a

spectrophotometer and the concentration of RNA was calculated with absorbance at 260

nm (A_{260}):

$$[RNA] = (A_{260}) \times (0.04 \ \mu g/\mu l) \times D$$

where D represents dilution factor. The total RNA samples were stored at -70 °C until

use.

4.3   Treatment of Total RNA with DNase I

To eliminate contaminating genomic DNA, total RNA samples were treated with

RNase-free DNase I (10 units/μl, Boehringer Mannheim Corporation, Indianapolis, IN).

10 μg of total RNA sample were incubated at 37 °C for 30 min with 30 units of DNase I

in DNase buffer (40 mM Tris-HCl, pH 7.5, 2 mM $CaCl_2$, 5 mM $MgCl_2$) in a 20 μl

volume. The reaction was stopped by heating the sample to 90 °C for 5 min.

4.4 Reverse Transcription (RT)

The reverse transcription was performed using a cDNA cycle kit (Invitrogen

Corporation, San Diego, CA). 5 μg total RNA was used for each reaction and the volume

was adjusted to 11.5 μl with sterile water. Then, the following RT reagents were added

to the reaction tubes: 1 μl random primers, 1 μl RNase inhibitor, 4 μl 5X RT buffer, 1 μl

100 mM dNTPs (dATP, dTTP, dGTP, dCTP), 1 μl 80 mM sodium pyrophosphate, and

0.5 μl AMV (avian myoblastosis virus) reverse transcriptase. Therefore, the final

reaction volume was 20 μl. Reaction tubes were incubated at 42 °C for 60 min in the

GeneAmp PCR System 9600 (Perkin Elmer, Foster City, CA). At the end of the

incubation, the reactions were stopped by heating at 95 °C for 2 min to denature the

RNA-cDNA hybrids and inactivate the reverse transcriptase. Then the reaction tubes

were placed on ice until the addition of PCR reagents or stored at -70 °C.

4.5 Polymerase Chain Reaction (PCR)

PCR were carried out using PCR Master (Boehringer Mannheim Corporation,

Indianapolis, IN) with specific primers for V2 receptor.

The sequences of primers for V2 receptor were as described in Chou et al. (1993).

V2 sense was 5'-ATC CGG AAG CTC CTC TGG AAA GAC C-3' corresponding to

bases 931-935 of the cloned full length sequence of cDNA (Lolait et al., 1992). V2

antisense was 5'-TGA GGC ATC TGT CCC AGT TGC TTC C-3' corresponding to bases

1355-1379. The expected size of the PCR product was 449 base pairs in length. A third

oligonucleotide (sense) including bases 1052-1073 of the cDNA was served as an

amplification product-specific probe. The sequence of this oligonucleotide was 5'-TCC

TCA AGA TGA ATC CTG TGC-3'.

PCR of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an

endogenous internal standard to control for variations due to differences in individual RT

and PCR reaction efficiencies. The sequences of GAPDH primers were as described in

Terada et al. (1993). GAPDH sense was 5'- TCC CTC AAG ATT GTC AGC AA-3'

corresponding to bases 506-525 of the cloned full length sequence of cDNA (Fort et al.,

1985). GAPDH antisense, was 5'-AGA TCC ACA ACG GAT ACA TT-3'

corresponding to bases 794-813. The expected size of the PCR product was 309 base

pairs in length. A third oligonucleotide (sense) including bases 629-648 was served as an

amplification product-specific probe. The sequence of this oligonucleotide was 5'-ACT

CAG AAG ACT GTG GAT GG-3'.

After reverse transcription, 5 μl of RT products was transferred to fresh tube and

used as template DNA for amplification. Total PCR reaction volume was 50 μl

containing 0.5 μM of each primer, 0.2 mM of each dNTP (dATP, dCTP, dGTP, dTTP),

10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl$_2$, 50 mM KCl, and 2.5 U of Taq DNA

polymerase.

The reaction mixture was incubated in the GeneAmp PCR System 9600 (Perkin

Elmer) at 94 °C for 3 min. Then, 30 cycles of amplification consisted of: denaturation,

94°C for 1 min; annealing, 60 °C for 1 min; and elongation, 72 °C for 3 min. A final

elongation was done at 72 °C for 7 min after last cycle. Then samples were kept at 4 °C

until further analysis.

4.6   Southern Blot and PCR Product Analysis

10 μl of PCR products were separated by electrophoresis in 1.8% agarose gel

(Sigma Chemical Co., St. Louis, MO) at 107 mV for 2 to 3 h using a horizontal gel

apparatus. After electrophoresis and ethidium bromide staining, DNA bands were

visualized with an ultraviolet transilluminator and photographed.

For Southern blot analysis, gels were denatured, neutralized and blotted onto

positively charge nylon membrane (Boehringer Mannheim Corporation, Indianapolis, IN)

according to the procedures described by Maniatis et al. (1989), with 20X SSC buffer

containing 3M NaCl and 0.3 M sodium citrate, pH 7.0 as the transfer buffer. UV-cross

linking was performed to bind the DNA to the membranes. Membranes were placed in

hybridization bottles containing 20 ml prehybridization solution per 100 cm^2 of

membrane surface area, and prehybridized at 68 °C for at least 1 h in a hybridization

oven/shaker (Amersham Life Science Inc., Cleveland, Ohio). Then, the prehybridization

solution was discarded and 20 ml hybridization solution containing 1-10 pmol/ml DIG-

labeled oligonucleotide probes was added. The DIG-labeled oligonucleotide probes were

prepared using DIG oligonucleotide 3'-end labeling kit (Boehringer Mannheim

Corporation, Indianapolis, IN). The hybridization was performed at 54 °C for 6 h. After

hybridization, membranes were washed 2 x 5 min at 54 °C with at least 50 ml 2X SSC,

0.1% SDS, per 100 cm^2 membrane and 2 x 5 min at 54 °C with 50 ml 0.1X SSC, 0.1%

SDS. Membranes might be used directly for detection of hybridized oligonucleotide or stored air-dried for later detection.

The detection of hybridized oligonucleotide was performed using DIG luminescent detection kit (Boehringer Mannheim Corporation, Indianapolis, IN). Membranes were rinsed briefly in washing buffer containing 0.1 M maleic acid, 0.15 M NaCl; pH 7.5; and 0.3% (v/v) Tween 20. Incubated membranes for 30 min in blocking solution containing 0.1 M maleic acid, 0.15 M NaCl; pH 7.5; and 1% blocking reagent followed by 30 min in blocking solution with 1:10,000 Anti-DIG-alkaline phosphatase. Discarded the antibody solution. Gently washed the membrane 2 x 15 min in washing buffer. Poured off washing buffer and equilibrated the membrane in detection buffer containing 100 mM Tris-HCl, 100 mM NaCl; pH 9.5 for 2 min. Diluted CSPD (disodium 3-(4-methoxyspiro{1,2-dioxetane3,2'-(5'-chloro) tricyclo[3.3.1.1^{3,7}]decan}-4-yl)phenyl phosphate) 1:100 in detection buffer. Incubated membranes in CSPD solution for 5 min. Then, the membrane was placed between two sheets of plastic page protectors and exposed to Lumi-Film Chemiluminescent Detection Film (Boehringer Mannheim Corporation, Indianapolis, IN) for various periods.

4.7   Relative Quantitation of V2 Receptor mRNA Levels

Lumi-Film Chemiluminescent Detection Films with bands were scanned by a laser densitometer and ImageQuaNT software (Molecular Dynamics, In., San Jose, CA). Expression of V2 receptor mRNA was relatively quantitated by comparing the densitometry value of the V2 receptor band with the densitometry value of the GAPDH band.

5.   Statistical Analysis

All results presented in the text and in tables and figures were expressed as means ± standard error of the mean (SEM). Significance of differences between strains and between gender groups within strains were assessed by Student's paired t-test or by analysis of variance (ANOVA) using the JMP package (SAS Institute Inc., Cary, NC). A p level of less than 0.05 was considered significant.
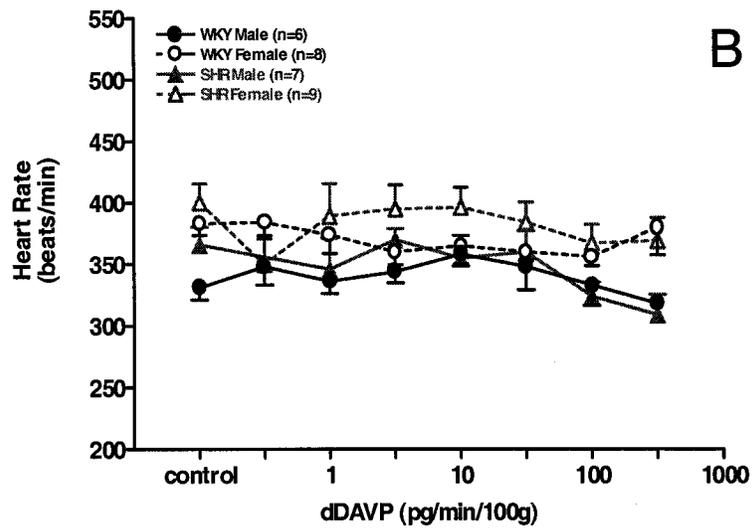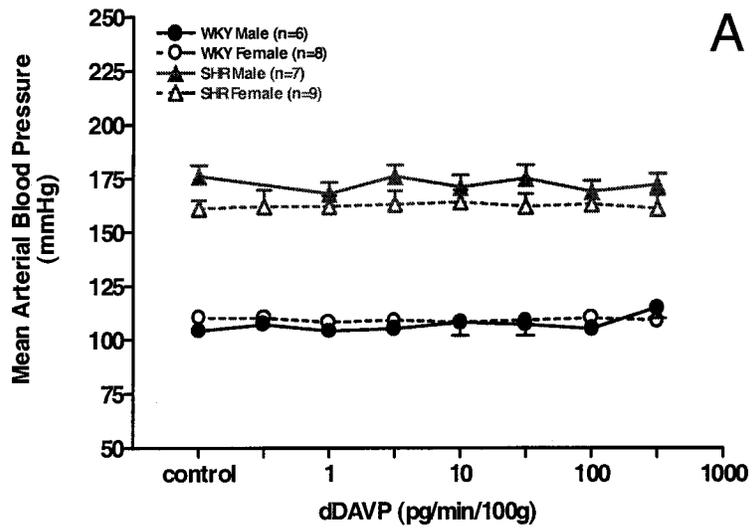
CHAPTER III

RESULTS

1.   In vivo Assessment of Maximum Urine Concentrating Ability of SHR

1.1   Antidiuretic Actions of Vasopressin V2 Receptor Agonist, [deamino-Cys^1,D-Arg^8]-Vasopressin (dDAVP) in Adult SHR Males (n=7) and SHR Females (n=9) and in WKY Males (n=6) and WKY Females (n=8).

Basal mean arterial blood pressure (figure 3A) in SHR male rats was significantly higher than that of WKY male rats. Likewise, basal mean arterial blood pressure in SHR female rats was significantly higher than that of WKY female rats. These results indicated a strain difference in the mean arterial blood pressure, which was higher in SHR rats than WKY rats. Basal mean arterial blood pressure was similar in WKY males and females, while basal mean arterial blood pressure was significantly higher in SHR males than in SHR females (ANOVA orthogonal contrasts, $p<0.05$). Mean arterial blood pressure remained stable throughout the experiment in all groups, indicating that water load and administration of dDAVP did not affect mean arterial blood pressure, nor disturb the resting state of the rats.

Basal heart rate (figure 3B) was not different among groups and remained stable throughout the experiment in all groups, indicating a smooth experimental course.

63

Figure 3A.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on mean arterial blood pressure in male and female WKY and SHR rats.

Figure 3B.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on heart rate in male and female WKY and SHR rats.

Basal values of body weight, hematocrit, plasma osmolality, and plasma concentrations of sodium and potassium after 90-120 min water load and before administration of dDAVP are shown in Table 1. Body weight, hematocrit, plasma osmolality, and plasma concentrations of sodium and potassium at the end of the experiment are also shown in Table 1.

The body weights of SHR rats were lower than that of WKY rats for both males and females. Males of both strains were heavier than females. Body weight remained stable throughout the experiment in all groups, indicating a stable fluid balance.

Basal hematocrits in SHR male rats were similar to that of WKY male rats. Basal hematocrits in SHR female rats were similar to that of WKY female rats. A gender difference existed in the hematocrits, which was higher in males than in females in both WKY and SHR rats. Hematocrits were stable throughout the experiment in all groups, indicating that a stable fluid balance and constant blood volume were maintained.

Basal plasma osmolality was similar among all groups, indicating that the effect of hydration procedure was similar among all groups. Plasma osmolality decreased significantly at the end of the experiment in all groups, however, there was no strain or gender difference.

Basal plasma concentration of sodium was similar among all groups, indicating that the effect of the hydration procedure was similar among all groups. Plasma concentration of sodium decreased significantly at the end of the experiment in all groups.

Table 1. <u>Basal Values After Water Load: Male and Female</u>. Body weight, hematocrit, plasma osmolality, plasma sodium concentration, and plasma potassium concentration before and after infusion of V2 agonist in male and female rats.

	WKY		SHR	
	Males n = 6	Females n = 8	Males n = 7	Females n = 9
Body weight (g)				
Beginning	411.2 ± 13.2	254.5 ± 8.7†	275.9 ± 11.6*	175.1 ± 3.4†*
End	416.5 ± 14.1	254.8 ± 8.1†	276.3 ± 11.5*	175.9 ± 3.2†*
Hematocrit (%)				
Beginning	45.4 ± 0.5	42.0 ± 0.5†	45.9 ± 0.7	40.8 ± 2.1†
End	43.5 ± 0.7	41.1 ± 0.5	44.5 ± 0.8	40.0 ± 1.6†
P_{osm} (mOsm/kg H_2O)				
Beginning	284.3 ± 2.1	284.0 ± 1.2	284.5 ± 0.9	283.2 ± 1.2
End	273.3 ± 2.0#	273.5 ± 1.3#	273.7 ± 0.8#	275.3 ± 2.3#
P_{Na} (mEq/L)				
Beginning	138.8 ± 0.8	139.4 ± 0.3	139.0 ± 0.5	137.7 ± 0.9
End	133.3 ± 1.0#	133.9 ± 0.7#	132.2 ± 0.5#	132.8 ± 1.5#
P_K (mEq/L)				
Beginning	3.9 ± 0.1	3.7 ± 0.1	3.8 ± 0.1	3.7 ± 0.1
End	3.7 ± 0.1	3.4 ± 0.1#	3.6 ± 0.1	3.5 ± 0.1

Data are means ± SE; n, number of rats. P_{osm}, plasma osmolality; P_{Na}, plasma sodium concentration; P_K, plasma potassium concentration. †$P<0.05$ vs. males of same strain; *$P< 0.05$ vs. WKY; #$P<0.05$ vs. beginning.

Basal plasma concentration of potassium was similar among all groups, indicating that the effect of hydration procedure was similar among all groups. Unlike plasma concentration of sodium, plasma concentration of potassium decreased slightly but without statistic significance. This indicated a stable potassium balance in the plasma during the experiment.

According to the above results, strain differences between WKY and SHR rats were shown in mean arterial blood pressure and body weight. Gender differences between male and female WKY and SHR rats were shown in body weight and hematocrit. No strain or gender difference was found in basal plasma osmolality, or plasma concentrations of sodium and potassium.
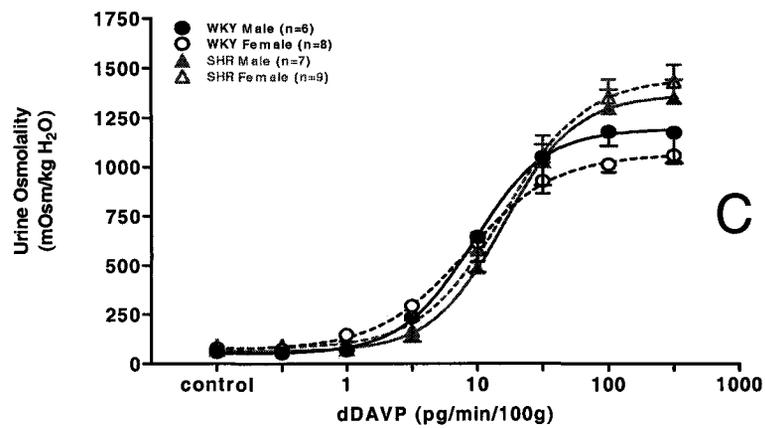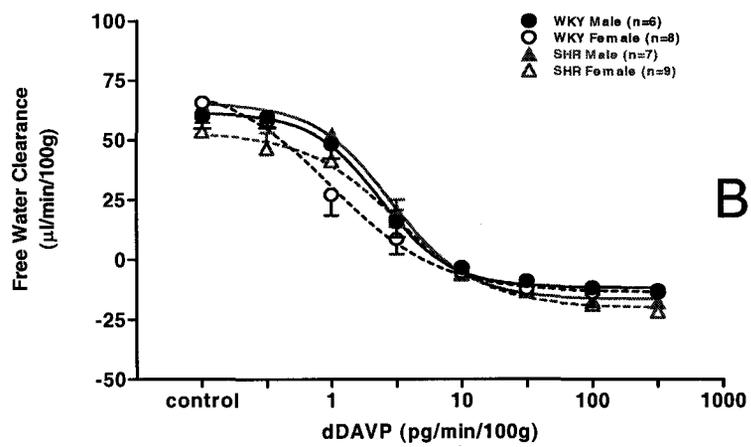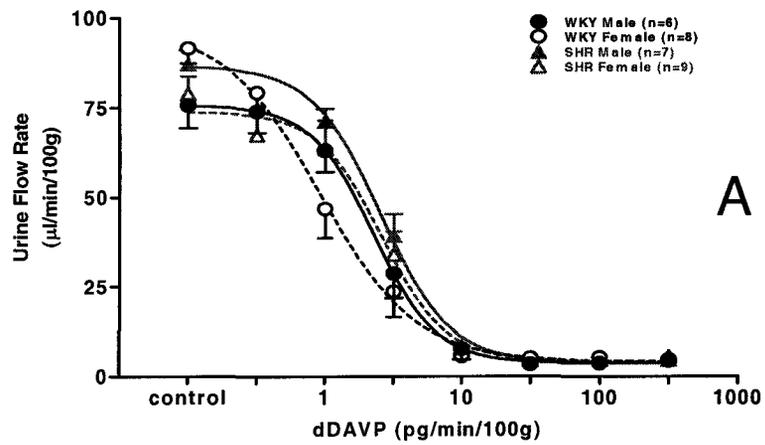
Basal urine flow rate (figure 4A) in SHR male rats was higher than that of WKY male rats. In contrast, basal urine flow rate in SHR female rats was lower than that of WKY female rats. Basal urine flow rate in WKY males was lower than that of WKY females. Basal urine flow rate in SHR males tended to be higher than that of SHR females although this difference was not statistically significant. These results indicated that with the suppression of endogenous vasopressin release and its antidiuretic activity in the kidney, the renal concentrating ability was lower in SHR males than in WKY males, which was opposite from that observed when comparing SHR and WKY females. The renal concentrating ability was also greater in WKY males compared to WKY females, which was not shown in SHR.

Basal free water clearance (C_{H2O}) (figure 4B) in SHR male rats was similar to that of WKY male rats. Basal free water clearance in SHR female rats was lower than that of

Figure 4A.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on urine flow rate in male and female WKY and SHR rats.

Figure 4B.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on free water clearance in male and female WKY and SHR rats.

Figure 4C.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on urine osmolality in male and female WKY and SHR rats.

WKY female rats. No difference was found between WKY males and females, whereas basal free water clearance in SHR males was higher than that of SHR females.

Basal urine osmolality (U_{osm}) (figure 4C) was not significantly different among all groups.

Basal osmolar clearance (C_{osm}) (figure 5) in SHR male rats was significantly higher than that of WKY male rats. Basal osmolar clearance in SHR female rats was similar to that of WKY female rats. Basal osmolar clearance in WKY males was significantly lower than that of WKY females. Basal osmolar clearance in SHR male and female rats was similar.

Basal urinary sodium excretion (figure 6A) in all groups could not be compared due to lack of data, since the urine concentration of sodium was not detectable using the current equipment available in the laboratory.

Basal urinary potassium excretion (figure 6B) in SHR male rats was significantly higher than that of WKY male rats. Basal urinary potassium excretion in SHR female rats was similar to that of WKY female rats. Basal urinary potassium excretion in WKY males was significantly lower than that of WKY females. Basal urinary potassium excretion in SHR males was similar to that of SHR females.

Basal urinary urea nitrogen excretion (figure 6C) in SHR male rats was higher than that of WKY male rats. Basal urinary urea nitrogen excretion in SHR female rats

71

Figure 5.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-
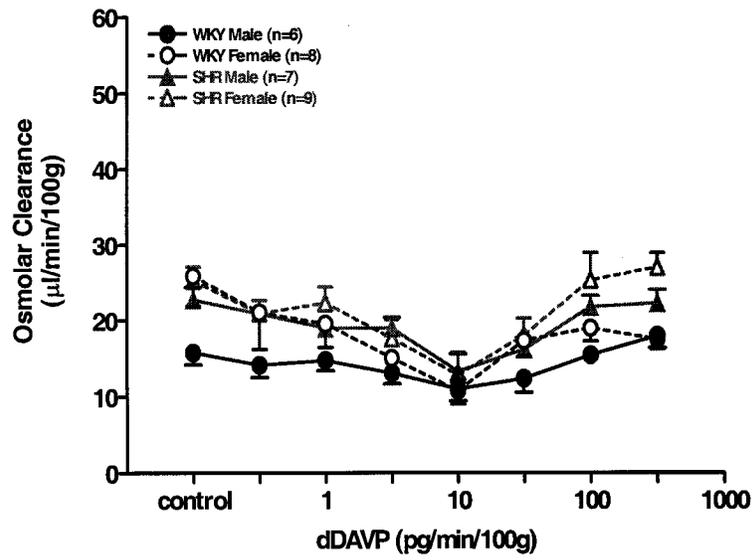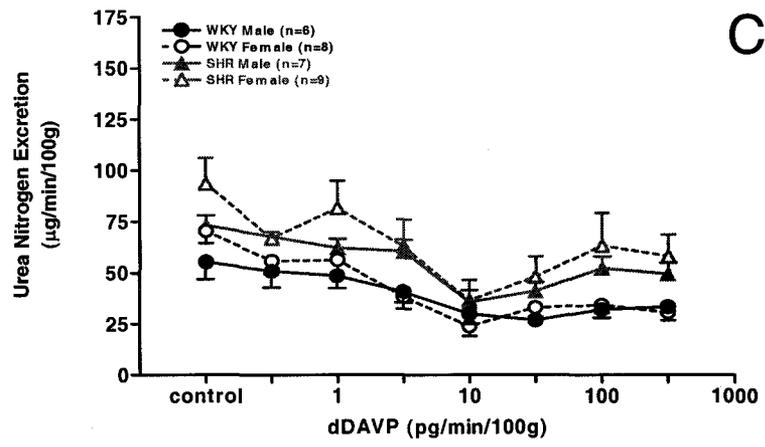vasopressin (dDAVP) on osmolar clearance in male and female
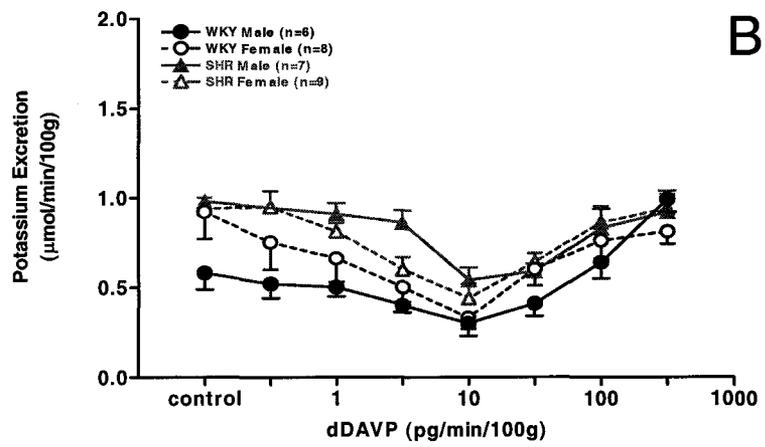WKY and SHR rats.

Figure 6A.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-
               vasopressin (dDAVP) on urinary sodium excretion in male and
               female WKY and SHR rats.

Figure 6B.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-
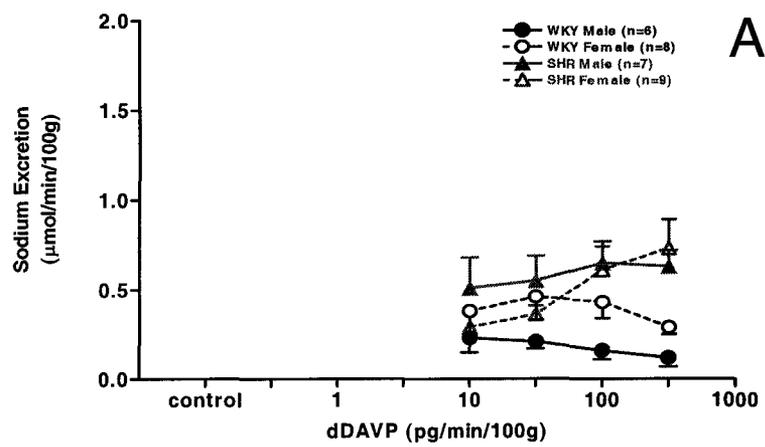   vasopressin (dDAVP) on urinary potassium excretion in male and
               female WKY and SHR rats.

Figure 6C.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-
               vasopressin (dDAVP) on urea nitrogen excretion in male and
               female WKY and SHR rats.

was similar to that of WKY female rats. Basal urinary urea nitrogen in WKY males was similar to that of WKY females. Basal urinary urea nitrogen in SHR males was also similar to that of SHR females.

Basal glomerular filtration rate (GFR) (figure 7A) was similar among all groups.

Although there was a tendency that basal effective renal blood flow (ERBF) (figure 7B) in SHR male and female rat was lower than that of WKY male and female rats, no significant difference was found.
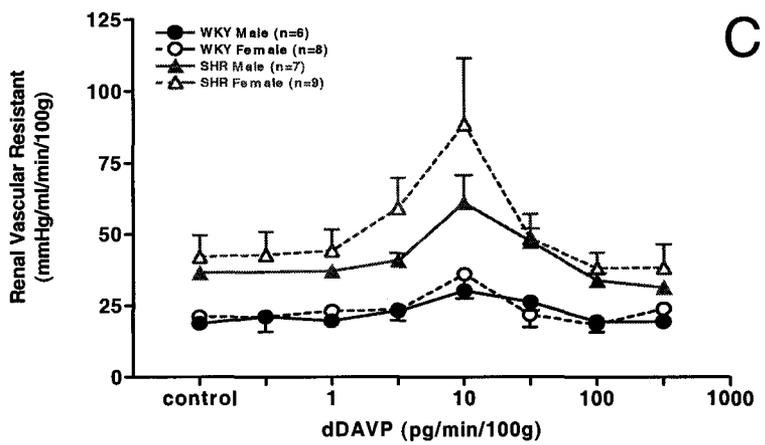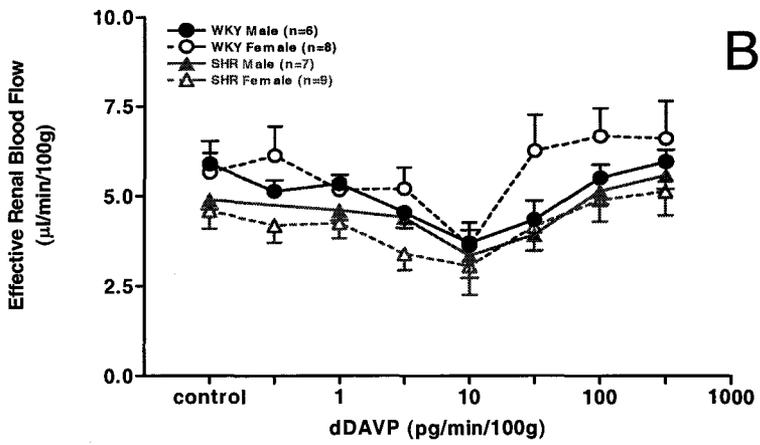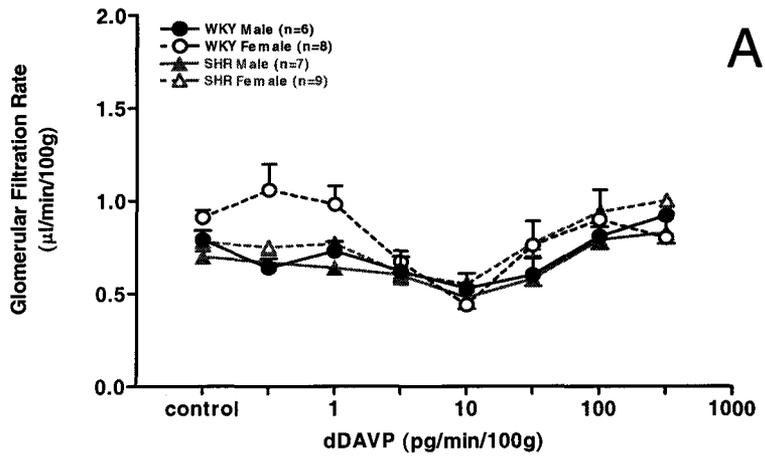
Basal renal vascular resistance (RVR) (figure 7C) was significantly higher in SHR male rats than that of WKY male rats. Basal renal vascular resistance was significantly higher in SHR female rats than that of WKY female rats. No differences were found between WKY males and females as well as SHR males and females.

According to the above results, strain differences were shown between SHR and WKY rats. SHR male rats had higher basal urine flow rate and free water clearance, which were accompanied by higher osmolar clearance due to higher urinary potassium excretion and urea nitrogen excretion. In contrast, strain difference between female SHR and WKY rats were shown where SHR female rats had lower basal urine flow rate and free water clearance, which were not accompanied by changes of osmolar clearance, urinary sodium excretion and urea nitrogen excretion. Gender difference were shown between WKY male and female rats where WKY male rats had lower basal urine flow rate, which were accompanied by lower osmolar clearance due to lower urinary potassium excretion. Gender difference was not shown between SHR male and female rats. Strain difference was also shown between SHR and WKY rats that basal renal vascular resistance in SHR rats was higher than that of WKY rats.

Figure 7A.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on glomerular filtration rate in male and female WKY and SHR rats.

Figure 7B.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on effective renal blood flow in male and female WKY and SHR rats.

Figure 7C.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on effective renal vascular resistant in male and female WKY and SHR rats.

A

Glomerular Filtration Rate (μl/min/100g)

WKY Male (n=6)
WKY Female (n=8)
SHR Male (n=7)
SHR Female (n=9)

dDAVP (pg/min/100g)

B

Effective Renal Blood Flow (μl/min/100g)

WKY Male (n=6)
WKY Female (n=8)
SHR Male (n=7)
SHR Female (n=9)

dDAVP (pg/min/100g)

C

Renal Vascular Resistant (mmHg/ml/min/100g)

WKY Male (n=6)
WKY Female (n=8)
SHR Male (n=7)
SHR Female (n=9)

dDAVP (pg/min/100g)

Following diuresis induced by intragastric water loading and infusion, intravenous infusion of vasopressin V2 receptor agonist dDAVP at increasing doses resulted in a significant dose-dependent antidiuresis in all groups of rats: decreasing urine flow rate and free water clearance, at the same time, increasing urine osmolality. The dose-response curves of urine flow rate, free water clearance, and urine osmolality are shown in figure 4A, 4B, and 4C.

dDAVP induced dose-dependent decrease in urine flow rate (figure 4A) was similar among all groups where ED_{50} and the minimum urine flow rate achieved was similar among all groups.

Similar to urine flow rate, dDAVP induced dose-dependent free water clearance (figure 4B) was not significantly different among all groups as indicated by similar ED_{50} and the minimum free water clearances achieved.

dDAVP induced dose-dependent increase in urine osmolality (figure 4C) was similar among all groups at low infusion rates of dDAVP (0.3-30 pg/min/100 g). However, high infusion rates of dDAVP (100-300 pg/min/100 g) resulted in a higher maximum urine osmolality in SHR rats than in WKY rats. No gender difference was observed in both WKY and SHR rats.

Low infusion rates of dDAVP (0.3-10 pg/min/100 g) decreased osmolar clearance (figure 5) gradually and to a minimum level at the infusion rate of 10 pg/min/100 g in all groups, which was significantly lower than their corresponding basal values. Osmolar clearance then rose gradually when dDAVP infused continuously at higher rates (30-300 pg/min/100 g). There was a tendency that osmolar clearance was higher in SHR male rats than in WKY male rats at all infusion rates. Osmolar clearance in SHR female rats

was not different from that in WKY female rats throughout the experiment, except in the highest infusion rate (300 pg/min/100 g) where osmolar clearance in SHR females was significantly higher than that of WKY females. No gender difference was found in both WKY and SHR.

Data was not available for the comparison of urinary sodium excretion (figure 6A) in low infusion rates of dDAVP (0.3-3 pg/min/100 g) since the urine concentrations of sodium was not detectable using the current equipment available in the laboratory. In high infusion rates of dDAVP (10-300 pg/min/100 g), urinary sodium excretion was significantly higher in SHR male rats than that of WKY male rats. Urinary sodium excretion was higher in SHR female rats than that of WKY female rats in the highest infusion rate of dDAVP (300 pg/min/100 g). No gender deference was found in urinary sodium excretion in WKY rats as well as in SHR rats.

Low infusion rates of dDAVP (0.3-10 pg/min/100 g) decreased urinary potassium excretion (figure 6B) gradually and to a minimum level at the infusion rate of 10 pg/min/100 g in all groups, which was significantly lower than their corresponding basal values. Urinary potassium excretion then rose gradually when dDAVP infused continuously at high rates (30-300 pg/100 g/min). Urinary potassium excretion was significantly higher in SHR male rats than in WKY male rats in low infusion rates of vasopressin (0.3-10 pg/min/100 g). No difference was found between SHR females and WKY females. No gender difference was found in both WKY and SHR rats.

Similar to urinary potassium excretion, low infusion rates of dDAVP (0.3-10 pg/min/100 g) decreased urinary urea nitrogen excretion (figure 6C) gradually and to a

80

minimum level at the infusion rate of 10 pg/min/100 g in all groups, which was

significantly lower than their corresponding basal values. Urinary urea nitrogen

excretion then rose gradually when dDAVP infused continuously at high rates (30-300

pg/min/100 g). Urinary urea nitrogen excretion was higher in SHR male rats than in

WKY male rats at all infusion rats. Urinary urea nitrogen excretion was higher in SHR

female rats than in WKY female rats at all infusion rats. No gender difference was found

in both WKY and SHR rats throughout the experiment.

Low infusion rates of dDAVP (0.3-10 pg/min/100 g) decreased glomerular

filtration rate (figure 7A) gradually and to a minimum level at the infusion rate of 10

pg/min/100 g in all groups, which was significantly lower than their corresponding basal

values. Glomerular filtration rate then rose gradually when dDAVP infused continuously

at high rates (30-300 pg/min/100 g). No strain or gender difference was found in all

groups throughout the experiment.

Low infusion rates of dDAVP (0.3-10 pg/min/100 g) decreased effective renal

blood flow (figure 7B) gradually and to a minimum level at the infusion rate of 10

pg/min/100 g in all groups, which was significantly lower than their corresponding basal

values. Effective renal blood flow then rose gradually when dDAVP infused

continuously at high rates (30-300 pg/min/100 g). No strain or gender difference was

found in all groups throughout the experiment.

Low infusion rates of dDAVP (0.3-10 pg/min/100 g) increased renal vascular

resistance (figure 7C) gradually and to a maximum level at the infusion rate of 10

pg/min/100 g in all groups, which was significantly higher than their corresponding basal

values. Renal vascular resistance then decreased gradually when dDAVP infused

continuously at high rates (30-300 pg/min/100 g). Renal vascular resistance was higher in SHR male and female rats than in WKY male and female rats. No gender difference was found in both WKY and SHR throughout the experiment.
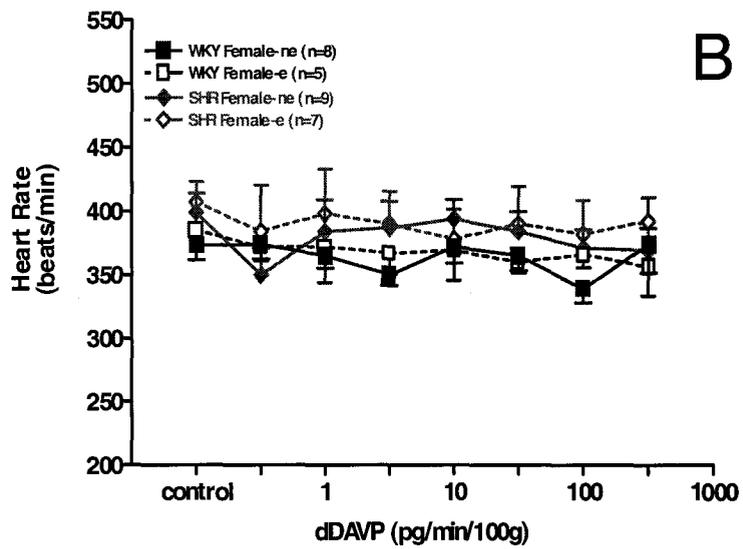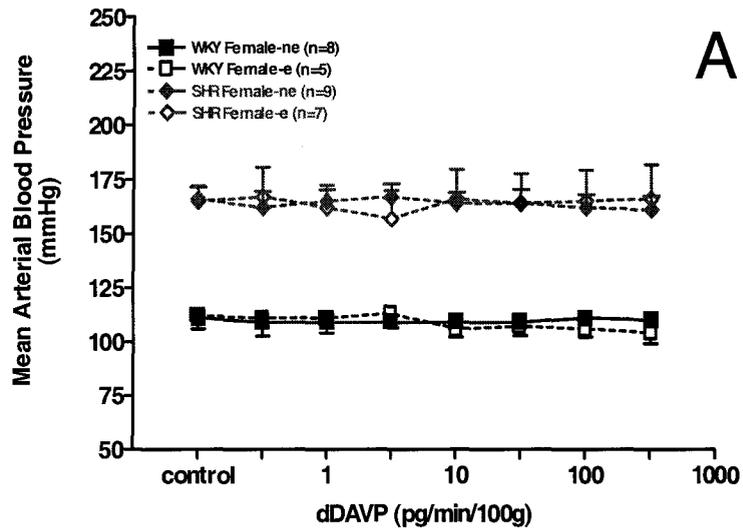
Taken together, the above findings showed that there was no gender difference in renal functions in response to dDAVP treatment. A strain difference was found between SHR and WKY rats where SHR had higher concentrating ability in response to dDAVP in the highest dose (300 pg/min/100 g). This was indicated by a higher osmolality, which was accompanied by higher osmolar clearance due to higher urinary sodium and urea nitrogen excretion.

1.2   Antidiuretic Actions of dDAVP in Female Rats during Estrous (SHR n=7, WKY n=5) and Nonestrous (SHR n=9, WKY n=8) Phases of the Estrous Cycle.

Basal mean arterial blood pressure (MABP) (figure 8A) in SHR estrous female rats was significantly higher than that of WKY estrous female rats. Likewise, basal mean arterial blood pressure in SHR nonestrous female rats was also significantly higher than that of WKY nonestrous female rats. These results indicated a strain different in mean arterial blood pressure, which was higher in SHR female rats than WKY female rats. Basal mean arterial blood pressure was not different between WKY estrous and nonestrous females as well as SHR estrous and nonestrous females, indicating no phase difference was found in both WKY and SHR female rats. Mean arterial blood pressure

Figure 8A. Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on mean arterial blood pressure in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

Figure 8B. Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on heart rate in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

A

B

remained stable throughout the experiment in all groups, indicating that water load and administration of dDAVP did not affect blood pressure, nor disturb the resting of the rats. Basal heart rate (figure 8B) was similar among all groups and remained stable throughout the experiment, indicating a smooth experimental course.

Basal values of body weight, hematocrit, plasma osmolality, and plasma concentrations of sodium and potassium after 90-120 min water load and before administration of dDAVP are shown in Table 2. Body weight, hematocrit, plasma osmolality, and plasma concentrations of sodium and potassium at the end of the experiment are also shown in Table 2.

The body weights of SHR estrous female rats were lower than that of WKY estrous female rats. The body weights of SHR nonestrous female rats were lower than that of WKY nonestrous female rats. The body weight between WKY estrous and nonestrous females was not different. Similarly, the body weight between SHR estrous and nonestrous females was not different. These results indicated a strain difference in the body weight, which was lower in SHR female rats than in WKY female rats. No phase difference was shown in both WKY and SHR female rats. Body weight remained stable throughout the experiment in all groups, indicating a stable fluid balance.

Basal hematocrits were similar among all groups, indicating that the effect of hydration procedure was the same in all groups. Hematocrits were stable throughout the experiment in all groups, indicating that a stable fluid balance and constant blood volume were maintained.

Table 2. <u>Basal Values After Water Load: Estrous and Nonestrous</u>. Body weight, hematocrit, plasma osmolality, plasma sodium concentration, and plasma potassium concentration before and after infusion of V2 agonist in female rats during the non-estrous and estrous phases of estrous cycle.

	WKY		SHR	
	Females Nonestrous phase n = 8	Females Estrous phase n = 5	Females Nonestrous phase n = 9	Females Estrous phase n = 7
Body weight (g)				
Beginning	261.5 ± 8.4	249.2 ± 11.4	174.3 ± 2.9*	176.2 ± 3.8*
End	261.4 ± 9.0	250.6 ± 11.1	175.7 ± 2.9*	177.3 ± 3.6*
Hematocrit (%)				
Beginning	41.6 ± 1.5	41.8 ± 0.6	41.4 ± 2.0	43.4 ± 2.0
End	40.5 ± 1.2	40.5 ± 0.9	40.1 ± 1.3	40.9 ± 2.1
P_{osm} (mOsmol/kg H_2O)				
Beginning	285.4 ± 1.0	281.8 ± 1.2	284.9 ± 0.9	284.6 ± 1.1
End	277.1 ± 2.5#	276.1 ± 2.5	280.7 ± 3.5	276.8 ± 1.2#
P_{Na} (mEq/L)				
Beginning	139.9 ± 0.4	139.1 ± 0.3	138.4 ± 0.6	137.9 ± 1.0
End	135.1 ± 0.7#	132.7 ± 1.0#	134.4 ± 0.7#	133.5 ± 1.3#
P_K (mEq/L)				
Beginning	3.7 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.9 ± 0.1
End	3.3 ± 0.1	3.6 ± 0.3	3.7 ± 0.2	3.6 ± 0.1

Data are means ± SE; n, number of rats. P_{osm}, plasma osmolality; P_{Na}, plasma sodium concentration; P_K, plasma potassium concentration. *$P < 0.05$ vs. WKY; #$P < 0.05$ vs. beginning.

Basal plasma osmolality was similar among all groups, indicating that the effect of hydration procedure among all groups was similar among all groups. Plasma osmolality decreased significantly at the end of the experiment in all groups, however, there was no strain or phase difference. Since hematocrit was stable in all groups throughout the experiment indicating a stable blood volume, the decrease of plasma osmolality was probably due to loss of osmotic molecules from the plasma during the experiment and the loss of osmotic molecules was similar among all groups.

Basal plasma concentration of sodium was similar among all groups, indicating that the effect of hydration procedure was similar among all groups. Plasma concentration of sodium decreased significantly at the end of the experiment in all groups. This decrease was probably due to the effect of water retention by the antidiuretic action of dDAVP, which diluted the plasma concentration of sodium at the end of the experiment.

Basal plasma concentration of potassium was similar among all groups, indicating that the effect of hydration procedure was similar among all groups. Unlike plasma concentration of sodium, plasma concentration of potassium decreased slightly without statistic significance. This indicated a stable potassium balance in the plasma during the experiment.

According to the above results, strain difference were shown between SHR and WKY female rats in basal mean arterial blood pressure, body weight. None phase difference was found between nonestrous and estrous female rats in both WKY and SHR rats.

Basal urine flow rate (figure 9A) was similar among all groups.

87

Basal free water clearance (figure 9B) was similar among all groups.

Basal urine osmolality (figure 9C) was similar among all groups.

Basal osmolar clearance (figure 10) was similar among all groups.

Basal urinary sodium excretion (figure 11A) in all groups was not comparable due to lack of data since the urine concentration of sodium using the current equipment available in the laboratory.

Basal urinary potassium excretion (figure 11B) was similar among all groups.

Basal urinary urea nitrogen excretion (figure 11C) was not significantly different among all groups.

Basal glomerular filtration rate (figure 12A) was similar among all groups.

Basal effective renal blood flow (figure 12B) was similar among all groups.

Basal renal vascular resistance (figure 12C) was similar among all groups.

After a steady state diuresis was induced by intragastric water loading and infusion, intravenous infusion of vasopressin V2 receptor agonist dDAVP at increasing doses caused a significant dose-dependent antidiuresis in all groups of rats. V2 receptor agonist dDAVP decreased urine flow rate and free water clearance, while at the same time, increased urine osmolality. The dose-response curves of urine flow rate, free water clearance, and urine osmolality are shown in figure 9A, 9B, and 9C.

dDAVP induced dose-dependent decrease in urine flow rate (figure 9A) was similar among all groups where ED_{50} and the minimum urine flow rate achieved was similar among all groups.

Figure 9A.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on urine flow rate in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

Figure 9B.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on free water clearance in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

Figure 9C.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on urine osmolality in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.
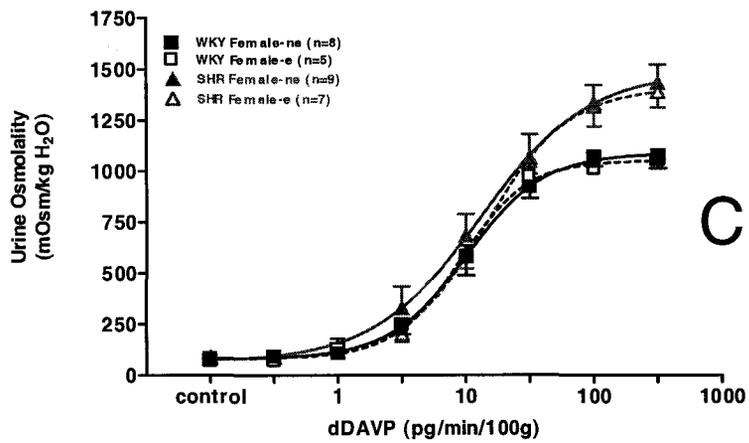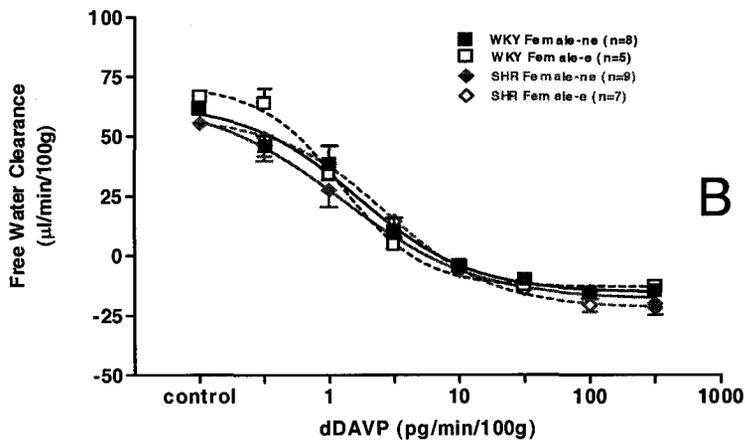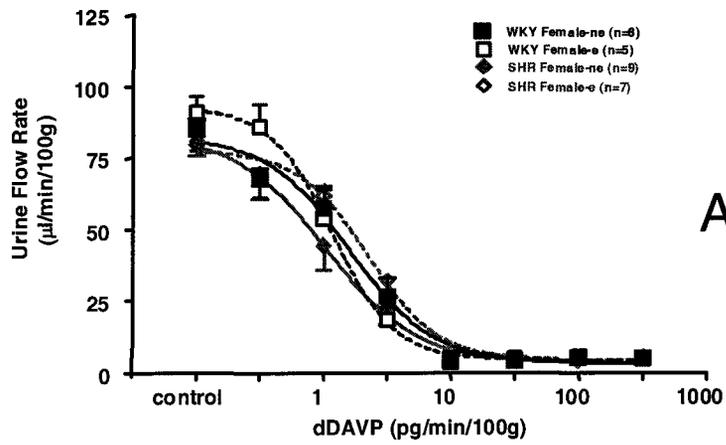
Figure 10.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-
vasopressin (dDAVP) on osmolar clearance in female WKY and
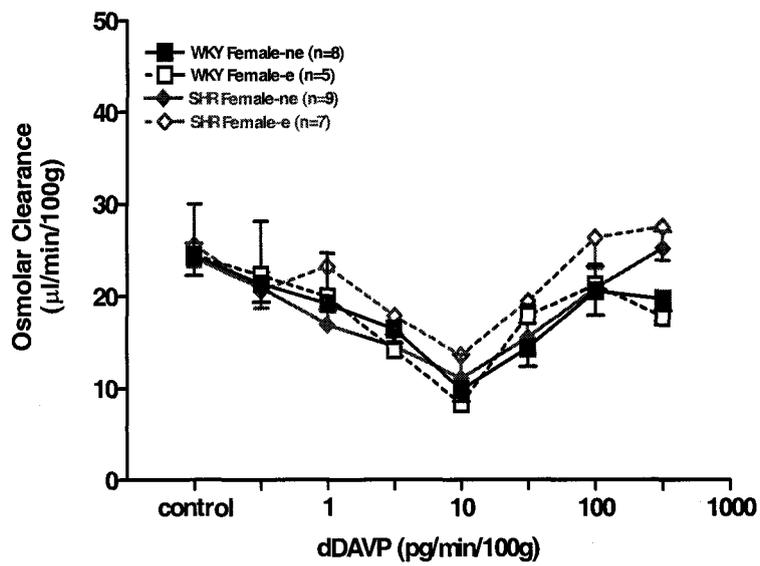SHR rats during estrous and nonestrous phases of the estrous
cycle.

Figure 11A.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on urinary sodium excretion in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

Figure 11B.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on urinary potassium excretion in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

Figure 11C.   Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on urea nitrogen excretion in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.
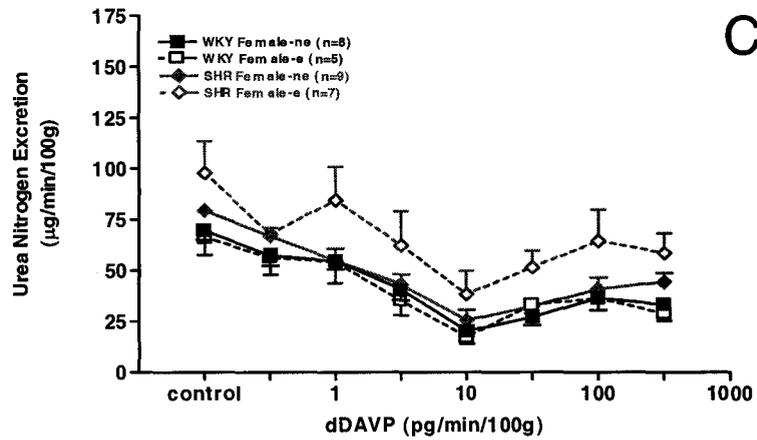
A

B

C

94

Figure 12A. Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on glomerular filtration rate in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

Figure 12B. Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on effective renal blood flow in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

Figure 12C. Effect of vasopressin V2 receptor agonist [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) on renal vascular resistance in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.
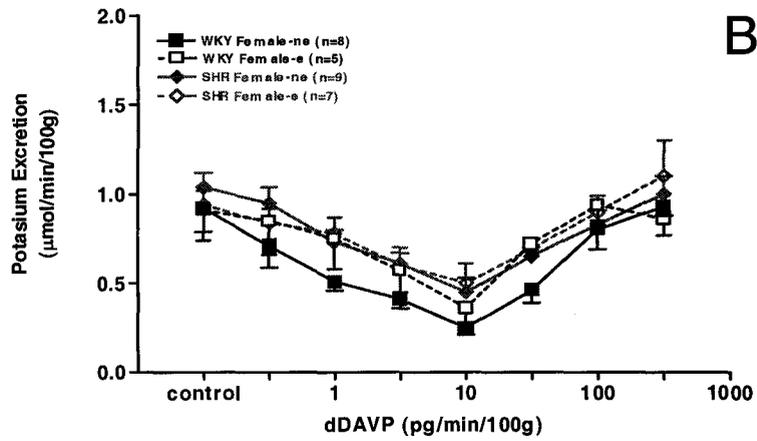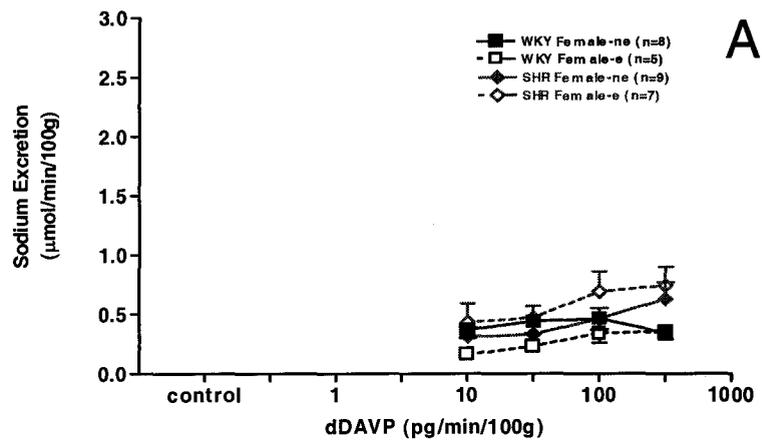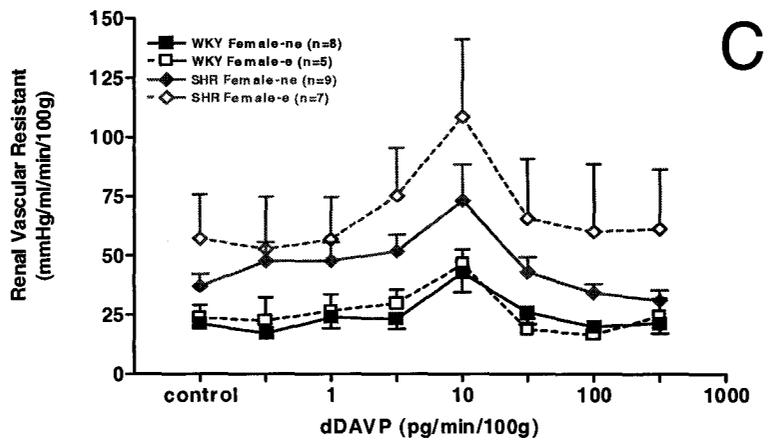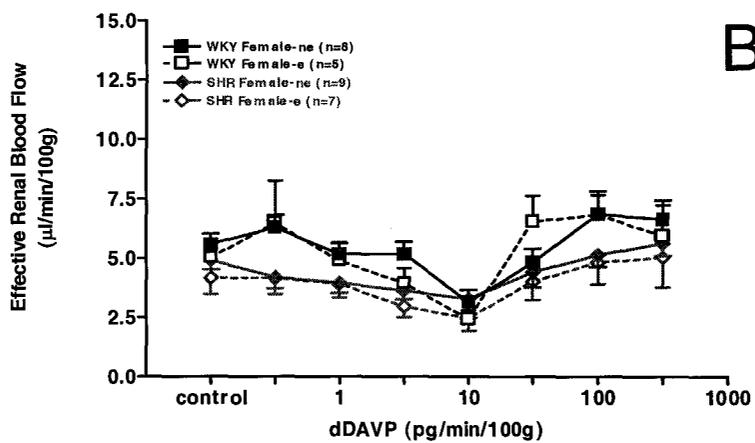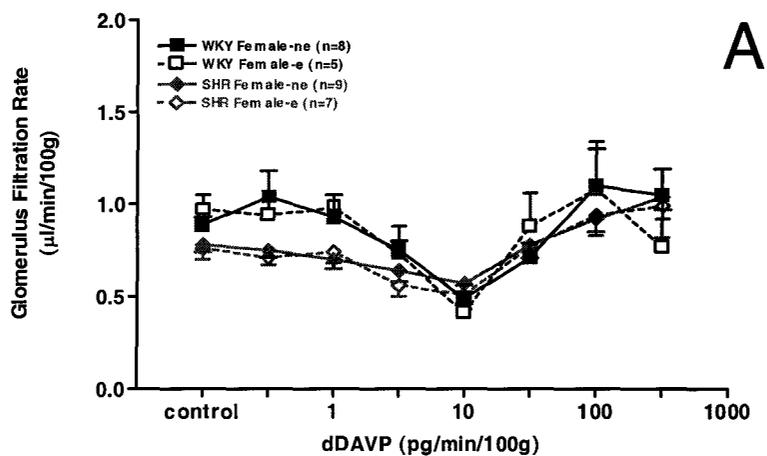
A

B

C

96

Similar to urine flow rate, dDAVP induced dose-dependent free water clearance (figure 9B) was not significantly different among all groups as indicated by similar ED_{50} and minimum free water clearances achieved.

dDAVP induced dose-dependent increase in urine osmolality (figure 9C) was similar among all groups at low infusion rates of dDAVP (0.3-30 pg/min/100 g). However, high infusion rates of dDAVP (100-300 pg/min/100 g) resulted in a higher maximum urine osmolality in SHR estrous female rats than in WKY estrous female rats as well as in SHR nonestrous female rats than in WKY nonestrous female rats. No phase difference was observed in both WKY and SHR female rats.

Low infusion rates of dDAVP (0.3-10 pg/min/100 g) decreased osmolar clearance (figure 10) gradually and to a minimum level at the infusion rate of 10 pg/min/100 g in all groups, which was significantly lower than their corresponding basal values. Osmolar clearance then rose gradually when dDAVP infused continuously at high rates (30-300 pg/min/100 g). Osmolar clearance in SHR female rats was not different from that in WKY female rats throughout the experiment, except in the highest infusion rate (300 pg/min/100 g) where osmolar clearance in SHR estrous female rats was significantly higher than that of WKY estrous female rats. Similarly, in the highest infusion rate (300 pg/min/100 g), osmolar clearance in SHR nonestrous female rats was significantly higher than that of WKY nonestrous female rats. No phase difference was shown in both WKY and SHR female rats.

Data was not available for the comparison of urinary sodium excretion (figure 11A) in low infusion rates of dDAVP (0.3-3 pg/min/100 g) since the urine concentrations of sodium was not detectable (<10 mEq/l) using the current equipment available in the

laboratory. In high infusion rates of dDAVP (10-300 pg/min/100 g), urinary sodium excretion was similar among all groups.

Low infusion rates of dDAVP (0.3-10 pg/min/100 g) decreased urinary potassium excretion (figure 11B) gradually and to a minimum level at the rate of 10 pg/min/100 g in all groups, which was significantly lower than their corresponding basal values. Urinary potassium excretion then rose gradually when dDAVP infused continuously at high rates (30-300 pg/min/100 g). Urinary potassium excretion was similar among all groups.

Similar to urinary potassium excretion, low infusion rates of dDAVP (0.3-10 pg/min/100 g) decreased urinary urea nitrogen excretion (figure 11C) gradually and to a minimum level at the infusion rate of 10 pg/min/100 g in all groups, which was significantly lower than their corresponding basal values. Urinary urea nitrogen excretion then rose gradually when dDAVP infused continuously at high rates (30-300 pg/min/100 g). Urinary urea nitrogen excretion was similar among all groups.

Low infusion rates of dDAVP (0.3-10 pg/min/100 g) decreased glomerular filtration rate (figure 12A) gradually and to a minimum level at the infusion rate of 10 pg/min/100 g in all groups, which was significantly lower than their corresponding basal values. Glomerular filtration rate then rose gradually when dDAVP infused continuously at high rates (30-300 pg/min/100 g). No strain or phase difference was found in all groups throughout the experiment.

Low infusion rates of dDAVP (0.3-10 pg/min/100 g) decreased effective renal blood flow (figure 12B) gradually and to a minimum level at the rate of 10 pg/min/100 g in all groups, which was significantly lower than their corresponding basal values. Effective renal blood flow then rose gradually when dDAVP infused continuously at high

rates (30-300 pg/min/100 g). No strain or phase difference was found in all groups throughout the experiment.

Low infusion rates of dDAVP (0.3-10 pg/min/100 g) increased renal vascular resistance (figure 12C) increased gradually and to a maximum level at the infusion rate of 10 pg/min/100 g in all groups, which was significantly higher than their corresponding basal values. Renal vascular resistance then decreased gradually when dDAVP infused continuously at high rates (30-300 pg/min/100 g). Renal vascular resistance was higher in SHR estrous female rats than in WKY estrous female rats as well as in SHR nonestrous female rats than in WKY nonestrous female rats. No phase difference was found in both WKY and SHR female rats throughout the experiment.

Taken together, the above results indicated a strain difference in the antidiuretic activity of dDAVP, which was enhanced in SHR female rats than in WKY female rats. No phase difference in the antidiuretic activity was shown in the estrous and nonestrous phases during the estrous cycle in WKY and SHR female rats.

2.   In vivo Assessment of Endogenous Vasopressin Renal Action in SHR

2.1   Renal Responses to Vasopressin V2 Receptor Antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-Vasopressin in Adult SHR Males (n=8) and SHR Females (n=8) and in WKY Males (n=10) and Females (n=10).

Basal mean arterial blood pressure (figure 13A) in SHR male rats was significantly higher than that of WKY male rats. Likewise, basal mean arterial blood pressure in SHR female rats was significantly higher than that of WKY female rats. These results indicated a strain difference in the mean arterial blood pressure, which was

higher in SHR rats than WKY rats. Basal mean arterial blood pressure was similar in WKY males and females, while basal mean arterial blood pressure was significantly higher in SHR males than in SHR females. Basal mean arterial blood pressure was not different between SHR males and females, although there was a tendency that SHR males had a higher mean arterial blood pressure than that of SHR females. Mean arterial blood pressure remained stable throughout the experiment in all groups, indicating that a stable fluid balance was maintained.

Basal heart rate (figure 13B) was not different among groups and remained stable throughout the experiment in all groups, indicating a smooth experimental course.

Basal values of body weight, hematocrit, plasma osmolality, and plasma concentrations of sodium and potassium after 90-120 min equilibration period and before administration of vasopressin V2 receptor antagonist [1-Adamantane acetyl^1, D-Tyr(Et)^2, Val^4, Abu^6, Arg^9]-arg^8-vasopressin are shown in Table 3. Body weight, hematocrit, plasma osmolality, and plasma concentrations of sodium and potassium at the end of the experiment are also shown in Table 3.

The body weights of SHR rats were lower than that of WKY rats for both males and females. Males of both strains were heavier than females. Body weight remained stable throughout the experiment in all groups, indicating a stable fluid balance.

Basal hematocrits in SHR male rats were not different to that of WKY male rats. Basal hematocrits in SHR female rats were similar to that of WKY female rats. A gender difference existed in the hematocrits, which was higher in males than in females in both WKY and SHR rats. Hematocrit was stable throughout the experiment in all groups, indicating that a stable fluid balance and constant blood volume were maintained.

100

Figure 13A.   Effect of vasopressin V2 antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin on mean arterial blood pressure in male and female WKY and SHR rats.

Figure 13B.   Effect of vasopressin V2 antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin on heart rate in male and female WKY and SHR rats.
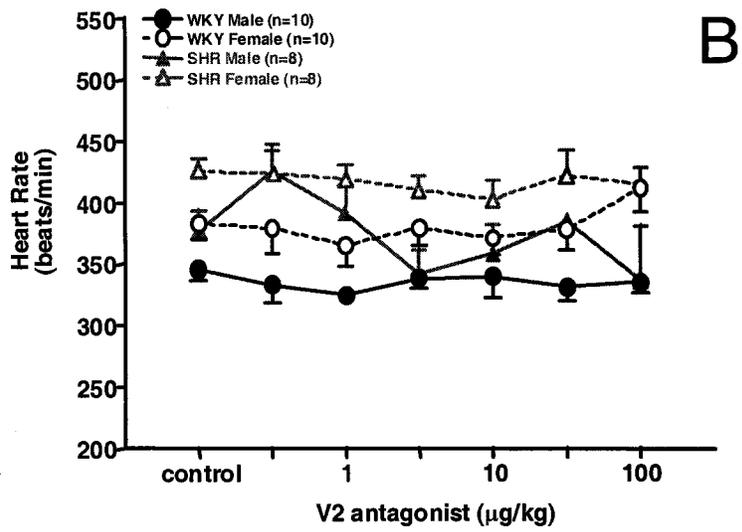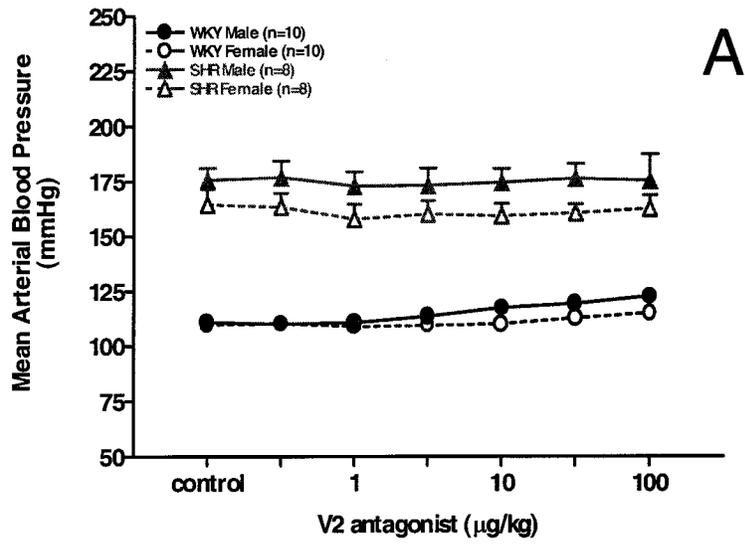
Table 3. <u>Basal Values in Euhydrated State: Male and Female.</u> Body weight, hematocrit, plasma osmolality, plasma sodium concentration, and plasma potassium concentration before and after injection of V2 antagonist in male and female rats.

	WKY		SHR	
	Males n = 10	Females n = 10	Males n = 8	Females n = 8
Body weight (g)				
Beginning	434.5 ± 10.2	$248.7 \pm 7.1†$	$283.8 \pm 6.2*$	$168.6 \pm 2.7†*$
End	429.5 ± 9.6	$246.3 \pm 6.7†$	$278.5 \pm 4.1*$	$168.5 \pm 2.8†*$
Hematocrit (%)				
Beginning	44.8 ± 0.5	$41.4 \pm 0.7†$	46.6 ± 1.0	$41.9 \pm 1.7†$
End	$46.7 \pm 0.6\#$	$42.7 \pm 0.8†$	46.5 ± 0.7	$41.6 \pm 1.2†$
P_{osm} (mOsmol/kg H_2O)				
Beginning	290.8 ± 1.2	$286.9 \pm 0.9†$	293.5 ± 0.7	$289.2 \pm 0.5†$
End	$297.9 \pm 1.9\#$	$296.6 \pm 2.8\#$	298.4 ± 3.1	$292.6 \pm 1.0\#$
P_{Na} (mEq/L)				
Beginning	143.4 ± 0.6	$141.3 \pm 0.2†$	144.1 ± 0.4	$142.1 \pm 0.3†$
End	$147.3 \pm 1.0\#$	$145.5 \pm 1.7\#$	$149.1 \pm 1.6\#$	143.5 ± 0.7
P_K (mEq/L)				
Beginning	4.0 ± 0.1	3.7 ± 0.1	3.9 ± 0.1	3.8 ± 0.1
End	3.9 ± 0.1	3.9 ± 0.1	3.8 ± 0.1	3.9 ± 0.1

Data are means $\pm$ SE; n, number of rats. P_{osm}, plasma osmolality; P_{Na}, plasma sodium concentration; P_K, plasma potassium concentration. $†P<0.05$ vs. males of same strain; $*P< 0.05$ vs. WKY; $\#P<0.05$ vs. beginning.

Basal plasma osmolality in SHR male rats was similar to that of WKY male rats. Basal plasma osmolality in SHR female rats was similar to that of WKY female rats. Basal plasma osmolality was higher in WKY males than in WKY females. Basal plasma osmolality was higher in SHR males than in SHR females. Therefore, a gender difference was evidenced in the plasma osmolality in both WKY and SHR rats, which was higher in males than in females. Plasma osmolality increased significantly at the end of the experiment in all groups, however, there was no strain or gender difference. Since hematocrits were stable in all groups throughout the experiment indicating a stable blood volume, the increase of plasma osmolality was probably due to increase of osmotic molecules in the plasma during the experiment.

Basal plasma concentration of sodium in SHR male rats was not different from that of WKY male rats. Basal plasma concentration of sodium in SHR female rats was not different from that of WKY female rats. Basal plasma concentration of sodium in WKY males was higher than that of WKY females. Basal plasma concentration of sodium in SHR males was higher than that of SHR females. Therefore, a gender difference was evident in the plasma concentration of sodium in both WKY and SHR rats, which was higher in males than in females. Plasma concentration of sodium increased at the end of the experiment in all groups. This is probably due to the loss of water from the urine. Basal plasma concentration of potassium was not different from all groups. Plasma concentration of potassium was stable throughout the experiment, indicating a stable potassium balance in the plasma during the experiment.

According to the above results, strain differences were shown between SHR and WKY rats in basal mean arterial blood pressure and body weight. Gender differences were shown between male and female rats in WKY and SHR rats in body weight, hematocrit, plasma osmolality and plasma concentration of sodium.

Basal urine flow rate (figure 14A) was similar among all groups.

Basal free water clearance (C_{H2O}) (figure 14B) was similar among all groups.

Basal urine osmolality (U_{osm}) (figure 14C) in SHR male rats was not different from that of WKY male rats. Basal urine osmolality in SHR female rats was significantly higher than that of WKY female rats. This indicated that a strain difference in the urine osmolality existed in female rats but not in male rats. Basal urine osmolality in WKY males was higher than that of WKY females. Basal urine osmolality in SHR males was similar to that of SHR females. This indicated that a gender difference in the urine osmolality existed in WKY rats but not in SHR rats.

Basal osmolar clearance (figure 15) was similar among all groups.

Basal urinary sodium excretion (figure 16A) was similar among all groups.

Basal urinary potassium excretion (figure 16B) was not different among groups, however, SHR tended to have a higher urinary potassium excretion than WKY rats.

It was not possible to compare basal urinary urea nitrogen excretion (figure 16C) between SHR male rats and WKY male rats since urinary urea nitrogen was measured in only one WKY male rat. Basal urinary urea nitrogen excretion in SHR female rats was higher than that of WKY female rats, but no significant difference. Basal urinary urea nitrogen excretion in WKY males was not possible to compare to that of WKY females

105

since lack of data in WKY males. Basal urinary urea nitrogen excretion in SHR males was similar to that of SHR females.

Intravenous injection of vasopressin V2 receptor antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin at increasing doses resulted in a significant dose-dependent diuresis: increasing urine flow rate and free water clearance, at the same time, decreasing urine osmolality. The dose-response curves of urine flow rate, free water clearance, and urine osmolality are shown in figure 14A, 14B, and 14C.
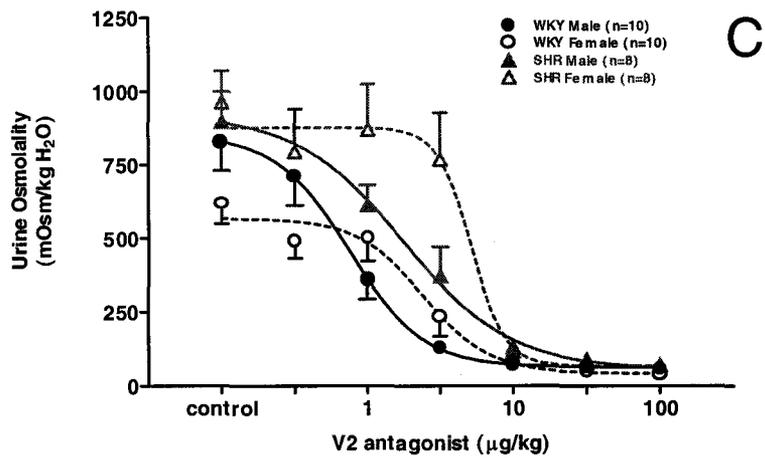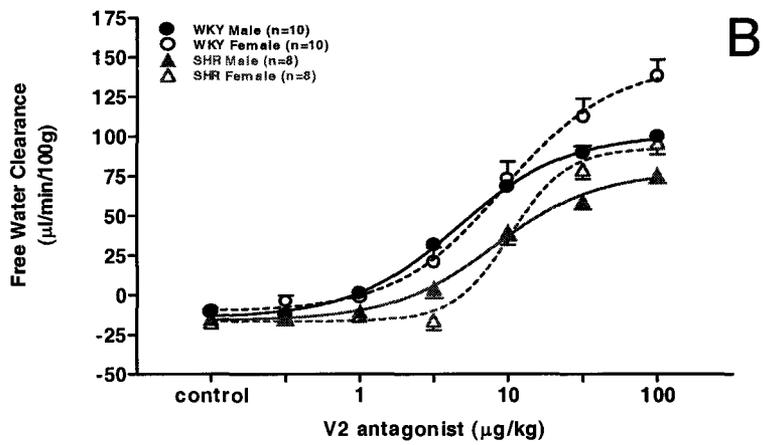
V2 antagonist induced dose-dependent increase in urine flow rate (figure 14A) was significantly lower in SHR male rats than in WKY male rats. V2 antagonist induced dose-dependent increase in urine flow rate was also significantly lower in SHR female rats than in WKY female rats. V2 antagonist induced dose-dependent increase in urine flow rate was significantly lower in WKY male rats than in WKY female rats in the higher doses (30-100 µg/kg). V2 antagonist induced dose-dependent increase in urine flow rate was also significantly lower in SHR male rats than in SHR female rats in doses of 30-100 µg/kg. These results indicated that there was not only a strain difference in urine flow rate, which was lower in SHR rats than in WKY rats, but also a gender difference in the urine flow rate, which was lower in male rats than in female rats.

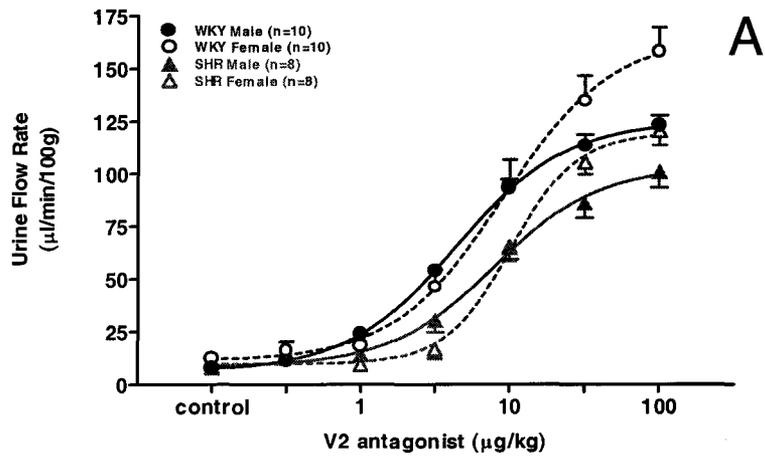Similar to urine flow rate, V2 antagonist induced dose-dependent free water clearance (figure 14B) was significantly lower in SHR male rats than in WKY male rats. V2 antagonist induced dose-dependent increase in free water clearance was also significantly lower in SHR female rats than in WKY female rats. V2 antagonist induced dose-dependent increase in free water clearance was significantly lower in WKY male

Figure 14A. Effect of vasopressin V2 antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin on urine flow rate in male and female WKY and SHR rats.

Figure 14B. Effect of vasopressin V2 antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin on free water clearance in male and female WKY and SHR rats.

Figure 14C. Effect of vasopressin V2 antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin on urine osmolality in male and female WKY and SHR rats.

rats than in WKY female rats in the higher doses (30-100 µg/kg). V2 antagonist

induced dose-dependent increase in free water clearance was also significantly

lower in SHR male rats than in SHR female rats in doses of 30-100 µg/kg. These

results indicated that there was not only a strain difference in free water clearance,

which was lower in SHR rats than in WKY rats, but also a gender difference in

free water clearance, which was lower in male rats than in female rats.

V2 antagonist induced dose-dependent decrease in urine osmolality (figure

14C) was significantly different among groups at the lower doses (0.3-3 µg/kg).

However, higher doses (10-100 µg/kg) resulted in similar minimum urine

osmolality in all groups.

Osmolar clearance (figure 15) was similar among all groups during the

experiment.

Urinary sodium excretion (figure 16A) was similar among all groups.

Urinary potassium excretion (figure 16B) was not different among groups,

however, SHR tended to have a higher urinary potassium excretion than WKY

rats.

It was not possible to compare urinary urea nitrogen excretion (figure

16C) between SHR male rats and WKY male rats since urinary urea nitrogen was

measured in only one male WKY rat. Urinary urea nitrogen excretion in SHR

female rats was significantly higher than that of WKY female rats. Urinary urea

nitrogen excretion in WKY males was not possible to compare to that of WKY

Figure 15.   Effect of vasopressin V2 antagonist [1-Adamantane acetyl^1, D-Tyr(Et)^2, Val^4, Abu^6, Arg^9]-arg^8-vasopressin on osmolar clearance in male and female WKY and SHR rats.

Figure legend:
- WKY Male (n=10)osm-
- WKY Female (n=10)
- SHR Male (n=8)
- SHR Female (n=8)

Y-axis: Osmolar Clearance (μl/min/100g)

X-axis: V2 antagonist (μg/kg)

control   1   10   100

Figure 16A.   Effect of vasopressin V2 antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin on urinary sodium excretion in male and female WKY and SHR rats.

Figure 16B.   Effect of vasopressin V2 antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin on urinary potassium excretion in male and female WKY and SHR rats.

Figure 16C.   Effect of vasopressin V2 antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin on urea nitrogen excretion in male and female WKY and SHR rats.

A

WKY Male (n=10)
WKY Female (n=10)
SHR Male (n=8)
SHR Female (n=8)

B

WKY Male (n=10)
WKY Female (n=10)
SHR Male (n=8)
SHR Female (n=8)

C

WKY Male (n=1)
WKY Female (n=10)
SHR Male (n=8)
SHR Female (n=8)

females since lack of data in WKY males. Urinary urea nitrogen excretion in SHR males was similar to that of SHR females.

From the results above, strain and gender differences were shown in urine flow rate and free water clearance. These differences were not accompanied by changes of osmolar clearance and excretion of sodium, potassium.

2.2   Renal Responses to Vasopressin V2 Receptor Antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-Vasopressin in Female Rats during Estrous (SHR n=5, WKY n=8) and Nonestrous (SHR n=8, WKY n=10) Phases of the Estrous Cycle

Basal mean arterial blood pressure (figure 17A) in SHR estrous female rats was significantly higher than that of WKY estrous female rats. Likewise, basal mean arterial blood pressure in SHR nonestrous female rats was significantly higher than that of WKY nonestrous female rats. These results indicated a strain difference in the mean arterial blood pressure, which was higher in SHR female rats than in WKY female rats. Basal mean blood pressure was not different between WKY estrous and nonestrous females as well as SHR estrous and nonestrous females. Therefore, no phase difference existed in both WKY and SHR female rats. Mean arterial blood pressure remained stable throughout the experiment in all groups, indicating that a stable fluid balance was maintained.

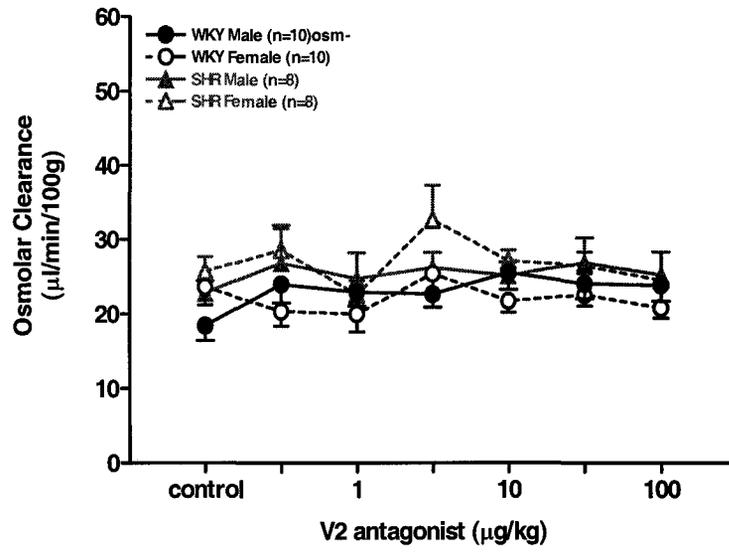Basal heart rate (figure 17B) was similar among all groups and remained stable throughout the experiment, indicating a smooth experimental course.
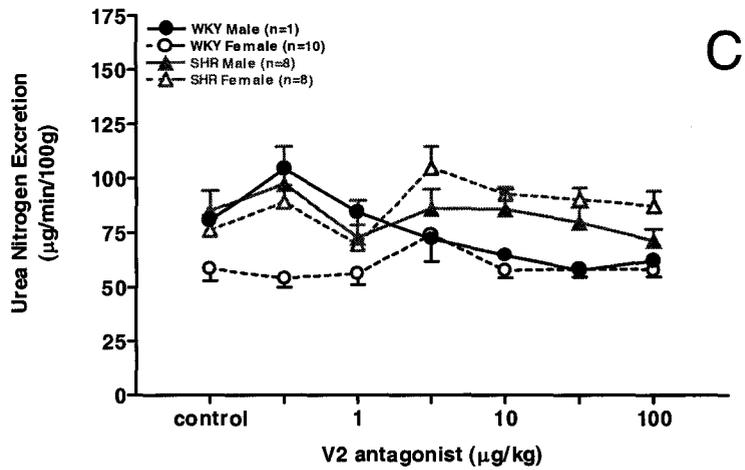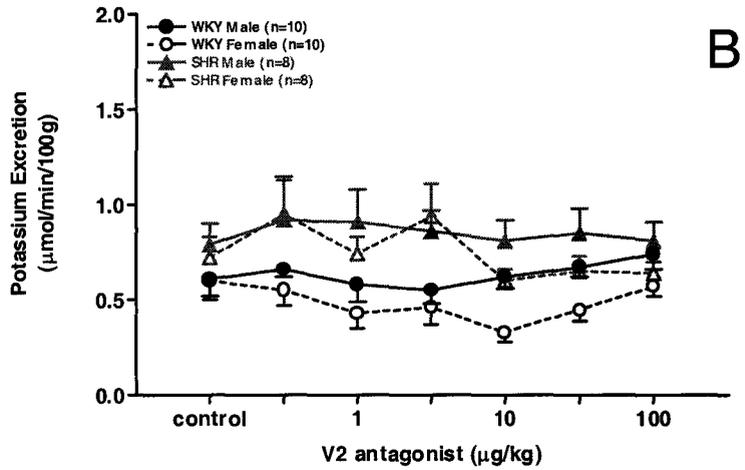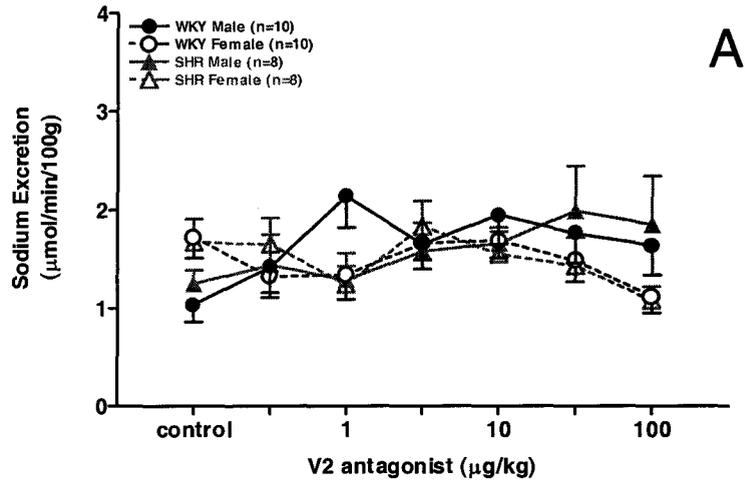
Figure 17A. Effect of vasopressin V2 antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin on mean arterial blood pressure in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

Figure 17B. Effect of vasopressin V2 antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin on heart rate in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

Basal values of body weight, hematocrit, plasma osmolality, and plasma concentrations of sodium and potassium after 90-120 min equilibration period and before administration of vasopressin V2 receptor antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin are shown in Table 4. Body weight, hematocrit, plasma osmolality, and plasma concentrations of sodium and potassium at the end of the experiment are also shown in Table 4.

The body weights of SHR estrous female rats were lower than that of WKY estrous female rats. The body weights of SHR nonestrous female rats were lower than that of WKY nonestrous female rats. The body weight between WKY eatrous and nonestrous females was not different. Similarly, the body weight between SHR estrous and nonestrous females was not different. These results indicated a strain difference in the body weights, which was lower in SHR female rats than in WKY female rats. There was no phase difference in the body weight in both WKY and SHR female rats. Body weight remained stable throughout the experiment in all groups, indicating a stable fluid balance.

Basal hematocrits were similar among all groups and stable throughout the experiment in all groups, indicating that a stable fluid balance and constant blood volume were maintained.

Basal plasma osmolality was similar among all groups. Plasma osmolality increased at the end of the experiment in all groups, however, there was no strain or phase difference in both WKY and SHR rats. Since hematocrits were stable in all groups throughout the experiment indicating a stable blood volume, the increase of plasma

Table 4. <u>Basal Values in Euhydrated State: Estrous and Nonestrous</u>. Body weight, hematocrit, plasma osmolality, plasma sodium concentration, and plasma potassium concentration before and after injection of V2 antagonist in female rats during the non-estrous and estrous phases of estrous cycle.

	WKY		SHR	
	Females Nonestrous phase $n = 10$	Females Estrous phase $n = 8$	Females Nonestrous phase $n = 8$	Females Estrous phase $n = 5$
Body weight (g)				
Beginning	250.5 ± 7.2	251.7 ± 9.4	$171.4 \pm 3.4^*$	$171.4 \pm 2.4^*$
End	247.7 ± 7.1	250.9 ± 9.4	$170.9 \pm 3.4^*$	$171.7 \pm 2.2^*$
Hematocrit (%)				
Beginning	40.9 ± 0.7	41.8 ± 1.3	41.1 ± 1.8	42.7 ± 0.7
End	41.6 ± 0.7	43.7 ± 1.4	40.9 ± 1.3	42.0 ± 0.4
P_{osm} (mOsm/kg H_2O)				
Beginning	287.2 ± 0.9	286.4 ± 1.2	288.3 ± 0.3	288.3 ± 1.4
End	$293.0 \pm 1.7\#$	$296.1 \pm 3.5\#$	$291.2 \pm 1.0\#$	$293.7 \pm 1.5\#$
P_{Na} (mEq/L)				
Beginning	141.3 ± 0.2	141.1 ± 0.6	140.7 ± 0.4	141.5 ± 1.0
End	145.5 ± 2.1	$147.2 \pm 2.1\#$	$142.3 \pm 0.5\#$	144.4 ± 1.1
P_K (mEq/L)				
Beginning	3.7 ± 0.1	3.7 ± 0.1	3.9 ± 0.1	3.8 ± 0.1
End	3.9 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	3.8 ± 0.1

Data are means $\pm$ SE; n, number of rats. P_{osm}, plasma osmolality; P_{Na}, plasma sodium concentration; P_K, plasma potassium concentration. $^*P < 0.05$ vs. WKY; $\#P<0.05$ vs. beginning.

118

osmolality was probably due to increase of osmotic molecules in the plasma during the experiment.

Basal plasma concentration of sodium was similar among all groups. Plasma concentration of sodium increased significantly at the end of the experiment in all groups however, there was no strain or phase difference. Since hematocrit was stable in all groups throughout the experiment indicating a stable blood volume, the increase of plasma concentration of sodium was probably due to conservation of sodium rather than loss of water, which might account for the increase of plasma osmolality.

Basal plasma concentration of potassium was similar among all groups. Plasma concentration of potassium was stable throughout the experiment in all groups, indicating a stable potassium balance in the plasma during the experiment.

According to the above results, strain differences were shown in basal mean arterial blood pressure and body weight. None of phase difference was found between nonestrous and estrous female rats in both WKY and SHR rats.

Basal urine flow rate (figure 18A) was similar among all groups.

Basal urine osmolality (U_{osm}) (figure 18B) was higher in SHR estrous female rats than in WKY estrous female. Basal urine osmolality was higher in SHR nonestrous female rats than in WKY nonestrous female rats. Basal osmolality in WKY estrous females was lower than that in WKY nonestrous females. Basal osmolality in SHR estrous females was not different from that in SHR nonestrous females. These results indicated that there was a strain difference in urine osmolality, which was higher in SHR

119

Figure 18A.   Effect of vasopressin V2 antagonist [1-Adamantane acetyl^1, D-Tyr(Et)^2, Val^4, Abu^6, Arg^9]-arg^8-vasopressin on urine flow rate in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

Figure 18B.   Effect of vasopressin V2 antagonist [1-Adamantane acetyl^1, D-Tyr(Et)^2, Val^4, Abu^6, Arg^9]-arg^8-vasopressin on free water clearance in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

Figure 18C.   Effect of vasopressin V2 antagonist [1-Adamantane acetyl^1, D-Tyr(Et)^2, Val^4, Abu^6, Arg^9]-arg^8-vasopressin on urine osmolality in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

A

B

C

121

female rats than in WKY female rats. There was also a phase difference in urine osmolality in WKY female rats, but not in SHR female rats.

Basal free water clearance (C_{H2O}) (figure 18C) was similar among all groups.

Basal osmolar clearance (figure 19) was similar among all groups.

Basal urinary sodium excretion (figure 20A) was similar among all groups.

Basal urinary potassium excretion (figure 20B) was similar among all groups.

Basal urinary urea nitrogen excretion (figure 20C) was not significantly different among all groups.

According to the above results, strain and phase differences were found in basal urine osmolality. No other strain and phase difference was found.

Intravenous injection of vasopressin V2 receptor antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin at increasing doses resulted in a significant dose-dependent diuresis: increasing urine flow rate and free water clearance, at the same time, decreasing urine osmolality. The dose-response curves of urine flow rate, free water clearance, and urine osmolality are shown in figure 18a, 18b, and 18c.

Low doses of V2 receptor antagonist (0.3-10 µg/kg) induced increase in urine flow rate (figure 18A) was not different between SHR estrous female rats and WKY estrous female rats. However, high doses of V2 receptor antagonist (30-100 µg/kg) induced increase in urine flow rate was significantly lower in SHR estrous female rats than in WKY estrous female rats. Similarly, low doses of V2 receptor antagonist (0.3-10 µg/kg) induced increase in urine flow rate was not different between SHR nonestrous female rats and WKY nonestrous female rats. In contrast, high doses of V2 receptor

Figure 19.   Effect of vasopressin V2 antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin on osmolar clearance in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

Figure showing Osmolar Clearance (μl/min/100g) versus V2 antagonist (μg/kg).

Legend:
- WKY Female-ne (n=10)
- WKY Female-e (n=8)
- SHR Female-ne (n=8)
- SHR Female-e (n=5)

X-axis labels: control, 1, 10, 100

Figure 20A.   Effect of vasopressin V2 antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin on urinary sodium excretion in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

Figure 20B.   Effect of vasopressin V2 antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin on urinary potassium excretion in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

Figure 20C.   Effect of vasopressin V2 antagonist [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin on urea nitrogen excretion in female WKY and SHR rats during estrous and nonestrous phases of the estrous cycle.

antagonist (30-100 µg/kg) induced increase in urine flow rate was significantly lower in SHR nonestrous female rats than in WKY nonestrous female rats. V2 receptor antagonist induced dose-dependent increase in urine flow rate was not different between WKY estrous and nonestrous females. V2 receptor antagonist induced dose-dependent increase in urine flow rate was significantly higher in SHR estrous females than that of SHR nonestraous females in high doses (30-100 µg/kg).

Similar to urine flow rate, V2 antagonist induced dose-dependent free water clearance (figure 18B) was significantly lower in SHR estrous female rats than in WKY estrous female rats. V2 antagonist induced dose-dependent increase in free water clearance was also significantly lower in SHR nonestrous female rats than in WKY nonestrous female rats. V2 antagonist induced dose-dependent increase in free water clearance was not different between WKY estrous female rats than in WKY nonestrous female rats. V2 antagonist induced dose-dependent increase in free water clearance was significantly higher in SHR estrous female rats than in SHR nonestrous female rats.

V2 antagonist induced dose-dependent decrease in urine osmolality (figure 18C) was significantly different among groups in low doses (0.3-3 µg/kg). However, higher doses (10-100 µg/kg) resulted in similar minimum urine osmolality in all groups.

Osmolar clearance (figure 19) was similar among all groups during the experiment.

Urinary sodium excretion (figure 20A) was similar among all groups.

Urinary potassium excretion (figure 20B) was not different among groups, however, SHR female rats tended to have a higher urinary potassium excretion than WKY female rats.

Urinary urea nitrogen excretion (figure 20C) in SHR female rats was significantly higher than that of WKY female rats. No phase difference was shown in both SHR and WKY females.

From the above results, strain difference was shown in V2 receptor induced increase in urine flow rate and free water clearance. No phase difference was shown in WKY females in urine flow rate and free water clearance, whereas phase difference was shown in SHR females.

3. V2 Receptor mRNA Expression in Renal Medullary Collecting Ducts

Relative quantitation of V2 receptor mRNA level in renal medullary collecting duct was shown in figure 21. V2 receptor mRNA level was not different between WKY and SHR rats at 4 weeks of age. V2 receptor mRNA level was significantly decreased in SHR rats at 12 to 16 weeks of age, which was also lower than that of WKY rats at 12 to 16 weeks of age.

Figure 21. V2 receptor mRNA from renal inner medulla of 4-week-old and adult (12- to 16-week-old) male WKY and SHR rats, normalized to mRNA levels of a housekeeper gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). (Data are means $\pm$ SEM. *$P<0.05$ vs. 4-week-old. †$P<0.05$ between age-matched WKY and SHR.)

V2 receptor mRNA measurement

CHAPTER IV

DISCUSSION

1. Comparison of Responses to Vasopressin V2 receptor Stimulation between SHR and WKY

1.1 Gender Differences

Gender and estrous cycle phase differences in the antidiuretic response to vasopressin were first reported by Toba et al. (1991) in ethanol-anesthetized rats that the reduction of urine volume in response to intravenous infusion of vasopressin was similar in males and estrus females but less in non-estrous females. Wang and colleagues extended the study in conscious, chronically instrumented hydrated Sprague-Dawley rats. Wang et al. (1993) demonstrated that the antidiuretic activity of vasopressin was three-fold greater in male than in female rats. They suggested that the greater antidiuretic activity of male rats was due to a greater number of vasopressin V2 receptors and an enhanced activity of adenylate cyclase in male rats. Further study by Wang and colleagues (1994) demonstrated that the antidiuretic activity of vasopressin in estrous female rats was similar to that in male rats but greater than that in nonestrous female rats. However, using the similar experimental protocol described by Wang et al. (1993, 1995, 1996), we could not demonstrate either a gender or a phase difference in the V2 receptor mediated antidiuretic activity of vasopressin in WKY and SHR rats.

The experimental protocol used in the present study was adapted from Wang et al. (1993, 1995, 1996) with slight modifications. Instead of Sprague-Dawley rats used by

Wang and colleagues, we used spontaneously hypertensive rats (SHR) and their

normotensive counterpart Wistar-Kyoto rats (WKY) in the present study. This allowed

us to study the gender difference in the antidiuretic response to vasopressin in not only

normal rats but also hypertensive rats, and therefore, the possible effect of gender

difference in blood pressure control in hypertension. Furthermore, a specific V2 receptor

agonist, [deamino-Cys^1,D-Arg^8]-vasopressin (dDAVP) was used in the present study,

instead of vasopressin which had both V1 and V2 effects used by Wang and colleagues.

This allowed us to examine specifically whether vasopressin V2 renal action contributed

to the gender difference in the antidiuretic response to vasopressin previously reported by

Wang et al. (1993).

Our results differed from the results of Wang and colleagues may be due to two

possibilities: First, the different genetic background of the rats that we used. Second, the

different effects of vasopressin and dDAVP.

It is well known that vasopressin is a peptide hormone with both vasopressor and

antidiuretic activities. Its antidiuretic to vasopressor ratio is approximately 1 (Manning et

al., 1973). In contrast, dDAVP is a selective V2 receptor agonist with marked increased

antidiuretic activity and reduced vasopressor activity. The antidiuretic to vasopressor

ratio of dDAVP is approximately 3000-fold greater than that of vasopressin. Thus,

application of dDAVP may result in the maximum stimulation of vasopressin V2 renal

activity with minimum effect of vasopressin V1 vascular activity. If a gender or a phase

difference in the antidiuretic response to vasopressin as previously shown in normal

Sprague-Dawley rats is indeed due to a gender or phase difference in vasopressin V2

receptor effects (Wang et al., 1993, 1994), a gender or a phase difference in the antidiuretic response to dDAVP should also be evident in the present study.

In our study, V2 agonist dDAVP administered in an amount equivalent to the amount of vasopressin that Wang and colleagues used in their studies showed neither a gender difference nor a phase difference in the antidiuretic activity in either WKY or SHR rats. Under sustained water load (2% body weight, i.g.) to suppressed endogenous release of vasopressin, intravenous infusion of dDAVP induced similar dose-dependent decreases in urine flow rate and free water clearance in all groups as indicated by ED_{50} and minimum levels achieved. It is likely that the gender difference in the antidiuretic response to vasopressin previously reported by Wang et al. (1993) was probably due to the V1 (vasopressor) effect of vasopressin in the kidney, rather than its V2 tubular action.

According to the results of Wang et al. (1993), infusion of AVP (3-1000 pg $\cdot$kg$^{-1}\cdot$ min^{-1}) resulted in a significant dose-dependent antidiuresis, indicated by a decrease in urine flow and free water clearance and an increase in urine osmolality. The differences in the antidiuretic activity of vasopressin between male and female rats were observed at lower infusion rates of AVP (3-100 pg $\cdot$kg$^{-1}\cdot$ min^{-1}), which was greater in male than in female rats, while there had no significant changes on mean arterial blood pressure (MABP) in both sexes. Accompanied with the significant decreases of urine flow and free water clearance, osmolar clearance decreased significantly in male rats while it remained unchanged in female rats. Their results suggested that not only water excretion but also solute excretion were affected in male rats. At high infusion rates of AVP (300-1000 pg $\cdot$kg$^{-1}\cdot$ min^{-1}), however, no differences in the antidiuretic activity of vasopressin

133

between male and female rats were observed. MABP pressure increased in both sexes, which was statistically significant in male rats. Osmolar clearance increased in both sexes without significant difference. It appears that high concentrations of circulating AVP at high infusion rates of AVP increased systemic blood pressure. Due to the effect of pressure-natriuresis-diuresis, water and solute excretion were increased. Since male rats had higher blood pressure than female rats did, higher renal perfusion pressure resulted in greater free water and osmolar clearance of the kidney in male rats, which would offset the greater reductions of free water and osmolar clearance of male rats observed at lower infusion rates of AVP. Therefore, at high infusion rates of AVP, the differences in the free water and osmolar clearance of the kidney between male and female rats no longer existed.

The reason responsible for the decreased osmolar clearance in male rats but not in female rats at low infusion rate of AVP in the study by Wang and colleagues (1993) was not clear. While vasopressin V2 receptors are expressed in the renal tubule system, vasopressin V1a receptors are expressed in not only the renal tubule system but also the renal vasculature. Studies have demonstrated that vasopressin vascular actions in the renal hemodynamics may also influence water and sodium excretion. In rats, the vascular effects of vasopressin are different in the renal cortex and medulla, where the renal medulla seems more sensitive to the vasoconstrictor effect of vasopressin. Franchini et al. (1997) reported that intravenous infusion of vasopressin, which increased plasma concentration of vasopressin within the physiological range, had no effect on mean arterial blood pressure, total renal blood flow, and blood flow in the renal cortex.

However, blood flow in the renal medulla was reduced and was accompanied by decrease in urinary sodium excretion. Since there is a greater pressor responsiveness to vasopressin in male rats than in female rats (Crofton et al., 1986, 1988). It is reasonable to believe that a gender difference in the vasoconstrictive effects of vasopressin in the renal hemodynamics via V1a receptors may result in differences in renal excretion of water and solute between male and female rats. However, whether a greater pressor responsiveness to vasopressin in male rats than in female rats is due to a greater number of V1 receptors or an enhanced sensitivity in the kidney is unclear.

1.2   Strain differences

Although the results from the present study did not support a significant role of gender in the V2 receptor-mediated regulation of renal water handling in both WKY and SHR rats, we confirmed our previous observation that there is an enhanced maximum concentrating ability in SHR compared to WKY (Ye et al., 1999). In response to the vasopressin V2 agonist dDAVP, urine flow rate and free water clearance were similar in all groups. However, SHR achieved a higher maximum urine osmolality than WKY rats, which was largely due to the higher excretion rates of sodium and urea nitrogen in SHR than in WKY rats at the higher dose of V2 stimulation. This result suggested that the maximal concentrating ability was different between SHR and WKY. While renal tubular water handling in SHR and WKY may not be different when all V2 receptors are maximally stimulated, the interstitial concentration gradient against which water can be

reabsorbed may be higher in SHR than in WKY as indicated by the increased ability to the concentrate urine.

2.   Comparison of Responses to Vasopressin V2 receptor Inhibition Between SHR and WKY

Although previous studies reported that exogenous vasopressin resulted in a greater antidiuretic action in male rats than in female rats (Wang et al., 1993, 1994), this present study failed to demonstrate such a difference in SHR and WKY rats, in response to a specific vasopressin V2 receptor agonist dDAVP. If a gender difference in the secretion of vasopressin does occur as reported by Crofton et al. (1985, 1986), one may expect that gender differences in its renal actions may also exist. To examine the gender effect of endogenous AVP in renal fluid handling in SHR, compared to WKY, a selective vasopressin V2 receptor antagonist, [1-Adamantane acetyl[1], D-Tyr(Et)[2], Val[4], Abu[6], Arg[9]]-arg[8]-vasopressin was used to inhibit the V2 action of endogenous vasopressin.

Vasopressin exerts its antidiuretic action through stimulation of vasopressin V2 receptors in the collecting ducts of the kidney. Without the presence of vasopressin, the entire collecting duct is impermeable to water. Binding of vasopressin to the V2 receptors trigger a series of intracellular responses in the collecting duct cells, which lead to the insert of aquaporin-2 (AQP-2) water channels in the apical membrane of the collecting duct cells, thereby increasing permeability and reabsorption of water. Vasopressin V2 receptor antagonist binds to the V2 receptors and inhibits the antidiuretic action of vasopressin, in turn, induces water diuresis. In the present study, we

136

demonstrated a lower water diuresis in males than in females in both SHR and WKY.

Meanwhile, a lower water diuresis in SHR was also observed, compared to WKY rats.

2.1   Gender Differences

Intravenous injection of vasopressin V2 receptor antagonist (0.1-100 µg/kg body

weight) resulted in a significant dose-dependent water diuresis in all groups (SHR male,

SHR female, WKY male, and WKY female). Urine flow and free water clearance were

similar in male and female rats in both strains at low doses of V2 antagonist (0.1-10

µg/kg). However, at higher doses of V2 antagonist (30-100 µg/kg), urine flow and free

water clearance were greater in female rats than in male rats in SHR and WKY. These

gender differences were not due to a direct vasopressin renal V2 effect on solute

excretion, since urine osmolality, osmolar clearance, sodium, potassium, and urea

excretion were similar in male and female SHR as well as in male and female WKY rats.

The reduced response in water diuresis in male rats following administration of

V2 antagonist indicates an enhanced antidiuretic activity of the kidney. Although not

measured in the present study, the plasma concentrations of vasopressin have been found

to be higher in male rats than in female rats, due to a higher rate of secretion from the

pituitary (Crofton et al., 1985, 1986). Higher levels of plasma circulating vasopressin in

male rats are likely to contribute to greater antidiuretic actions of the kidneys. Therefore,

blockade of vasopressin V2 antidiuretic actions in male rats requires higher plasma

concentrations of V2 antagonist than in female rats. In other words, administration of

equal doses of V2 antagonist to male and female rats will lead to less inhibition of

vasopressin V2 antidiuretic actions in male rats, and therefore, produce a lower water diuresis response. In addition, vasopressin vascular actions on renal hemodynamics may also influence renal excretion function. It has been demonstrated that there was a greater pressor responsiveness to vasopressin in male rats than in female rats (Crofton et al., 1986, 1988; Laszlo et al., 1993), due to a greater increase in total peripheral resistance in male rats (Crofton et al., 1988). Laszlo et al. (1993) reported a greater number of vasopressin binding sites in the aorta membrance in male rats. It is not known whether a greater number of vasopressin binding site exits in the renal vasculature in male rats. Parekh et al. (1993) found that both cortical and outer medullary blood flows were auto-regulated in male rats. However, in female rats, cortical blood flow but not outer medullary blood flow was auto-regulated, due to a higher level of vasodilator prostaglandins in the medulla which interfere with blood flow autoregulation in the female rats. Therefore, reduced renal perfusion pressure and/or medullary blood flow may also contribute to the reduced renal water excretion in male rats.

The initial urine osmolality before the administration of V2 antagonist was higher in male WKY than in female WKY. This result suggested that in physiological condition, vasopressin responsiveness in the kidney is different between male and female WKY, probably due to a higher concentration of circulating vasopressin or a higher medullary concentrating ability in male WKY rats. This gender difference was not shown in SHR indicated that the development of hypertension might blunt the difference in vasopressin responsiveness in the kidney between male and female SHR.

There was a significant difference in the maximum increase in urine flow rate and free water clearance between estrous and non-estrous females in SHR, which was greater in estrous than in non-estrous females. This estrous cycle phase difference was not shown in WKY. The mechanism responsible for this difference in SHR and WKY was not clear.

2.2   Strain Differences

The ability of the V2 antagonist to induce water diuresis was less in SHR than that in WKY rats. Urine flow and free water clearance were significantly lower in male and female SHR after V2 antagonist treatment compared to male and female WKY rats, indicating that the ability of the V2 antagonist to induce water diuresis was less in SHR than in WKY rats. This result was similar with the previous findings by Li et al. (1993), who also reported a lower renal response in SHR than in WKY rats after administration of a V2 receptor antagonist ($[d(CH_2)_5,D-Ile^2,Ile^4]$-arginine vasopressin). As mentioned earlier, a reduced response in water diuresis following administration of V2 receptor antagonist might indicate an enhanced antidiuretic activity of the kidney. Elevated levels of vasopressin have been found in SHR compared with those in WKY rats (Crofton et al., 1978; Morhing et al., 1979). Higher levels of plasma circulating vasopressin in SHR are likely to contribute to greater antidiuretic actions in the kidney, and lead to lower water diuresis in response to a V2 antagonist. The reduced response in SHR might be also due to an alteration of renal hemodynamics where renal medullary vascular resistance was elevated in SHR (Roman and Kaldunski, 1988; Gebremedhin et al., 1990). In addition,

an enhanced medullary concentrating ability in SHR might also contribute to the reduced

response to the V2 antagonist in SHR (Ye et al., 1999).

3. Comparison of Blood Pressure and Renal Hemodynamics Between SHR and

   WKY

   In the present studies, the mean arterial blood pressures (MABP) of both SHR and

WKY remained stable throughout the experiments, indicating that acute, short-term

administration of vasopressin V2 receptor agonist dDAVP and antagonist [1-Adamantane

acetyl^1, D-Tyr(Et)^2, Val^4, Abu^6, Arg^9]-arg^8-vasopressin had no effect on blood pressure in

both SHR and WKY rats.

3.1   Gender Differences

   While the MABP was similar in male and female WKY rats, the MABP was 10 -

15 mmHg higher in male SHR than in female SHR. Our results are in agreement with the

findings by others that male SHR develop higher blood pressure than female SHR (Iams

and Wexler, 1977; Masubuchi et al., 1982; Cambotti et al, 1984; Chen and Meng, 1991;

Reckelhoff et al., 1998, 2000). Reckelhoff et al. (1998) reported that the blood pressure

in male SHR became significantly higher than that in females at 12 weeks of age.

   The mechanism responsible for higher blood pressure in male SHR is not clear.

A number of studies have provided evidence that the gonadal hormones are important in

the development of hypertension in SHR. Gonadectomy as well as treatment of gonadal

hormones alter the natural course of hypertension development (Iams and Wexler, 1977;

Masubuchi et al., 1982; Cambotti et al, 1984; Chen and Meng, 1991; Reckelhoff et al., 1998, 2000). However, the mechanisms whereby gonadal hormones may increase blood pressure remain uncertain. It is particularly interesting that gonadectomy performed at 4 weeks of age when the rats were still normotensive attenuated the development of hypertension when they matured (Iams and Wexler, 1977, 1979). In contrast, gonadectomy performed at 6 weeks of age when the blood pressures of the rats had already increased had no effect (Aoki, 1963; Dahl et al., 1975).

These results suggest that the age of the rat at the time of gonadectomy is of great importance in the development of hypertension in SHR, where the gonadal hormones may be involved, particularly, between 4 and 6 weeks of age or younger. Experiments conducted by Cambotti et al. (1984) demonstrated that the neonatal gonadal hormone environment is a major determinant to the sexually dimorphic pattern of hypertension development in the SHR. Results from Uyehara and Gellai (1993) also support the importance of age in the development of hypertension in SHR. The blood pressure of SHR at 4 weeks of age was still within the normal range but then increased significantly at 6 weeks of age.

Reckelhoff et al. (1998; 2000) have demonstrated a shift in the renal pressure-natriuresis relationship in male and female SHR, with males excreting less sodium than females at similar renal perfusion pressures. Castration of the males resulted in the restoration of the pressure-natriuresis relationship and lowered blood pressure levels similar to those in the females. These results suggested a possible relationship between

the excretion function of the kidneys and blood pressure where gonadal hormones may be involved, especially in the early stage of hypertension development in SHR.

In the present study with vasopressin V2 receptor antagonist, there was a gender difference in the excretion of osmoles, shown in the urine osmolality dose response curves, with female SHR exhibiting a much steeper slope than male SHR, which was not exhibited in the WKY. Reduced vasopressin responsiveness in SHR females, along with the maintenance of lower blood pressure in SHR females, indicate that perhaps because SHR females are able to excrete a more concentrated urine than males, the shift of the pressure natriuresis curve does not have to be as great, and blood pressure can be maintained at a lower level than males.

3.2   Strain Differences

The basal MABP was significantly higher in SHR than in WKY rats and tended to be higher in male SHR compared to female SHR. Although renal vascular resistance (RVR) was significantly higher in SHR, glomerular filtration rate (GFR) and effective renal blood flow (ERBF) were not different among groups. Results from Uyehara and Gellai (1993) have shown that GFR and ERBF were reduced as early as 4 weeks of age in SHR, preceding a sharp increase in blood pressure between 4 and 6 weeks of age. GFR and ERBF returned to normal at 6 weeks of age and remained unchanged thereafter. In contrast, RVR was already increased at 4 weeks of age and stayed elevated. Their results suggested that the renal function is impaired preceding the development of hypertension in SHR and that an increased blood pressure was needed to increase GFR

142

and RBF and compensate for impaired renal function. Thus it is not surprising that in the adult rats studied, GFR and ERBF already appeared to be corrected by the elevated blood pressures in the SHR.

Actually, it was nearly 30 years ago, in 1974, when for the first time, Guyton and associates proposed the kidneys are central to the establishment and maintenance of hypertension. Since then, numerous studies conducted regarding the role of the kidney in blood pressure control and hypertension support their view. Renal transplantation studies in rats and humans have provided strong evidence for a pivotal role of the kidney in the pathogenesis of hypertension and this is probably due to a genetic defect influencing renal function.

The most important mechanism by which the kidney contributes to long-term blood pressure regulation is its ability to excrete sodium and water so that a balance between sodium and water intake and sodium and water excretion is maintained. This in turn maintains body fluid volume and blood pressure (Guyton et al., 1974). According to the pressure-natriuresis-diuresis theory proposed by Guyton and associates, renal perfusion pressure is a major determinant of sodium and water excretion. An elevation of renal perfusion pressure results in increased excretion of sodium and water. In normotensive subjects, elevation of arterial blood pressure leads to an increase in renal perfusion pressure, which promotes sodium and water excretion, and consequently reduces blood volume and returns arterial blood pressure to normal. In hypertensive subjects, however, the inability of kidney to excrete sodium and water requires an increased arterial blood pressure to reestablish and maintain sodium and water balance.

Any factor that impairs the ability of the kidney to excrete sodium and water and shifts the relationship between sodium excretion and arterial blood pressure toward higher pressures may induce hypertension.

In fact, all forms of experimental models of hypertension studied to date use some maneuver to reduce the ability of the kidney to excrete sodium and water. Studies have demonstrated that many factors, including neural, hormonal, and humoral factors, may influence the ability of kidney to excrete sodium and water hence arterial blood pressure. Furthermore, many of these factors have been shown to play a role in various forms of hypertension, such as arterial baroreflexes, renin-angiotensin-aldosterone, vasopressin, adrenal catecholamines, atrial natriuretic peptide, endothelial and nitric oxide, kallikrein kinin system, and prostaglandins (Navar, 1997).

It has long been recognized that vasopressin, with its antidiuretic action on renal tubular system via V2 receptors and its vasoconstrictor effect on renal vasculature via V1 receptors, may influence renal excretion of sodium and water, and participate in body fluid homeostasis and blood pressure control. Elevated levels of vasopressin have been found in SHR (Crofton et al., 1978; Morhing et al., 1979). Intravenous injection of vasopressin antiserum lowered blood pressure in SHR indicated that vasopressin plays a role in this model of hypertension. Furthermore, studies using specific antagonists which block the vascular or renal action of vasopressin, alone or combined, lowered blood pressure in SHR indicated that both the vascular and renal actions of vasopressin are involved in the development and maintenance of hypertension.

Thus, in this study renal responsiveness to vasopressin, specifically the V2 mediated effects of vasopressin, was examined. Acute short-term administration of vasopressin V2 receptor agonist and antagonist had no effect on blood pressure in adult SHR with established hypertension, however, no difference would be expected because the role of V2 mediated influence on blood pressure would be via long-term regulation of fluid and electrolyte handling by the kidney working to reset the set point for blood pressure regulation, and not via immediate short-term vasoconstriction effects. Thus, instead, we demonstrated an enhanced antidiuretic activity in SHR, probably due to higher level of endogenous circulation vasopressin and/or greater medullary concentrating ability in SHR. Indeed, the V2 agonist experiments demonstrated that while there was no difference in renal sensitivity to V2 stimulation, there was a difference in maximal urine concentrating ability as exhibited by higher V2 agonist efficacy in increasing urine osmolality in SHR compared to WKY.

We also found that, the relative levels of renal inner medullary V2 receptor mRNA were lower in adult SHR than in WKY, indicating a lower expression of V2 receptors in the kidney of SHR once hypertension was established. These findings suggest that established high blood pressure in SHR is associated with an increased stimulation and down-regulation of renal V2 receptors.

Taken together, all forms of hypertension studied to date have shown to be caused by an inability of the kidneys to excrete sodium and water. Vasopressin, with its antidiuretic action on renal tubular system via V2 receptors and its vasoconstrictor effect on renal vasculature via V1 receptors, may influences renal excretion of sodium and

145

water, and participate in body fluid homeostasis and blood pressure control. There are gender differences in the release and excretion of vasopressin, as well as the cardiovascular action and antidiuretic action of vasopressin. Gender difference has also been found in the development of hypertension in SHR where high blood pressure develops more rapidly and to a higher level in males than in females. It is likely that the gender differences of vasopressin indeed contribute to the gender difference of hypertension development in SHR.

In conclusion, vasopressin influence on renal fluid handling appears to be altered in established hypertension. Pharmacological stimulation of V2 receptors revealed an enhanced maximal antidiuretic activity in male and female SHR rats compared to WKY rats. This difference may be largely due to the higher level of endogenous circulating vasopressin and/or higher medullary concentrating ability in SHR compared to WKY rats. While there were differences in endogenous vasopressin renal action between males and females in both strains, there were no significant differences in the gender effect of endogenous vasopressin between normotensive rats and hypertensive rats. Thus, whether gender differences in fluid handling contribute to gender differences in hypertension development remains to be determined.

If gender differences in vasopressin mediated renal fluid handling do contribute to the lesser development of hypertension in females than males, it may be that effect may be evident before hypertension is fully established, and further study of prehypertensive animals is warranted.

REFERENCES

Abe, K., and S. Ito. The kindney and hypertension. *Hypertens. Res.* 20:75-84, 1997.

Aki, Y., T. Tamaki, H. Kiyomoto, H. He, H. Yoshida, H. Iwao, and Y. Abe. Nitric oxide may participate in V2 vasopressin-receptor-mediated renal vasodilation. *J. Cardiovasc. Pharmacol.* 23:331-336, 1994.

Altura, B. M., and B. T. Altura. Vascular smooth muscle and neurohypophyseal hormones. *Fed. Proc.* 36:1853-1860, 1977.

Aoki, K., H. Tanakawa, T. Fujinami, O. Miyazaki, and Y. Hashimoto. Phthologic studies on the endocrine organs of the spontaneously hypertensive rats. *Jpn. Heart J.* 4:426, 1963.

Baylis, P. H., B. A. Spruce, and J. Burd. Osmoregulation of vasopressin secretion during the menstrual cycle. In: Schrier, R. W., ed. Vasopressin. Raven Press, New York. pp241, 1985.

Berecek, K. H., R. D. Murray, F. Gross, and M. J. Brody. Vasopressin and vascular reactivity in the development of DOCA hypertension in rats with hereditary diabetes insipidus. *Hypertension* 4:3-12, 1982.

Bianchi, G., U. Fox, G. F. Di Francesco, A. M. Giovanetti, and D. Pagetti. Blood pressure changes produced by kidney cross-transplantation between spontaneously hypertensive rats and normotensive rats. *Clin. Sci. Mol. Med.* 47:435-448, 1974.

Bianchi, G., P. G. Bear, U. Fox, and E. Guidi. The role of the kidney in the rat with genetic hypertension. *Postgrad. Med. J.* 53:123-138, 1977.

Blizard, D. A., W. N. Peterson, S. S. Iskandar, Z. K. Shihabi, and N. Adams. The effect of a high salt diet and gender on blood pressure, urinary protein excretion and renal pathology in SHR rats. *Clin. Exp. Hypertens.* A13:687-697, 1991.

Burrell L. M., P. A. Phillips, J. Risvanis, K. L. Aldred, A.-M. Hutchins, and C. I. Johnston. Attenuation of genetic hypertension after short-term vasopressin V1A receptor antagonism. *Hypertension* 26:828-834, 1995.

Burt, V. L., F. Whelton, E. J. Roccella, C. Brown, J. A. Cutler, M. Higgins, M. J. Horan, and D. Labarthe. Prevalence of hypertension in the US adult population: results from the Third National Health and Nutrition Examination Survey, 1988-1991. *Hypertension* 25:305-313, 1995.

Byrom, F. B. The effect of oestrogenic and other sex hormones on the response of the rat to vasopressin. *Lancet* 234:129-131, 1938.

Cambotti, L. J., F. E. Cole, A. A. Gerall, E. D. Frohlich, and A. A. MacPhee. Neonatal gonadal hormones and blood pressure in the spontaneously hypertensive rat. *Am. J. Physiol.* 247:E258-E264, 1984.

Charlton, J. A., and P. H. Baylis. Stimulation of rat renal medullary Na+/K(+)-ATPase by arginine vasopressin is mediated by the V2 receptor. *J. Endocrinol.* 127:213-216, 1990.

Chen, Y. F., and Q. C. Meng. Sexual dimorphism of blood pressure in spontaneously hypertensive rats is androgen dependent. *Life Sci.* 48:85-96, 1991.

Chou, C.-L., S. R. DiGiovanni, A. Luther, S. J. Lolait, and M. A. Knepper. Oxytocin as an antidiuretic hormone. II. Role of V_2 vasopressin receptor. *Am. J. Physiol.* 269:F78-F85, 1995.

Christy, N. P., and J. C. Shaver. Estrogens and the Kidney. *Kidney Int.* 6:366-376, 1974.

Churchill, P. C., M. C. Churchill, and A. K. Bidani. Kidney cross transplants in Dahl salt-sensitive and salt-resistant rats. *Am. J. Physiol.* 262:H1809-H1817, 1992.

Cornoni-Hhuntley, J., A. Z. LaCroix, and R. J. Havlik. Race and sex differentials in the impact of hypertension the United States. *Arch. Intern. Med.* 149:780-788, 1989.

Cowley, A. W, Jr. Role of the renal medulla in volume and arterial pressure regulation. *Am. J. Physiol.* 273: R1-R15, 1997.

Cowley, A. W., E. Monos and A. C. Guyton. Interaction of vasopressin and the baroreceptor reflex system in the regulation of arterial blood pressure in the dog. *Circ. Res.* 34:505-514, 1974.

Cowley, A. W., Jr., E. Szczepanska-Sadowska, K. Stepniakowski, and D. L. Mattson. Chronic intravenous administration of V1 arginine vasopressin agonist results in sustained hypertension. *Am. J. Physiol.* 267:H751-H756, 1994.

Cowley, A. W., Jr., E. W. Quillen, Jr., and M. M. Skelton. Role of vaspressin in cardiovascular regulation. *Fed. Proc.* 42:3170-3176, 1983.

Cowley, A. W., Jr., M. M. Skelton, and T. M. Kurth. Effects of long-term vasopressin receptor stimulation on medullary blood flow and arterial pressure. *Am. J. Physiol.* 275:R1420-R1424, 1998.

Cowley, A. W., Jr., W. C. Cushman, E. W. Quillen, Jr., M. M. Skelton, and H. G. Langford. Vasopressin elevation in essential hypertension and increased responsiveness to sodium intake. *Hypertension* 3 (suppl I):I-93-I-100, 1981.

Cowley, A. W., M. M. Skelton and M. T. Velasque. Sex difference in the endocrine predictors of esssential hypertension. Vasopressin versus renin. *Hypertension* 7 (Suppl I): I-151-I-160, 1985.

Crofton, J. T., and L. Share. Gonadal hormones modulate deoxycorticosterone-salt hypertension in male and female rats. *Hypertension* 29:494-499, 1997.

Crofton, J. T., and L. Share. Osmotic control of vasopressin in male and female rats. *Am. J. Physiol.* 257:R738-R743, 1989.

Crofton, J. T., D. L. Ratliff, D. P. Brooks, and L. Share. The metabolic clearance rate of and pressor responses to vasopressin in male and female rats. *Endocrinology* 118: 1777-1781, 1986.

Crofton, J. T., L. Share, B. C. Wang, and R. E. Shade. Pressor responsiveness to vasopressin in the rat with DOC-salt hypertension. *Hypertension* 2:424-431, 1980.

Crofton, J. T., L. Share, D. P. Brooks. Gonadectomy abolishes the sexual dimorphism in DOC-salt hypertension in the rat. *Clin. Exp. Hypertens.* 11:1249-1261, 1989.

Crofton, J. T., L. Share, R. E Shade, W. J. Lee-Kwon, M. Manning, and W. H. Sawyer. The importance of vasopressin in the development and maintenance of DOC-salt hypertension in the rat. *Hypertension* 1:31-38, 1979.

Crofton, J. T., L. Share, R. E. Shade, C. Allen, and D. Tarnowski. Vasoopressin in the rat with spontaneous hypertension. *Am. J. Physiol.* 235:H361-H366, 1978.

Crofton, J. T., L., Share, and D. P. Brooks. Pressor responsiveness to and secretion of vasopressin during the estrous cycle. *Am. J. Physiol.* 255:R1041-R1048, 1988.

Crofton, J. T., M. Ota, and L. Share. Role of vasopressin, the renin-angiotensin system and sex in Dahl salt-sensitive hypertension. *J. Hypertens.* 11:1031-1038, 1993.

Crofton, J. T., P. G. Baer, L. Share, and D. P. Brooks. Vasopressin release in male and female Rats: effect gonadectomy and treatment with gonadal steroid hormones. *Endocrinology* 117:1195-1200, 1985.

Curtis, J. J., R. G. Luke, and H. P. Dustan, M. Kashgarian, J. D. Whelchel, P. Jones, A. G. Diethelm. Remission of essential hypertension after renal transplantation. *N. Engl. J. Med.* 309:1009-1015, 1983.

Dahl, L. K., and M. Heine. Primary role of renal homografts in setting chronic blood pressure levels in rats. *Circ. Res.* 36:692-696, 1975.

Dahl, L. K., K. D. Knudsen, E. V. Ohanian, M. Muirhead, and R. Tuthill. Role of the gonads in hypertension-prone rats. *J. Exp. Med.* 142:748-759, 1975.

Dai, W. J., L. M. Lu, and T. Yao. Effects of gonadal steroid hormones on hypothalamic vasopressin mRNA level in male and female rats. *Sheng Li Hsueh Pao* 48:557-563, 1996.

Dreifuss, J. J. A review of neurosecretory granules: their contents and mechanism of release. *Ann. N. Y. Acad. Sci.* 248:184-201, 1975.

Ellis, M. E., and A. Grollman. The antidiuretic hormone in the urine in experimental and clinical hypertension. *Endocrinology* 44:415-419, 1949.

Forsling, M. L., M. Akerlund, and P. Stromberg. Variations in plasma concentrations of vasopressin during the menstrual cycle. *J. Endocrinol.* 89:263-266, 1981.

Forsting, M. L., P. Stromberg, and M. Akerlund. Effect of ovarian steroids on vasopressin secretion. *J. Endocrinol.* 95:147-151, 1982.

Fort, P., L. Marty, M. Picchsczyk, S. E. Sabrouly, C. Dani, P. Jeanteur, and J. M. Blanchard. Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigene family. *Nucleic Acids Res.* 13:1431-1442, 1985.

Franchini, K. G., and A. W. Cowley, Jr. Sensitivity of the renal medullary circulation to plasma vasopressin. *Am. J. Physiol.* 271:R647-R653, 1996.

Franchini, K. G., D. L. Mattson, and A. W. Cowley, Jr. Vasopressin modulation of medullary blood flow and pressure-natriuresis-diuresis in the decerebrated rat. *Am. J. Physiol.* 272:R1472-R1479, 1997.

Frey, B. A., O. Grisk, N. Bandelow. S. Wussow, P. Bie, and R. Rettig. Sodium homeostasis in transplanted rats with a spontaneously hypertensive rat kidney. *Am. J. Physiol.* 279:R1099-R1104, 2000.

Frieden, J., and A. D. Keller. The release of vasopressin in response to haemorrhage and its role in the mechanism of blood pressure regulation. *Circ. Res.* 2:214-220, 1954.

Führ, J., J. Kaczmarczyk, and C. D. Krüttgen. Eine einfache colorimetrische Methode zur Inulinbestimmung für Nieren-clearanceuntersuchungen bei Stoffwechselgesunden und Diabetikern. *Klin. Wochenschr.* 33:729-730, 1955.

Ganten, U., G. Schroder, M. Witt, F. Zimmermann, D. Ganten, and G. Stock. Sexual dimorphism of blood pressure in spontaneoualy hypertensive rats: effects of anti-androgen treatment. *J. Hypertens.* 7:721-726, 1989.

Gauer, O. H. and J. P. Henry. Circulating basis of fluid volume control. *Physiol. Rev.* 43:423-481, 1963.

Gebremedhin, D., F. J. Fenoy, D. R. Harder, and R. J. Roman. Enhanced vascular tone in the renal vasculature of spontaneously hypertensive rats. *Hypertension* 16:648-654, 1990.

Gellai, M., and H. Valtin. Chronic vascular constrictions and measurements of renal function in conscious rats. *Kidney Int.* 15:419-426, 1979.

Goodman, L. S., and A. Gilman. The Pharmacological Basis of Therapeutics. Macmillan, New York. 1955.

Graf, C., C. Maser-Gluth, W. de Muinck-keizer, and R. Rettig. Sodium retention and hypertension after kidney transplantation in rats. *Hypertension* 21:724-730, 1993.

Guidi, E., G. Bianchi, E. Rivolta, C. Ponticelli, F. Quarto di Palo, L. Minetti, and E. Polli. Hypertension in man with a kidney transplant: role of familial versus other factors. *Nephron* 41:14-21, 1985.

Guyton, A. C., T. G. Coleman, A. W. Cowley, R. D. Manning, R. A. Norman, and J. D. ferguson. A systems analysis approach to understanding long-range arterial blood pressure control and hypertension. *Circ. Res.* 35:159-176, 1974.

Hafez, E. S. E. (ed.), Reproduction and Breeding Techniques for Laboratory Animals, Detroit, Les an Febiger. pp115-118, 1970.

Hashimoto, J., Y. Imai, N. Minami, M. Munakata, and K. Abe. Effects of vasopressin V1 and V2 receptor antagonists on the development of salt-induced hypertension in Dahl rats. *J. Cardiovasc. Pharmacol.* 26:548-554, 1995.

Hatano, T., K. Ogawa, K. Kanda, H. Seo, and N. Matsui. Effect of ovarian steroids on cyclic adenosine 3',5'-monophosphate production stimulated by arginine vasopressin in rat monolayer cultured cells. *Endocrinol. Jpn.* 35:267-274, 1988.

Hayashi, M., S. Sasaki, H. Tsuganezawa, T. Monkawa, W. Kitajima, K. Konishi, K. Fushimi, F. Marumo, and T. Saruta. Expression and distribution of aquaporin of collecting duct are regulated by vasopressin V2 receptor in rat kidney. *J. Clin. Invest.* 94:1778-1783, 1994.

Hebert, S. C., and T. E. Andreoli. Control of NaCl transport in the thick ascending limb. *Am. J. Physiol.* 246:F745-F756, 1984.

Heller, J., G. Schubert, J. Havlickova, and K. Thurau. The role of the kidney in the development of hypertension: a transplantation study in the Prague hypertensive rat. *Pflugers Arch.* 425:208-12, 1993.

Hinojosa-Laborde, C., D. L. Lange, and J. R. Haywood. Role of female sex hormones in the development and reversal of dahl hypertension. *Hypertension* 35:484-489, 2000.

Hirasawa, A., K. Hashimoto, and G. Tsujimoto. Distribution and developmental change

of vasopressin V_{1A} and V_2 receptor mRNA in rats. *Eur. J. Pharmacol.* 267:71-75, 1994.

Hirsch, A. T., V. J. Dzau, J. A. Majzoub, and M. A. Creager. Vasopressin-mediated forearm vasodilation in normal humans. Evidence for a vascular vaspressin V2 receptor. *J. Clin. Invest.* 84:418-426, 1989.

Hosoya, M., T. Ogura, H. Watanable, Z. Ota, and Z. Kageyama. Autoradiographic localization and age-related changes in vasopressin receptors in spontaneously hypertensive rats. *Nephron* 72:281-287, 1996.

Howell, W. E. The physiological effects of extracts of the hypophysis cerebri and infundibular body. *J. Exp. Med.* 3:245-258, 1898.

Hypertension Detection and Follow-up Program Cooperative Group. Blood pressure studies in 14 community. *JAMA* 237:2385-2391, 1977.

Iams, S. G., and B. C. Wexler. Inhibition of the development of spontaneous hypertension in SH rats by gonadectomy or estradiol. *J. Lab. Clin. Med.* 94:608-616, 1979.

Iams, S. G., and B. C. Wexler. Retardation in the development of spontaneous hypertension in SH rats by gonadectomy. *J. Lab. Clin. Med.* 90:997-1003, 1977.

Jard, S., R. C. Gaillard, G. Guillon, J. Marie, P. Schoenenberg, A. F. Muller, M. Manning, and W. H. Sawyer. Vasopressin antagonists allow demonstration of a novel type of vasopressin receptors in the rat adenohypophysis. *Mo/. Pharinacol.* 30:171-177, 1986.

Jard, S. Vasopressin isoreceptors in mammals: relation to cyclic AMP-dependent and cyclic AMP-independent transduction mechanisms. *Curr. Top. Membr. Transp.* 18:255-282, 1983.

Johnson, J. A., J. E. Zehr, and W. W. Moore. Effects of separate and concurrent osmstic and volume stimuli on plasma ADH in sheep. *Am. J. Physiol.* 218:1273-1280, 1970.

Jorgensen, P. L. The function of (Na+, K+)-ATPase in the thick ascending limb of Henles loop. *Curr. Probl. Clin. Biochem.* 6:190-199, 1976.

Kawabe, K., T. X. Watanabe, K. Shiono, and H. Sokabe. Influence on blood pressure of renal isografts between spontaneously hypertensive and normotensive rats, utilizing the F_1 hybrids. *Jpn. Heart J.* 20:886-894, 1978.

Khokhar, A. M., and J. D. H. Slater. Increased renal excretion of arginine-vasopressin during mild hydropenia in young men with mild essential benign hypertension. *Clin. Sci. Mol. Med.* 51:691s-694s, 1976.

Kim, G. H., C. A. Ecellbarger, C. Mitchell, R. K. Packer, J. B. Wade, and M. A. Knepper. Vasopressin increases Na-K-2Cl cotransporter expression in thick ascending limb of Henle's loop. *Am. J. Physiol.* 276:F96-F103, 1999.

Klingler, C., L. Preisser, M. B. Barrault, P. Lluel, L. Horgen, L. Teillet, N. Ancellin, and B. Corman. Vasopressin V2 receptor mRNA expression and cAMP accumulation in aging rat kidney. *Am. J. Physiol.* 272:R1775-R1782, 1997.

Knepper, M. A. Molecular physiology of urinary concentrating mechanism: regulation of aquaporin water channels by vasopressin. *Am. J. Physiol.* 272:F3-F12, 1997.

Kopf, D., R. Waldherr, and R. Rettig. Source of kidney determines blood pressure in

young renal transplanted rats. *Am. J. Physiol.* 265:F104-F111, 1993.

Kotchen, J. M., H. E. McKean, and T. A. Kotchen. Blood pressure trends with aging. *Hypertension* 4:128-134, 1982.

Landgraf, R., P. Szot, and D. M. Dorsa. Vasopressin receptors in the brain, liver and kidney of rats following osmotic stimulation. *Brain Research* 544:287-290, 1991.

Laszlo, F. A., C. Varga, A. Papp, I. Pavo, and F. Fahrenholz. Difference between male and female rats in vasopressor response to arginine vasopressin. *Acta Physiol. Hung.* 81:137-145, 1993.

Leng, G., W. T. Mason, and R. G. Dyer. The supraoptic nucleus as an osmoreceptor. *Neuroendocrinology* 34:75-82, 1982.

Li P., D. D. Smith. Decreased renal activity of vasopressin in spontaneously hypertensive rats. *J. Hypertens.* 11:41-48, 1993.

Lloyd, S. The vascular responses of the rat during the reproductive cycle. *J. Physiol. (Lond.)* 148:625-632, 1959.

Lolait, S. J., A.-M. O'Carroll, O. W. McBride, M. Konig, A. Morel, and M. J. Brownstein. Cloning and characterization of a vasopressin V2 receptor and possible link to nephrogenic diabetes insipidus. *Nature* 357:336-339, 1992.

Maniatis, T., E. Fritsch, and J. Sambrook. Molecular Cloning: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 1989.

Mason, W. T. Supraoptic neurones of rat hypothalamus are osmosensitive. *Nature* 287:154-157, 1980.

Masubuchi, Y., T. Kumai, A. Uematsu, K. Komoriyama, and M. Hirai. Gonadectomy-induced reduction of blood pressure in adult spontaneously hypertensive rats. *Acta Endocrinol.* 101:154-160, 1982.

Mattson, D. L., R. J. Roman, and A. W. Cowley, Jr. Role of nitric oxide in renal papillary blood flow and sodium excretion. *Hypertension* 19:766-769, 1992.

Mattson, D. L., S. Lu, and A. W. Cowley, Jr. Role of nitric oxide in the control of the renal medullary circulation. *Clin. Exp. Pharmacol. Physiol.* 24:587-590, 1997.

Mattson, D. L., S. Lu, K. Nakanishi, P. E. Papanek, and A. W. Cowley, Jr. Effect of chronic renal medullary nitric oxide inhibition on blood pressure. *Am. J. Physiol.* 266:H1918-H1926, 1994.

Medina, P., G. Segarra, J. M. Vila, P. Chuan, C. Domenech, and S. Lluch. V2-receptor-mediated relaxation of human renal arteries in response to desmopressin. *Am. J. Hypertens.* 12:188-193, 1999.

Merkelbach, U., P. Czernichow, R. C. Gaillard, and M. B. Vallatton. Radioimmunoassay of [8-arginine]-vasopressin. II. Application to determination of antidiuretic hormone in urine. *Acta Endocrinol.* 80:453-464, 1975.

Michell, R. H., C. J. Kirk, and M. M. Billah. Hormonal stimulation of phosphatidylinositol breakdown with particular reference to the hepatic effects of vasopressin. *Biochem. Soc. Trans.* 7:861-865, 1979.

Mohring, J., B. Mohring, M. Petri, and D. Haack. Vasopressor role of ADH in the pathogenesis of malignant DOC hypertension. *Am. J. Physiol.* 232:F260-F269, 1977.

Mohring, J., B. Mohring, M. Petri, and D. Haack. Plasma vasopressin concentrations and effects of vasopressin antiserum on blood pressure in rats with malignant two-kidney Goldblatt hypertension. *Circ. Res.* 42: 17-22, 1978.

Mohring, J., J. Kintz, and J. Schoun. Studies on the role of vasopressin in blood pressure control of spontaneously hypertensive rats with established hypertension (SHR, stroke-prone strain). *J. Cardiovasc. Pharmacol.* 1:593-608, 1979.

Montani, J. P., J. F. Liard, J. Schoun, and J. Mohring. Hemodynamic effects of exogenous and endogenous vasopressin at low plasma concentrations in conscious dogs. *Circ. Res.* 47:346-355, 1980.

Morgan, D. A., G. F. DiBona, and A. L. Mark. Effects of interstrain renal transplantation on NaCl-induced hypertension in Dahl rats. *Hypertension* 15:436-442, 1990.

Morton, J. J., C. Garcia del Rio, and M. J. Hughes. Effect of acute vasopressin infusion on blood pressure and plasma angiotensin II in normotensive and DOCA-salt hypertensive rats. *Clin. Sci. (Colch)* 62:143-149, 1982.

Naitoh, M., H. Suzuki, M. Murakami, A. Matsumoto, A. Ichihara, H. Nakamoto, Y. Yamamura, and T. Saruta. Arginine vasopressin produces renal vasodilation via V2 receptors in conscious dogs. *Am. J. Physiol.* 265:R934-R942, 1993.

Nakanishi, K., D. L. Mattson, V. Gross, R. J. Roman, and A. W. Cowley, Jr. Control of renal medullary blood flow by vasopressin V1 and V2 receptors. *Am. J. Physiol.* 269:R193-R200, 1995,

Nakano, J. Cardiovascular responses to neurohypophysial hormones. In: Handbook of Physiology, Section 7: Endocrinology. Vol. IV: The Pituitary Gland and Its Neuroendocrine Control, Part 1 (R. O. greep and E. B. Astwood, eds.), American Physiogical Society, Washington, D. C. pp395-442, 1974.

Navar, L. G. The kidney in blood pressure regulation and development of hypertension. *Med. Clin. North Am.* 81:1165-1198, 1997.

Nonoguchi, H., A. Owada, N. Kobayashi, M. Takayama, Y. Terada, J. Koike, K. Ujiie, F. Marumo, T. Sakai, and K. Tomita. Immunohistochemical localization of V2 vasopressin receptor along the nephron and functional role of luminal V2 receptor in terminal inner medullary collecting ducts. *J. Clin. Invest.* 96:1768-1778, 1995.

North, W. G. Biosynthesis of vasopressin and Neurophysins. In: Gash, D., and G. J. Boer (ed.) Vasopressin: principles and properties. Plenum Press, New York. pp175-176, 1987.

Okada, H., H. Suzuki, Y. Kanno, and T. Saruta. Effect of nonpeptide vasopressin receptor antagonists on developing, and established DOCA-salt hypertension in rats. *Clin. Exp. Hypertens.* 17:469-483, 1995.

Okada, H., H. Suzuki, Y. Kanno, Y. Yamamura, and T. Saruta. Chronic and selective vasopressin blockade in spontaneously hypertensive rats. *Am. J. Physiol.* 267:R1467-R1471, 1994.

Okuniewski, R., E. A. Davis, B. Jarrott, and R. E. Widdop. A comparison of the development of renal hypertension in male and female rats. *Clin. Sci. (Colch)* 95:445-451, 1998.

Oliver, H., and E. A. Schafer. On the physiological action of extracts of the pituitary body and certain other glandular organs. *J. Physiol. (Lond.)* 18:277-279, 1895.

Ostrowski, N. L., S. J. Lolait, D. J. Bradley, A.-M. O'Carroll, M. J. Brownstein, and W. S. Young, III. Distribution of V1a and V2 vasopressin receptor messenger ribonucleic acids in rat liver, kidney, pituitary and brain. *Endocrinology* 131:533-535, 1993.

Ouchi, Y., L. Share, J. T. Crofton, K. Iitake, and D. P. Brooks. Sex difference in the development of deoxycorticosterone-salt hypertension in the rat. *Hypertension* 9:172-177, 1987.

Ouchi, Y., L. Share, J. T. Crofton, K. Iitake, and D. P. Brooks. Sex difference in pressor responsiveness to vasopressin and baroreflex function in DOC-salt hypertensive rats. *J. Hypertens.* 6:381-387, 1988.

Padfield, P. L., J. J. Brown, A. F. Lever, J. J. Morton, and J. I. S. Robertson. Changes of vasopressin in hypertension: cause or effect? *Lancet* 1:1255-1257, 1976.

Padfield, P. L., J. J. Brown, A. F. Lever, J. J. Morton, and J. I. S. Robertson. Blood pressure in acute and chronic vasopressin excess. Studies of malignant hypertension and the syndrome of inappropriate antidiuretic hormone secretion. *N. Engl. J. Med.* 304:1067-1070, 1981.

Page, I. H., and J. E. Sweet. The effect of hypophysectomy on arterial blood pressure of dogs with experimental hypertension. *Am. J. Physiol.* 120:238-247, 1937.

Parekh, N., A. P. Zou, I. Jungling, K. Endlich, J. Sadowski, M. Steinhausen. Sex differences in control of renal outer medullary circulation in rats: role of prostaglandins. *Am. J. Physiol.* 264:F629-F636, 1993.

Park, F., A. P. Zou, and A. W. Cowley, Jr. Arginine vasopressin-mediated stimulation of nitric oxide within the rat renal medulla. *Hypertension.* 32:896-901, 1998.

Park, F., D. L. Mattson , M. M. Skelton, and A. W. Cowley, Jr. Localization of the vasopressin V1a and V2 receptors within the renal cortical and medullary circulation. *Am. J. Physiol.* 273:R243-R251, 1997.

Patschan, O., B. Kuttler, U. Heemann, A. Uber, and R. Retting. Kidneys from normotensive donors lower blood pressure in young transplanted spontaneously hypertensive rats. *Am. J. Physiol.* 273:R175-R180, 1997.

Pettinger, W. A., R. Fallet, Y. Wang, L.-T. Tam, and W. B. Jeffries. Enhanced cAMP response to vasopressin in the CCT of DOCA-Na hypertensive rats. *Am. J. Physiol.* 251:F1096-F1100, 1986.

Rabito, S. F., O. A. Carretero, and A. G. Scicli. Evidence against a role of vasopressin in the maintenance of high blood pressure in mineralocorticoid and renovascular hypertension. *Hypertension* 3:34-38, 1981.

Rascher. W., E. Weidmann, and F. Gross. Vasopressin in the plasma of stroke-prone spontaneously hypertensive rats. *Clin. Sci.* 61:295-298, 1981.

Rascher. W., R. E. Lang, E. Weidmann, D. Ganten, T. Unger, and F. Gross. Evidence against a role of circulating vasopressin in the pathogenesis of spontaneous hypertension in rats. In: Rascher, W., D. Clough, and D. Ganten. (ed.) Hypertensive mechanisms. Schattauer, Stuttgart. pp574-576, 1982.

Reckelholf, J. F., H. Zhang, and J. P. Granger. Testosterone exacerbates hypertension and

reduces pressure-natriuresis in male spontaneously hypertensive rats. *Hypertension* 31: 435-439, 1998.

Rector, F. C., Jr. Renal concentrating mechanisms. In: Andreoli, T. E., J. J. Grantham, and F. C. Rector, Jr. (ed.) Disturbances in Body Fluid Osmolalily. American Physiological Society, Bethesda. pp125-148, 1977.

Rettig, R., C. G. Folberth, H. Stauss, D. Kopf, R. Waldherr, and T. Unger. Role of the kidney in primary hypertension: a renal transplantation study in rats. *Am. J. Physiol.* 258:F606-F611, 1990.

Robertson, G. L., R. L. Shelton, and S. Athar. The osmoregulation of vasopressin. *Kidney Int.* 10:25-37, 1976.

Roman, R. J., and M. L. Kaldunski. Renal cortical and papillary blood flow in spontaneously hypertensive rats. *Hypertension* 11:657-663, 1988.

Rowland M., and J. Roberts. Blood pressure levels and hypertension in persons aged 6-74 years: United States, 1976-80. NCHS Advance Data from Vital and Health Statistics of the National Center of Health Statistics 84. Washington D.C.: USA Government Publication, 1982.

Rowland, N. E., and M. J. Fregly. Role of gonadal hormones in hypertension in the Dahl salt-sensitive rat. *Clin. Exp. Hypertens.* A14:367-375, 1992.

Saameli, K. The circulatory actions of the neurohypophysial hormones and similar polypeptides. In: Handbook of Experimemtal Pharmacology. Springer-Verlag, Heidelberg. Vol. 23:pp545-611, 1968.

Saito, T., and Y. Yajima. Development of DOCA-salt hypertension in the Brattleboro rat. *Ann. N. Y. Acad. Sci.* 394:309-318, 1982.

Sasaki, S., and M. Imai. Effects of vasopressin on water and NaCl transport across the in vitro perfused medullary thick ascending limb of Henle's loop of mouse, rat, and rabbit kidneys. *Pflugers Arch.* 383:215-221, 1980.

Sawyer, W. H. Neurohypophysial hormones. *Pharmacol. Rev.* 13:225-227, 1971.

Shayakul, C., A. Steel, and M. A. Hediger. Molecular cloning and characterization of the vasopressin-regulated urea transporter of rat kidney collecting ducts. *J. Clin. Invest.* 98:2580-2587, 1996.

Skowsky, W. R., L. Swan, and P. Smith. Effects of sex steroid hormones on arginine vasopression in intact and castrated male and female Rats. *Endocrinology* 104:105-108, 1979.

Sladek, C. D., M. L. Blair, C. Sterling, and M. L. Mangiapane. Attenuation of spontaneous hypertensive in rats by a vasopressin antagonist. *Hypertension* 12:506-512, 1988.

Smith H. W., N. Finkelstein, L. Aliminosa, B. Crawford, and M. Graber. The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. J. Clin. Invest. 24:388-404, 1945.

Stallone, J. N. Mesenteric vascular responses to vasopressin during development of DOCA-salt hypertension in male and female rats. *Am. J. Phystol.* 268:R40-R49, 1995.

Stamler, J., R. Stamler, W. F. Riedlinger, G. Algera, and R. H. Roberts. Hypertension screening of 1 million Americans. Community hypertension evaluation clinic

(CHEC) program, 1973-1975. *JAMA* 235:2299-2306, 1976.

Starling, E. H., and E. B. Verney. The secretion of urine as studied on the isolated kidney. *Proc. R. Soc. Lond. (Biol.)* 97:321-363, 1924.

Szczepanska-Sadowska, E., K. Stepniakowski, M. M. Skelton, and A. W. Cowley, Jr. Prolonged stimulation of intrarenal V1 vasopressin receptors results in sustained hypertension. *Am. J. Physiol.* 267: R1217-R1225, 1994.

Szentivanyi, M., Jr., F. Park, C. Y. Maeda, and A. W. Cowley, Jr. Nitric oxide in the renal medulla protects from vasopressin-induced hypertension. Hypertension 35:740-745, 2000.

Tamaki, T., K. Kiyomoto, H. He, A. Tomohiro, A. Nishiyama, Y. Aki, S. Kimura, and Y. Abe. Vasodilation induced by vasopressin V2 receptor stimulation in afferent arterioles. *Kidney Int.* 49:722-729, 1996.

Teitelbaum I., and S. McGuinness. Vasopressin resistance in chronic renal failure. Evidence for the role of decreased V_2 receptor mRNA. *J. Clin. Invest.* 96:378-385, 1995.

Terada, Y., K. Tomita, H. Nonoguchi, T. Yang, and F. Marumo. Different localization and regulation of two types of vasopressin receptor messenger RNA in microdissected rat nephron segments using reverse transcription polymerase chain reaction. *J. Clin. Invest.* 92:2339-2345, 1993.

Terris, J., C. A. Ecelbarger, S. Nielsen, and M. A. Knepper. Long-term regulation of four renal aquaporins in rats. *Am. J. Physiol.* 271:F414-F422, 1996.

Toba, K., J. T. Crofton, M. Inoue, and L. Share. Effects of vasopressin on arterial blood pressure and cardiac output in mate and female rats. *Am. J. Physiol.* 261:R1118-R1125, 1991.

Uyehara, C. F. T., and M. Gellai. Impairment of renal function precedes establishment of hypertension in spontaneously hypertensive rats. *Am. J. Physiol.* 265:R943-R950, 1993.

Vallotton, M.B., U. Merkelbach, and R. C. Gaillard.. Studies of the factors modulating antidiuretic hormone excretion in man in response to the osmolar stimulus: effects of oestrogen and angiotensin II. *Acta Endocrinol.* 104:295-302, 1983.

van Lieburg, A. F., N. V.A.M. Knoers, L. A.H. Monnens, and P. Smits. Effects of arginine vasopressin and 1-desamino-8-D arginine vasopressin on forearm vasculature of healthy subjects and patients with a V2 receptor defect. *J. Hypertens.* 13:1695-1700, 1995.

Verney, E. B. Absorption and excretion of water. *Lancet* 2:739-744, 781-783, 1946.

Verney, E. B. The antidiuretic hormone and the factors which determine its release. *Proc. R. Soc.* 135:25-106, 1947.

Von den Velden, R. Die Nierenwirkung von Hypophysen extrakten beim Menschen. *Berl. Klin. Wochenschr.* 50:2083-2086, 1913.

Wagner, H. N., and E. Braunwald. The pressor effect of the antidiuretic principle of the posterior pituitary in orthostatic hypotension. *J. Clin. Invest.* 35:1412-1418, 1956.

Wang, Y. X., J. T. Crofton, and L. Share. Sex differences in the cardiovascular and renal actions of vasopressin in conscious rats. *Am. J. Physiol.* 277:R370-R376, 1997.

Wang, Y. X., J. T. Crofton, H. W. Liu, D. P. Brooks, and L. Share. Effects of gonadectomy on sexually dimorphic antidiuretic action of vasopressin in conscious rats. *Am. J. Physiol.* 267:R536-R541, 1994.

Wang, Y. X., J. T. Crofton, H. W. Liu, K. Sato, D. P. Brooks, and L. Share. Estradiol attenuates the antidiuretic action of vasopressin in ovariectomized rats. *Am. J. Physiol.* 268:R951-R957, 1995.

Wang, Y. X., J. T. Crofton, S. L. Bealer, and L. Share. Sexual dimorphism in regional blood flow responses to vasopressin in conscious rats. *Am. J. Physiol.* 273:R1126-R1131, 1997.

Wang, Y. X., R. M. Edwards, P. Nambi, E. J. Stack, M. Pullen, L. Share, J. T. Crofton, and D. P. Brooks. Sex difference in the antidiuretic activity of vasopressin in the rat. *Am. J. Physiol.* 265:R1284-R1290, 1993.

Woods, R. L., and C. I. Johnston. Importance of antidiuretic properties of vasopressin in experimenttal renal hypertension. *Clin. Exp. Pharmacol.* 8:519-523, 1981.

Ye, D., C. A. Burghardt, G. M. Hasiro, and C. F. T. Uyehara. Vasopressin renal V2 action during established hypertension in spontaneously hypertensive rats. *FASEB J.* 13(5):A721, 1999.

Yong, L. C., L. H. Kuller, G. Rutan and C. Bunker. Longitudinal study of blood pressure: changes and determinants from adolescence to middle age. The Dormont high school follow-up study, 1957-1963 to 1989-1990. *Am. J. Epidemiol.* 138:973-83, 1993.