ROLE OF ALDOSTERONE AND THE ALDOSTERONE BINDING GLOBULIN IN EXPERIMENTAL AND SPONTANEOUS HYPERTENSION

By

MARK JOHN JOSEPH NOWACZYNSKI
B.Sc. (Honours), Queen's University, 1981

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY in THE FACULTY OF GRADUATE STUDIES (Department of Physiology)

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
April 1986
© Mark John Joseph Nowaczynski, 1986
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of **PHYSIOLOGY**

The University of British Columbia  
2075 Wesbrook Place  
Vancouver, Canada  
V6T 1W5

Date **23 April 1986**
It is known that the binding capacity of the plasma aldosterone binding globulin (ABG) for aldosterone is increased in 52% of subjects with essential hypertension (EH), but not in individuals with secondary forms of hypertension. It was also previously shown that this elevation in the ABG binding capacity for aldosterone is transmitted as an autosomal dominant trait in families with a history of EH, which suggested that augmented ABG binding capacity could serve as a genetic marker of EH.

To examine whether or not ABG played a role in the development of EH, the urinary homologue of ABG (ABG-TsU) was isolated from pooled human urine and administered to normotensive Sprague-Dawley rats to see if hypertension could be induced. ABG-TsU was purified by differential ultrafiltration, ion exchange chromatography, and gel filtration to electrophoretic homogeneity and was shown to bind aldosterone and dehydroepiandrosterone sulfate with high affinity. ABG-TsU administered once daily i.p. produced a sustained increase in blood pressure (BP) in 5-8 days. After 12 days ABG-TsU treated rats were hypertensive and had increased heart weights but showed no changes in plasma electrolytes, aldosterone, or plasma renin activity (PRA). This hypertension was however aldosterone dependent since it was prevented by adrenalectomy or administration of a spironolactone but not by adrenalectomy when physiological amounts of aldosterone were concomitantly administered with ABG-TsU. The elevated BP in rats treated with ABG-TsU for two weeks was due to increased cardiac output since total peripheral resistance remained inappropriately normal. ABG-TsU induced hypertension thus resembled borderline EH both hormonally and hemodynamically, which suggested that ABG may be playing a pathophysiological role in some subjects with EH.

ABG-TsU was isolated from individual normotensive subjects (normal ABG binding capacity), from a patient with renovascular hypertension (normal ABG binding capacity), and from EH subjects with either high (EHH) or normal (EHN) ABG binding capacity in order to see if the
hypertensinogenicity of ABG-TsU represented a qualitative difference in some EH subjects. Equivalent doses of ABG-TsU were administered over a two week period to Sprague-Dawley rats. Only ABG-TsU from subjects with EH having increased ABG binding capacity (EHH) induced hypertension. ABG-TsU may thus differ qualitatively in a subgroup of subjects with EH and may be of etiological importance in those with increased ABG binding capacity for aldosterone (EHH).

The role of mineralocorticoids in the development and maintenance of hypertension in the spontaneously hypertensive rat (SHR) was investigated starting at 7, 10, and 12 weeks of age by continuously administering spironolactone for one or two weeks. Spironolactone treatment of SHR from 7-9 weeks of age attenuated the rise in BP, but spironolactone treatment of SHR starting at 10 or 12 weeks of age failed to affect BP. The importance of aldosterone in the initial development of the hypertension was examined in 7 week old SHR which were either sham operated (SHAM), adrenalectomized (ADRX), or adrenalectomized and given physiological amounts of aldosterone from 7-9 weeks of age (ADRX+ALDO). At 9 weeks of age SHAM rats were very hypertensive, ADRX rats were normotensive, and ADRX+ALDO rats were hypertensive (in ADRX+ALDO rats BP was half way back to SHAM levels). No difference in plasma aldosterone, Na⁺, K⁺, or PRA existed between SHAM and ADRX+ALDO rats, indicating that the replacement dose of aldosterone chosen was indeed physiological. It is concluded that mineralocorticoids play a role in the development but not in the maintenance of hypertension in the SHR since spironolactone attenuates the BP increase at 7-9 weeks of age but not once the hypertension is more established. Aldosterone therefore appears to be a major initiator of this hypertension, accounting for approximately half of the adrenal dependent component of spontaneous hypertension in the rat during its development.
# Table of Contents

Abstract

List of Figures

List of Tables

List of Abbreviations

Acknowledgements

Introduction

(A) HYPERTENSION; A BRIEF OVERVIEW

(i) Definition of Hypertension

(ii) Prevalence of Hypertension

(iii) Consequences of Hypertension

(iv) Benefits of Treating Hypertension

(v) Concluding Remarks

(B) ROLE OF HEREDITY AND THE ENVIRONMENT IN THE DEVELOPMENT OF ESSENTIAL HYPERTENSION (EH)

(i) Genetic Aspects of EH

(ii) Dietary Factors; Sodium and Potassium

(C) HEMODYNAMICS OF EH
# Table of Contents

**D) MECHANISMS OF SODIUM INDUCED BP ELEVATION IN EH**

1. BP and the Homeostasis of Sodium and Potassium in EH. ........................................... 9
2. Cellular Ion Transport in EH. ......................................................................................... 10
3. Salt Sensitivity in EH. ................................................................................................. 14

**E) PHYSIOLOGY OF ALDOSTERONE**

1. Mineralocorticoid Activity. .......................................................................................... 15
2. Control of Aldosterone Secretion. ................................................................................ 16
3. Metabolism of Aldosterone. ........................................................................................ 17
4. Mineralocorticoid Induced BP Elevation. ...................................................................... 19
5. Cardiac Effects of Aldosterone. ................................................................................... 22
6. Effects of Aldosterone on Arterial Baroreceptors. ....................................................... 25
7. Vascular Effects of Aldosterone. ................................................................................... 25
8. Effects of Aldosterone on Ion Transport in Vascular Smooth Muscle. ...................... 26
9. Aldosterone Receptors and Hypertension. ................................................................... 29

**F) SPIRONOLACTONES AND BLOOD PRESSURE**

1. General Aspects. ........................................................................................................... 32
2. Spironolactones in the Management of EH. ................................................................. 33

**G) ALDOSTERONE IN ESSENTIAL HYPERTENSION**

1. Interactions Between Aldosterone and BP. ................................................................. 35
2. Aldosterone Dynamics in Essential Hypertension. ....................................................... 37
3. Plasma Protein Binding of Aldosterone. ....................................................................... 40
4. Modifications in the Plasma Protein Binding of Aldosterone in Essential Hypertension. 43

**H) THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR)**

1. A Model of Essential Hypertension. ............................................................................. 45
2. Transmembrane Ion Transport in the SHR. ............................................................... 47
3. Role of Aldosterone in the Development of Hypertension in the SHR. ...................... 49

## Aims of the Thesis

52

## Methods: Biochemical Studies

54

**A) ISOLATION OF AB8-TsU**

1. Source of Urine. ........................................................................................................... 54
2. Ultrafiltration. .............................................................................................................. 54
3. Ion Exchange Chromatography. ................................................................................ 56
4. Gel Filtration. ............................................................................................................. 56

**B) CHARACTERIZATION OF AB8-TsU**

1. Determination of Molecular Weight. .......................................................................... 58
2. Electrophoretic Mobility. ............................................................................................ 60
3. Isoelectric Focussing. ................................................................................................. 60
4. Binding Affinity. ......................................................................................................... 61
Results: Biochemical Studies

(A) ISOLATION OF ABG-TsU .................................................. 63

(B) CHARACTERIZATION OF ABG-TsU ...................................... 63
   (i) Determination of Molecular Weight .................................. 63
   (ii) Electrophoretic Mobility .............................................. 64
   (iii) Isoelectric Focussing ............................................... 64
   (iv) Binding Affinity ..................................................... 64

(C) PARTIAL CHARACTERIZATION OF RAT PLASMA ABG ............... 64

Methods: Physiological Studies .............................................. 68

(A) CARE AND HOUSING OF ANIMALS ...................................... 68

(B) SURGICAL PROCEDURES .................................................. 68
   (i) Adrenalectomy ......................................................... 68
   (ii) Implantation of Osmotic MiniPumps ............................... 69
   (iii) Post Operative Care ................................................. 69

(C) METHOD OF ABG-TsU ADMINISTRATION ............................... 69

(D) DETERMINATION OF BLOOD PRESSURE ............................... 70
   (i) Indirect SBP Determination With Preheating .................... 70
   (ii) Indirect SBP Determination Without Preheating ................ 70
   (iii) Direct BP Determination .......................................... 71

(E) MEASUREMENT OF CARDIAC OUTPUT .................................. 71

(F) DETERMINATION OF PRESSOR RESPONSIVENESS TO PHENYLEPHRINE (PD25) .......................................................... 72

(G) PLASMA ASSAYS ............................................................ 73

(H) STATISTICS .................................................................. 75

Methods: Experimental Protocols ........................................... 76

(A) ABG-TsU INDUCED HYPERTENSION IN THE SPRAGUE–DAWLEY RAT .......................................................... 76
(B) ADRENAL DEPENDENCE OF ABG-TsU INDUCED HYPERTENSION IN THE SPRAOUE-DAWLEY RAT .............................................. 78

(C) HEMODYNAMICS OF ABG-TsU INDUCED HYPERTENSION ................................................................. 80

(D) EFFECT OF ABG-TsU ISOLATED FROM INDIVIDUAL HYPERTENSIVE AND NORMOTENSIVE HUMANS ON THE BP OF THE SPRAOUE-DAWLEY RAT ......................................................... 80

(E) MINERALOCORTICOID CONTRIBUTION TO THE INITIATION OF HYPERTENSION IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR) ................................................................. 82
   (i) Animals .................................................................................. 82
   (ii) Comparison of Baseline Variables Between 12 Week Old WKY and SHR ......................................................... 82
   (iii) Spironolactone Treatment of SHR of Different Ages ........... 82
   (iv) Adrenalectomy and Aldosterone Replacement in 7 Week Old SHR .............................................................. 83

Results: Physiological Studies & Experimental Protocols ........................................................................... 89

(A) ABG-TsU INDUCED HYPERTENSION IN THE SPRAOUE-DAWLEY RAT .............................................. 89

(B) ADRENAL DEPENDENCE OF ABG-TsU INDUCED HYPERTENSION IN THE SPRAOUE-DAWLEY RAT ................................................................. 92

(C) HEMODYNAMICS OF ABG-TsU INDUCED HYPERTENSION ................................................................. 95

(D) EFFECT OF ABG-TsU ISOLATED FROM INDIVIDUAL HYPERTENSIVE AND NORMOTENSIVE HUMANS ON BP IN THE RAT ................................................................. 98
   (i) Effect of ABG-TsU Isolated from a Renovascular Hypertensive Subject Having Normal ABG Binding Capacity (RHN) ......................................................... 98
   (ii) Effect of ABG-TsU Isolated From Subject With EH Having Normal ABG Binding Capacity (EHN) ................................................................. 98
   (iii) Effect of ABG-TsU Isolated from Subjects With EH Having Increased ABG Binding Capacity (EHH) ................................................................. 98
   (iv) Effect of ABG-TsU Isolated from Normotensives Having Normal ABG Binding Capacity (NTN) ................................................................. 99

(E) DETERMINATION OF PRESSOR RESPONSIVENESS TO PHENYLEPHRINE (PD_{25}) ................................................................. 109

(F) PRESSOR RESPONSIVENESS TO PHENYLEPHRINE (PD_{25}) IN ABG-TsU TREATED RATS ................................................................. 109
### (G) MINERALOCORTICOID CONTRIBUTION TO THE INITIATION OF HYPERTENSION IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Comparison of Baseline Variables Between 12 Week Old WKY and SHR</td>
<td>109</td>
</tr>
<tr>
<td>(ii) Spironolactone Treatment of SHR of Different Ages</td>
<td>111</td>
</tr>
<tr>
<td>(iii) Adrenalectomy and Aldosterone Replacement</td>
<td>117</td>
</tr>
</tbody>
</table>

### Discussion

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) CHARACTERIZATION OF ABG-TsU</td>
<td>125</td>
</tr>
<tr>
<td>(B) PARTIAL CHARACTERIZATION OF RAT PLASMA ABG</td>
<td>127</td>
</tr>
<tr>
<td>(C) ABG-TsU INDUCED HYPERTENSION IN THE SPRAGUE–DAWLEY RAT</td>
<td>128</td>
</tr>
<tr>
<td>(D) MINERALOCORTICOID CONTRIBUTION TO THE INITIATION OF HYPERTENSION IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR)</td>
<td>143</td>
</tr>
</tbody>
</table>

### References

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>154</td>
</tr>
</tbody>
</table>
List of Figures

FIGURE 1: Isolation of ABG-TsU ................................................................. 55
FIGURE 2: Chromatography of ABG-TsU .................................................. 57
FIGURE 3: Molecular Weight Determination of ABG-TsU ......................... 59
FIGURE 4: Electrophoresis and Isoelectric Focussing of ABG-TsU ............... 65
FIGURE 5: ABG-TsU Binding Affinity for Aldosterone and DHEA-SO₄ ...... 66
FIGURE 6: Partial Characterization of Rat Plasma ABG .......................... 67
FIGURE 7: Pressor Responsiveness (PD₂₅) to Phenylphrine in Untreated Rats ................................................................. 73
FIGURE 8: Protocol for ABG-TsU Induced Hypertension ......................... 77
FIGURE 9: Protocol for Determining the Adrenal Dependence of
ABG-TsU Induced Hypertension in Sprague-Dawley Rats ....................... 79
FIGURE 10: Soldactone Treatment of SHR from 7 to 9 Weeks of Age ........ 84
FIGURE 11: Soldactone Treatment of SHR from 7 to 8 Weeks of Age ........ 85
FIGURE 12: Soldactone Treatment of SHR from 10 to 12 Weeks of Age ..... 86
FIGURE 13: Soldactone Treatment of SHR from 12 to 13 Weeks of Age .... 87
FIGURE 14: Protocol for Determining the Adrenal Dependence
of Hypertension in the SHR ................................................................. 88
FIGURE 15: Effect of ABG-TsU (POOL 1) on SBP in Sprague-Dawley Rats ... 90
FIGURE 16: Plasma Aldosterone, K⁺, and PRA following 12 Days of
ABG-TsU (POOL 1) or Vehicle Administration .................................... 92
FIGURE 17: Mineralocorticoid Dependent Effect of ABG-TsU on SBP in Sprague-Dawley Rats .................................................. 94

FIGURE 18: Hemodynamics of ABG-TsU Induced Hypertension ............................................. 96

FIGURE 19: Hypertensinogenicity of ABG-TsU Isolated from Individual Normotensive or Hypertensive Humans ........................................ 100

FIGURE 20: Time Course of SBP Changes in ABG-TsU (EHH) Treated Rats ........................... 103

FIGURE 21: Plasma Aldosterone, K⁺, and PRA following 14 Days ABG-TsU (EHH) or Vehicle Administration ........................................... 104

FIGURE 22: Pressor Responsiveness to Phenylephrine in ABG-TsU Treated Rats .................... 108

FIGURE 23: Effect of Spironolactone on BP of SHR at Different Ages .................................. 112

FIGURE 24: Effect of Adrenalectomy, Aldosterone Replacement, and Spironolactone on the Development of Hypertension in SHR .................. 120

FIGURE 25: Systolic and Diastolic BP Two Weeks following Sham Operation or Adrenalectomy with and without Aldosterone Replacement ....... 121

FIGURE 26: Plasma Renin Activity Two Weeks following Sham Operation or Adrenalectomy with and without Aldosterone Replacement ...... 122

FIGURE 27: Plasma Potassium Two Weeks following Sham Operation or Adrenalectomy with and without Aldosterone Replacement .......... 123

FIGURE 28: Free and Bound Plasma Aldosterone Two Weeks following Sham Operation or Adrenalectomy with and without Aldosterone Replacement ............ 124
List of Tables

TABLE 1: Effect of ABG-TsU (POOL 1) or Vehicle on Various Variables after 12 Days of Treatment..........................91

TABLE 2: Effect of ABG-TsU (POOL 2) or Vehicle on Various Variables after 14 Days of Treatment..........................97

TABLE 3: Effect of ABG-TsU (RHN) or Vehicle on Various Variables after 14 Days of Treatment..........................101

TABLE 4: Effect of ABG-TsU (EHN) or Vehicle on Various Variables after 14 Days of Treatment..........................102

TABLE 5: Effect of ABG-TsU (EHH) or Vehicle on Various Variables after 14 Days of Treatment..........................105

TABLE 6: Effect of ABG-TsU (NTN) or Vehicle on Various Variables after 14 Days of Treatment..........................106

TABLE 7: SBP in Rats Treated With Vehicle for Two Weeks or With ABG-TsU isolated from Various Sources..........................107

TABLE 8: Baseline Variables in Twelve Week Old WKY and SHR..........................................................110

TABLE 9: Effect of Spironolactone (1.5 mg/rat/day) on Various Variables in SHR (Group 1) Treated from 7 to 9 Weeks of Age..........................113

TABLE 10: Effect of Spironolactone (5 mg/rat/day) on Various Variables in SHR (Group 2) Treated from 7 to 8 Weeks of Age..........................114

TABLE 11: Effect of Spironolactone (1.5 mg/rat/day) on Various Variables in SHR (Group 3) Treated from 10 to 12 Weeks of Age..........................115

TABLE 12: Effect of Spironolactone (5 mg/rat/day) on Various Variables in SHR (Group 4) Treated from 12 to 13 Weeks of Age..........................116

TABLE 13: Effect of Sham Operation, Adrenalectomy, or Adrenalectomy with Aldosterone Replacement at 7 Weeks of Age on Various Variables in 9 Week Old SHR..........................................................119
List of Abbreviations

ABG.......................... thermolabile plasma aldosterone binding globulin
ABG-S.......................... thermolabile salivary homologue of ABG
ABG-Ts.......................... thermostable plasma ABG
ABG-TsU.......................... thermostable urinary homologue of ABG
ABG-TsU (EHH).................. ABG-TsU isolated from subjects with EH having increased ABG binding capacity for aldosterone
ABG-TsU (EHN).................. ABG-TsU isolated from subject with EH having normal ABG binding capacity for aldosterone
ABG-TsU (RHN).................. ABG-TsU isolated from a renovascular hypertensive subject having normal ABG binding capacity for aldosterone
ACTH.......................... adrenocorticotropic hormone
ADRX.......................... bilaterally adrenalectomized rats
ADRX+ALDO..................... bilaterally adrenalectomized rats given physiological amounts of aldosterone
ASF............................. aldosterone stimulating factor
AV3V........................... anteroventral third ventricle
BP.............................. arterial blood pressure
cyclic AMP..................... cyclic adenosine monophosphate
CBO............................ corticosteroid binding globulin
CO.............................. cardiac output
DBP............................ diastolic blood pressure
DHEA-SO4...................... dehydroepiandrosterone sulfate
DLF............................. digitalis like factors
DOC............................ deoxycorticosterone
DOCA........................... deoxycorticosterone acetate
ECFV........................... extracellular fluid volume
EH.............................. essential hypertension
EH.............................. individuals with EH having increased ABG binding capacity for aldosterone
EHN............................. subject with EH having normal ABG binding capacity for aldosterone
HR............................. heart rate
ip.................................intraperitoneally
LVWI..............................left ventricular work index
MAP.................................mean arterial pressure
MCR.................................metabolic clearance rate
MSH.................................melanocyte stimulating hormone
mw.................................molecular weight
NTN.................................normotensive individuals having normal plasma ABO binding
                                 capacity for aldosterone
OC.................................oral contraceptives
PD.................................pressor dose
PD25...............................pressor dose of phenylephrine required to elevate
                                 DBP by 25 mmHg
pi.................................isoelectric point
PRA.................................plasma renin activity
RHN.................................renovascular hypertensive patient having normal ABO binding
                                 capacity for aldosterone
SBP.................................systolic blood pressure
SHAM...............................sham operated rats
SHBG...............................sex hormone binding globulin
SHR.................................spontaneously hypertensive rats
SR.................................secretion rate
WKY.................................Wistar-Kyoto rats
I would like to express my thanks to Dr. F. Lioy for his continuous direction and supervision of this thesis project. I would also like to thank Dr. J. Ledsome for allowing me to pursue graduate studies in the Department of Physiology. I am indebted to Dr. J. Ruedy for his consistent encouragement and for the opportunity to conduct experiments and use the facilities of the Department of Medicine, St-Paul's Hospital. I am grateful to Dr. G.E. Wilkins of the Department of Medicine, St-Paul's Hospital, for his support, constructive discussions, and his contribution of biological materials from fully characterized hypertensive patients. I am thankful to Dr. J. Gregg for willingly allowing the use of the UBC Animal Care Centre where the first experiments on ABG-TsU induced hypertension were conducted. I am obligated to Dr. W. Boyko of the Department of Pathology, St-Paul's Hospital, for the histological examination of tissues from rats with ABG-TsU induced hypertension. The determinations of Plasma Renin Activity by Miss Agnes Tchao of the Department of Laboratories, St-Paul's Hospital, are greatly appreciated. I would like to thank Dr. Donatien Mavoungou for the many stimulating discussions that I had with him and for his keen and helpful advice throughout these studies. The invaluable assistance of the Steroid Research Laboratory, St-Paul's Hospital, in furnishing me with the facilities to isolate ABG-TsU and conduct assays of plasma variables is thankfully acknowledged as is the help that was provided by measuring some plasma variables in part of the plasma samples generated during the course of these studies. The selflessness of Mrs. Maleq Hirji in assisting in the preparation of this manuscript is appreciatively recognized. I am deeply grateful to Dr. W. Nowaczynski for his dedicated guidance throughout my studies.
Introduction

(A) HYPERTENSION: A BRIEF OVERVIEW:

(i) **Definition of Hypertension**: Because arterial blood pressure (BP) values in man follow a continuous frequency distribution, the upper limit of the normal BP range is selected arbitrarily. The World Health Organization has suggested criteria for normotension (BP<140/90 mmHg), borderline hypertension (140-160/90-95 mmHg), and established hypertension (BP>160/95 mmHg) [1]. "The operational definition of hypertension is the level at which the benefits, risks, and costs of action exceed those of inaction" [2].

Secondary forms of hypertension (usually correctable) are relatively rare. The bulk of hypertensive patients (89%) are diagnosed as having essential hypertension (EH), i.e. hypertension of unknown etiology [3]. EH probably encompasses a family of hypertensive disorders with differing pathophysiology.
(ii) **Prevalence of Hypertension:** The incidence of hypertension increases with age, usually first manifesting itself in early middle age. Severe hypertension is uncommon, 80% of afflicted patients having diastolic BP (DBP) between 90-109 mmHg [4].

In the landmark Framingham Study, begun in 1948, 5200 men and women 32-64 years of age at entry were observed every two years [1, 5]. At the time of entry, 16-18% of subjects had established hypertension (BP>160/95 mmHg) [5]. However, if borderline hypertensives (BP>140/90 mmHg) were also included, 40% of men and 48% of women were hypertensive [5]. When one considers the devastating consequences of high BP, these numbers have frightening implications.

(iii) **Consequences of Hypertension:** "Cardiovascular disease is the leading cause of death worldwide. Hypertension -the leading cause of heart attack, stroke, and kidney failure- occurs in more than 20% of adults in most modern societies" [6]. The Framingham Study showed that the incidence of cardiovascular morbidity and mortality rose with increasing BP, strokes being the major cause of death at higher BP. When all risk factors were considered, hypertension emerged as the single most potent contributor to cardiovascular mortality [5]. Actuarial data demonstrated an increase in mortality (mostly due to coronary heart disease) with increasing BP, an augmented risk of death being already associated with BP>127/83 mmHg [7]. The incidence of hypertension associated with various cardiovascular conditions in persons less than 65 years of age were sobering: sudden coronary death 70%, angina pectoris 75%, acute myocardial infarction 75%, aortic aneurism 80-95%, cerebrovascular accident 70-95%, renal failure 90% [49].
(iv) **Benefits of Treating Hypertension:** In moderate and severe hypertension antihypertensive drug therapy reduced cardiovascular morbidity by up to 75% compared to placebo [8]. The benefits of treating mild hypertension are controversial. It is very apparent however that deaths and complications due to strokes, coronary heart attacks, and congestive heart failure are much more common in mildly hypertensive subjects than in non-hypertensives [9]. Two large scale clinical trials on the benefits of antihypertensive drug therapy in mild hypertension showed that after 4-5 years of treatment cardiovascular morbidity was reduced (compared to placebo) by 26-30% [8].

Though this data is highly encouraging, there is great room for improvement: Studies in the United Kingdom have shown that only half of hypertensives have ever been diagnosed; of these only half have ever been treated; and only half of these have normalized BP. In other words only one in eight hypertensives have their BP under control [10].

(v) **Concluding Remarks:** A better understanding of the pathophysiology of EH will lead to better detection and treatment of this group of disorders. For the time being we can only detect and treat the symptoms of EH (high BP) and not its causes. With a better understanding of EH mechanisms, people predisposed to developing EH could be identified (before their BP rises) due to recognition of subtle biochemical clues (a strong family history of EH is suggestive but certainly not diagnostic) and steps taken to prevent the development of hypertension. This would have a decisive impact on cardiovascular morbidity and mortality.
(B) ROLE OF HEREDITY AND THE ENVIRONMENT IN THE DEVELOPMENT OF ESSENTIAL HYPERTENSION (EH):

(i) Genetic Aspects of EH: Systolic BP (SBP) is higher in the offspring of hypertensives than in those of normotensives [11, 12]. EH tends to run in families yet both genetic and environmental factors appear to be necessary to initiate the disease process [13, 14]. It has been hypothesized that the inheritable component of EH (genetic predisposition) is expressed only when high risk genotypes are exposed to certain environmental stimuli [15]. Blacks and whites living in a similar environment do not have the same BP levels, and members of the same racial group living in different environments also have differing BP levels suggesting that both genetic and environmental factors do indeed affect BP [16].

(ii) Dietary Factors: Sodium and Potassium: Dietary salt intake is a major environmental factor leading to the development of hypertension [6, 17, 18, 19]. The high sodium/low potassium diet of “civilized” man is the major environmental factor leading to the development of EH in genetically predisposed individuals [17]. The incidence of hypertension in populations with an elevated dietary salt intake is high and an age related increase in BP is observed [17, 18]. In populations with a low sodium intake, hypertension is rare and no age related increase in BP occurs [17, 18]. However when these populations are exposed to western diets high in sodium, BP increases with age and hypertension becomes prevalent [6].

The Yanomama Indians, who inhabit the Amazonian jungle, are an extreme example of sodium restriction. Their urinary excretion of sodium averaged only 1.3 mEq/24 hrs. while that of potassium averaged over 200
5 mEq/24 hrs. (white expedition members studying them excreted typical “civilized” amounts of 200 mEq/24 hrs. of sodium and 56 mEq/24 hrs. of potassium, the average BP being 126/81 mmHg). In the Yanomama no hypertension was detected and beyond adolescence no age related increase in BP was observed, BP averaging only 101/55 mmHg in adults [19].

A very strong correlation exists between sodium intake and the incidence of hypertension between populations [20] but such a correlation within populations has been very difficult to demonstrate. Only one study (conducted in a race homogeneous group of Neapolitan men) has shown a positive correlation between daily sodium intake and diastolic BP within a population [22].

Restriction of dietary sodium intake in EH results in a fall in BP [6, 21, 23, 24, 25]. In a group of 90 mildly hypertensive subjects dietary sodium restriction controlled BP as adequately as antihypertensive drugs in one third of subjects, and allowed for the reduction of drug dosage in 4 out of 5 subjects [25]. Moderate dietary sodium restriction in young subjects with borderline EH greatly blunted stress induced BP increases [26].

Dietary potassium supplementation significantly reduced the BP of subjects with EH [21, 27, 28, 29, 31]. A highly significant negative correlation between daily potassium intake and both SBP and diastolic BP (DBP) was found within a race homogeneous population of Belgians [30]. In another study the antihypertensive effect of dietary potassium supplementation was accompanied by an increase in plasma potassium concentration [K⁺ directly relaxes vascular smooth muscle(by stimulating Na⁺/K⁺-ATPase?), resulting in vasodilation] [28]. The hypotensive effect of dietary K⁺ supplementation in EH was accompanied by a decrease in the pressor responsiveness to infused norepinephrine and angiotensin II,
suggesting that vascular smooth muscle sensitivity to vasopressors was somehow altered by dietary K⁺ [31]. Interestingly, the incidence of hypertension in different untreated population groups in Japan decreased with increasing plasma potassium concentrations (there being a highly significant negative correlation) [32].

The effects of dietary potassium supplementation and sodium restriction on BP in EH are additive and may eliminate or reduce the need for drugs [21, 27]. Conservative management of hypertension (including a low sodium high potassium diet) can adequately control BP in half of subjects with mild EH [6]. Though following the example of the Yanomama (very low Na⁺ intake/very high K⁺ intake) is highly impractical, some imitation of their dietary habits could reduce BP in EH partway towards the very healthy levels of the Yanomama. Before discussing how sodium increases BP, the hemodynamics of EH will be briefly reviewed.

(C) HEMODYNAMICS OF EH:

BP is a secondary parameter, and thus any discussion of changes in mean arterial pressure (MAP) must involve the primary determinants of MAP, namely, cardiac output (CO) and total peripheral resistance (TPR) since MAP = CO x TPR.

The hemodynamics of EH differ greatly according to age. In young subjects with borderline EH, the BP increase is often due to augmented CO with TPR remaining normal (i.e. equal to that of age matched normotensives) [33-37]. It must be stressed however that the numerical normality of TPR in borderline EH is physiologically inappropriate for the level of CO (and MAP), i.e. there is an inability to vasodilate in the face of increased flow. Thus
these subjects are vasoconstricted relative to what would be observed at the same CO in age matched normotensives.

Increasing heart rate, cardiac contractility, and filling pressure (preload) via blood volume expansion are all factors which would lead to elevated CO. In borderline EH both heart rate and contractility are augmented, and these increases may be partly neurogenically mediated since sympathetic tone is increased and vagal tone is diminished [34, 37]. There is no evidence of blood, plasma, or extra-cellular fluid volume expansion in either borderline or established EH, even when CO is elevated [33, 35-41]. In fact these volumes are often found to be lower in EH than in age matched normotensive subjects [33, 35, 40], even in borderline EH, when CO is high [41]. In borderline EH cardiopulmonary blood volume is however increased due to redistribution of blood from the systemic to the pulmonary circulation (due to sympathetic overactivity?) [34, 35]. Total vascular compliance is decreased in EH and is accompanied by an increase in central venous pressure [38]. Increased right atrial filling pressure due to augmented sympathetic venous tone (and/or decreased compliance of the capacitance vessels) could explain the centralization of blood volume seen in EH, which would raise filling pressure and hence CO without any real hypervolemia [36, 41].

Guyton has proposed that hypertension is initiated by volume expansion (due to a defect in renal Na+ and water excretion) which increases mean circulatory filling pressure and hence CO. The raised CO results in an increment in regional blood flows and "whole-body autoregulation" occurs (augmenting TPR) to bring regional flows back to normal. By this mechanism the hypertension is supposed to evolve from a high CO state into one of increased TPR [42]. Two fundamental perturbations are necessary for this
theory to be substantiated in EH: volume expansion leading to a rise in CO, and blood flow in excess of tissue requirements ("luxury" perfusion) triggering a secondary increase in TPR. As discussed above, blood volume is normal or low in borderline EH (blood volume even being contracted when CO is high [41]). There is also no evidence of luxury perfusion in borderline EH [34, 35, 37]. Four independent studies have shown that oxygen consumption at rest is increased in borderline EH [34, 35]. A direct correlation between CO and oxygen consumption demonstrated that CO was normal (for the level of oxygen consumption) as was the arteriovenous oxygen difference [35]. Thus no luxury perfusion occurs in borderline EH and there is consequently no need to protect the tissues from overperfusion through an autoregulatory increase in TPR.

In established EH BP is high due to increased TPR, CO being normal or somewhat reduced [33-37]. Whether or not high CO hypertension evolves into a high TPR state remains controversial. However in 5-10 year follow-up studies of patients with initial borderline EH, reductions in CO and elevations in TPR have been demonstrated which may have been due to structural changes (left ventricular hypertrophy decreasing cardiac compliance, increased arteriolar wall thickness elevating TPR) [35]. Humoral factors, altered vascular reactivity, structural changes, and heightened neurogenic tone could all be responsible for the raised TPR in established EH. Arterial compliance is reduced in EH, reflecting structural adaptive changes in the arterial wall [43]. Vascular reactivity and sympathetic tone are augmented in established EH and there is enhanced post-junctional α-adrenoceptor mediated vasoconstriction [44, 45].

In human EH heart rate slows down less following infusion of angiotensin II than it does in normotensive subjects, even when
hypertension is mild, indicating that the baroreflex is less efficient [35]. The greater the severity of the hypertension, the less efficient the baroreflex becomes [35]. Decreased baroreflex sensitivity in EH occurs together with increased pressor responsiveness to vasoconstrictors and augmented sympathetic outflow [199, 200]. The baroreceptors become reset in humans with EH and BP is buffered around a new set point [112].

(D) **MECHANISMS OF SODIUM INDUCED BP ELEVATION IN EH:**

(i) **BP and the Homeostasis of Sodium and Potassium in EH:** Dietary sodium restriction decreased intralymphocytic sodium content and blunted stress induced BP elevation in borderline EH [26]. Increasing dietary sodium intake elevated BP and red cell sodium concentrations in patients with EH (when compared to normotensives), which suggested that altered cellular sodium transport may have been involved in the hypertensive process [46].

Blood volume and exchangeable sodium were normal in EH, and total body sodium was even decreased in young subjects with borderline EH [39]. Mean plasma sodium concentrations were significantly lower in subjects with EH than in normotensive controls, and mean exchangeable sodium was significantly lower than normal in young patients with borderline EH [47]. In older hypertensive subjects total body sodium and exchangeable sodium correlated positively with both SBP and DBP, no correlation existing in normotensive subjects or young patients with EH [47]. Plasma potassium concentrations were not related to BP in normotensive subjects, but in patients with EH they were negatively correlated with both SBP and DBP as were exchangeable and total body potassium [47]. In a large scale study involving 3824 patients with EH, plasma potassium correlated negatively
with both SBP and DBP, and plasma sodium correlated positively with SBP only [48].

Sodium and potassium homeostasis and BP regulation are thus somehow interrelated in EH. Cellular sodium and potassium transport may be abnormal in EH due to either the influence of humoral factors, or due to primary alterations in cellular ion transport mechanisms.

(ii) Cellular Ion Transport in EH: The "sodium hypothesis" has been proposed to explain how excessive dietary sodium intake leads to elevated BP in individuals with EH. The release of a putative natriuretic hormone (an endogenous digitalis like factor, i.e. a Na⁺/K⁺-ATPase inhibitor) in response to volume expansion (due to an inherited defect in the kidney's ability to excrete sodium) is presumed to occur [50, 51, 52]. The inhibition of sodium transport in the kidney would correct the volume expansion but in vascular smooth muscle the inhibition of Na⁺ transport would increase intracellular levels of Na⁺ and Ca²⁺. The increased availability of Ca²⁺ would elevate muscle tone and lead to increased contractile activity in arterial, venous and cardiac muscle.

Short term in-vitro experiments have demonstrated that inhibition of Na⁺/K⁺-ATPase leads to vasoconstriction, and stimulation of Na⁺/K⁺-ATPase leads to vasodilation [53]. However, the long term in-vivo effects of Na⁺/K⁺-ATPase inhibitors on vascular resistance have not been elucidated [53]. Increased serum Na⁺/K⁺-ATPase inhibiting activity has been demonstrated in subjects with EH (as compared to normotensive controls) and in monkeys with primary hypertension (as compared to normotensive monkeys) [54, 55]. Subjects with EH excreted an intravenously infused Na⁺ load sooner and more readily than did normotensive controls, this exaggerated natriuresis in
EH having been perhaps due to increased circulating levels of a Na\(^+\)/K\(^+\)-
ATPase inhibitor [198]. Whether or not these changes in circulating levels of
Na\(^+\)/K\(^+\)-ATPase inhibitors in EH are primary or secondary (or indeed due to
volume expansion) is open to debate. No evidence of volume expansion
exists in established EH, or during its development [see section (C)], and
although there is evidence for the existence of a natriuretic hormone, no
such hormone has been fully characterized.

Tobian first demonstrated that vascular sodium and water content
were elevated in both human and experimental hypertension, and suggested
that the resultant “water-logging” effect would increase the vessel
wall/lumen ratio thereby elevating TPR [56]. Altering membrane
permeability to Na\(^+\) or inhibiting the extrusion of cell sodium (by inhibiting
Na\(^+\)/K\(^+\)-ATPase) would lead to an accumulation of Na\(^+\) in the cell [57].
Rapidly acting vasoconstrictors increase membrane permeability (in vascular
smooth muscle cells) so that Na\(^+\) moves down its concentration gradient into
the cell [57]. It has been proposed that the transmembrane sodium gradient
provides the driving force for Ca\(^{2+}\) extrusion from the cell [51]. By
decreasing the transmembrane sodium gradient the intracellular Ca\(^{2+}\)
concentration rises [51]. This is also mediated by the opening of voltage
dependant Ca\(^{2+}\) channels in the membrane when the transmembrane
potential becomes less negative [59]. When sympathetic nerve terminals
depolarize, influx of Ca\(^{2+}\) into the cell results in the fusing of storage vesicles
with the cell membrane and hence release of norepinephrine. If the
intracellular concentration of Ca\(^{2+}\) prior to depolarization is higher due to a
decrease in the transmembrane sodium gradient, then during depolarization
the Ca\(^{2+}\) that enters the cell will cause an even greater peak intracellular
Ca\(^{2+}\) concentration to be reached, resulting in a greater release of
norepinephrine and hence a greater degree of vasoconstriction [51]. In vascular smooth muscle, increasing the intracellular Ca\(^{2+}\) concentration results in an increase in activity of the calmodulin regulated enzyme myosin-light-chain-kinase resulting in increased phosphorylation of the myosin light chain which leads to augmented actin-myosin interaction and hence a greater degree of contraction [58]. Rapidly acting vasoconstrictors (angiotensin II and \(\alpha\)-agonists) cause an increase in cytoplasmic [Ca\(^{2+}\)] through the opening of receptor operated Ca\(^{2+}\) channels in the cell membrane [59]. The Ca\(^{2+}\) that has entered the cell to cause contraction must be extruded to decrease intracellular [Ca\(^{2+}\)] to prevent the interaction of actin and myosin and therefore cause relaxation [58]. Ca\(^{2+}\) is extruded passively through exchange with Na\(^{+}\) and actively by a membrane Ca\(^{2+}\)-ATPase [59]. It has also been proposed that a primary defect in vascular smooth muscle Ca\(^{2+}\)-ATPase leads to increased intracellular [Ca\(^{2+}\)] and hence hypertension independently of any defect in cellular sodium transport [59].

When Na\(^{+}\)-loaded/K\(^{+}\)-depleted erythrocytes were incubated in a physiological medium, they tended to recover their normal low-Na\(^{+}\)/high-K\(^{+}\) content [60]. The erythrocytes of patients with EH showed a decrease in the rate of Na\(^{+}\) extrusion compared to those of normotensive control subjects. This defect was also present in a high proportion of the normotensive offspring of subjects with EH (but not in secondary forms of hypertension) which suggested that the defect in Na\(^{+}\) transport was inherited [60]. Such a genetic defect has been confirmed in patients with EH by familial studies showing abnormal in-vitro Na\(^{+}\) transport (Na\(^{+}\)-Li\(^{+}\) countertransport) in the erythrocytes of subjects with EH and in normotensive individuals with a family history of EH, but not in normotensive subjects without any family history of EH (i.e. bimodal frequency distribution in families with a history of
EH, unimodal distribution in others) [61]. The level of Na\textsuperscript{+}-Li\textsuperscript{+}
countertransport correlated between mother and offspring, between father
and offspring, but not between husband and wife, suggesting that this
biochemical abnormality in EH was indeed inherited [61]. The defect in Na\textsuperscript{+}
transport did not appear to be a primary one since a dialyzable plasma
factor was shown to be responsible for the abnormality in red cell Na\textsuperscript{+}-Li\textsuperscript{+}
countertransport observed in patients with EH [62].

Interference with the basic mechanisms involved in the regulation of
cellular ion concentration and transport directly affects vascular smooth
muscle tone. There is no doubt that increasing vascular smooth muscle [Na\textsuperscript{+}]
leads to vasoconstriction. Sodium channel blockers (amiloride) cause
relaxation of vascular smooth muscle and also cause a large drop in BP in the
spontaneously hypertensive rat (SHR) by decreasing the inward current of
Na\textsuperscript{+} ions [63].

The calcium channel blockers (verapamil, nifedipine) inhibit calcium
entry into vascular smooth muscle and into the myocardium. They
effectively lower BP in experimental and clinical hypertension through both
direct myocardial effects (reducing contractility, heart rate and hence CO)
and direct vascular effects (reducing venous tone and hence venous return,
and reducing arteriolar tone and hence TPR) [64]. The blood concentrations
of Ca\textsuperscript{2+} channel blockers achieved in clinical use are not high enough to block
receptor operated Ca\textsuperscript{2+} channels [59]. Resting Ca\textsuperscript{2+} entry is not blocked but
the voltage sensitive channels are effectively blocked when the cell is
subjected to depolarizing stimuli [59]. The pressor responsiveness to
norepinephrine, angiotensin II, and vasopressin is greatly diminished by
treatment with Ca\textsuperscript{2+} channel blockers [59, 65]. This suggests that the final
common pathway for increased vascular tone is the trans-cellular Ca\textsuperscript{2+} flux.
A primary abnormality in the control of trans-cellular Ca\textsuperscript{2+} flux in EH could exist. The effects of verapamil and sodium nitroprusside (a non-specific vasodilator) on increasing forearm blood flow were compared in patients with EH and in age matched normotensive controls [66]. Verapamil increased forearm blood flow to a far greater extent in patients with EH than in normotensive controls, whereas sodium nitroprusside affected both groups equally [66]. This does suggest that a greater dependency of arteriolar tone on Ca\textsuperscript{2+} influx exists in EH, which may be due to a fundamental derangement in the regulation of transmembrane ion fluxes [66].

(iii) Salt Sensitivity in EH: Patients with EH have been classified as "salt-sensitive" or "salt-resistant" according to their BP response when going from a low to a high sodium intake [67, 68]. Following seven days on a low sodium intake (9 mEq/24 hrs) patients were subjected to a sodium loaded diet (249 mEq/24 hrs) and their BP increase in the first 24 hours following sodium loading was noted. If MAP rose by more than 10 mmHg, they were classified as salt-sensitive, and the non-responders were categorized as salt-resistant (an equal number of patients fell into each group) [67, 68]. Salt-sensitive patients retained more sodium following sodium loading and showed a greater increment in CO than did salt-resistant patients [67, 68]. The difference in sodium retention was not however related to differences in serum Na\textsuperscript{+}, plasma volume, or extracellular fluid volume [69]. This suggests that Na\textsuperscript{+} was sequestered somewhere in the body. Indeed, following Na\textsuperscript{+} loading, salt-sensitive patients had a higher intralymphocytic sodium concentration than did salt-resistant patients [37]. Salt-sensitive patients appeared to have an impaired ability to excrete Na\textsuperscript{+} which was either due to
a renal abnormality or altered humoral control of sodium homeostasis [70].
The greater increase in CO following sodium loading in salt-sensitive patients
was not accompanied by a compensatory decrease in TPR [70]. The
inappropriate level of TPR may have been due to the release of Na+/K+-ATPase inhibitors (to correct the sodium retention) with their resultant
vasoconstrictive effects [see section (D)(ii)]. Dietary potassium
supplementation reduced the pressor effects of sodium loading perhaps
partly through the stimulatory effects of K* on vascular Na+/K+-ATPase.
Support for this comes from the fact that intra-arterial infusion of K* caused
vasodilation, and pre-treatment with ouabain (a Na+/K+-ATPase inhibitor)
reduced the vasodilatory response to K* infusion [70].

A lesser decrement in plasma aldosterone concentration was observed
in salt-sensitive patients following Na+ loading than in salt-resistant patients
[67, 71]. The non-suppressibility of plasma aldosterone in salt-sensitive
patients probably contributed to the sodium retention and to the enhanced
pressor responsiveness to norepinephrine observed [71].

Before discussing the role of aldosterone in the pathogenesis of EH,
aspects of the physiology and pathophysiology of aldosterone will be
reviewed.

(B) PHYSIOLOGY OF ALDOSTERONE:

(i) Mineralocorticoid Activity: Aldosterone is intimately involved in
both sodium and potassium homeostasis. Aldosterone is a steroid hormone
produced by the cells of the zona glomerulosa of the adrenal cortex and it is
the most potent mineralocorticoid present in man, accounting for 60% of total
mineralocorticoid activity [72, 73]. The definition of a mineralocorticoid
hormone is a steroid which promotes unidirectional sodium transport across epithelial surfaces [74]. Classical mineralocorticoid target tissues are kidney, salivary glands, and the gastrointestinal tract, all of which contain high levels of high affinity receptors for aldosterone and related mineralocorticoids [75]. Mineralocorticoids act on the distal renal tubule to promote sodium reabsorption and the secretion of potassium and hydrogen ions [73]. Mineralocorticoids are essential for life: Aldosterone deficiency leads to a decrease in distal tubular sodium reabsorption and potassium excretion, resulting in potassium accumulation and sodium loss from the body. Eventually volume depletion, circulatory collapse and cardiac arrest ensue in death [72]. Mineralocorticoid excess leads to sodium retention, potassium depletion, and hypertension [73].

(ii) Control of Aldosterone Secretion: Aldosterone secretion is primarily stimulated by sodium depletion, the renin-angiotensin system (via angiotensin II), plasma potassium, and adrenocorticotropic hormone (ACTH) [73]. The circadian rhythm of aldosterone is predominantly under the control of ACTH, with plasma concentrations of aldosterone being highest in the early morning [76]. ACTH is a potent short term stimulator of aldosterone secretion [77]. The most potent regulators of aldosterone secretion with long term effects are angiotensin II and plasma potassium [77].

Other factors potentially controlling aldosterone secretion are dopamine, β-lipotropin, β-melanocyte stimulating hormone (β-MSH), α-MSH, a novel aldosterone stimulating factor (ASF), and atrial natriuretic factor (ANF). Dopamine appears to have a tonic inhibitory influence on aldosterone secretion and may blunt the stimulatory effects of angiotensin II on aldosterone secretion [78, 79]. Unlike ACTH, the potent stimulatory effect of
β-lipotropin and that of β-MSH on aldosterone secretion are not mediated via stimulation of cyclic adenosine monophosphate (cyclic AMP), in contrast to the stimulatory effect of α-MSH, suggesting different modes of action on aldosterone stimulation [80, 81]. A glycoprotein of anterior pituitary origin, (ASF), apparently not related to ACTH or β-lipotropin, was shown to be a very potent stimulator of aldosterone secretion whose effects were not blocked by angiotensin II antagonists or ACTH antagonists [82]. Atrial extracts as well as synthetic ANF inhibited both angiotensin II and ACTH stimulated secretion of aldosterone [83].

Many factors may interact at any given time to control aldosterone secretion and modulate the effects of the major stimuli to its secretion. The effects of the renin-angiotensin system however are of predominant importance in the regulation of aldosterone secretion and are indispensable in the control of extracellular fluid volume and hence BP.

(iii) Metabolism of Aldosterone: The plasma concentration of aldosterone is dependant on both its secretion rate (SR) and its metabolic clearance rate (MCR, which is the blood turnover rate and is therefore dependant on metabolic degradation and tissue uptake) such that: plasma concentration = SR/MCR [84]. The bi-exponential character of the plasma disappearance curve of injected 3H-aldosterone indicates that aldosterone is distributed between at least two compartments (inner and outer pools) [84]. In man the half-life of the first component of the disappearance curve of aldosterone is approximately 15 minutes, and that of the second component 30-35 minutes [85]. Aldosterone is dispersed quickly into the inner pool (with a volume of 25 L) and then more gradually into the outer pool (with a volume of about 40 L) [85]. The total volume of distribution of aldosterone
exceeds the volume of total body water which indicates that in addition to being taken-up (and concentrated) in certain tissues, aldosterone is protein bound in plasma (and therefore protected from being metabolized) [84].

In dogs injected with $^3$H-aldosterone, rapid uptake into kidney, liver, heart, and aorta was observed within 5-15 minutes of the aldosterone injection. Peak tissue radioactivity exceeded plasma radioactivity and remained higher in heart, aorta, kidney, and liver suggesting that all of these tissues were target tissues for aldosterone [86]. Interestingly, it has been reported that aldosterone concentrating neurons exist in the central nervous system (primarily in limbic structures) which may also be important for pituitary-adrenal feedback [87].

Eventually, 80% of aldosterone is distributed to the liver, and 15% to the kidney [85]. About 40% of aldosterone is reduced in the liver to tetrahydroaldosterone-3-monoglucuronide [72]. A further 10% of aldosterone is conjugated to glucuronic acid in the kidney resulting in the formation of aldosterone-18-glucuronide (18-oxoconjugate) [72]. Both tetrahydroaldosterone-3-monoglucuronide and the 18-oxoconjugate are very polar and thus easily excreted into the urine.

Following the intravenous administration of $^3$H-aldosterone to dogs, the urinary metabolites of aldosterone were identified: Only 0.2% of the administered hormone appeared as free (unmetabolized) aldosterone, 5-12% as the 18-oxoconjugate, and 20-40% as tetrahydroaldosterone-3-monoglucuronide [84]. Urine is the major route of excretion of aldosterone in man. Following the intravenous injection of $^3$H-aldosterone in man, 80% of the administered radioactivity appeared in urine within 24 hours and 90% within 48 hours (mostly in the form of polar conjugates) [85].
(iv) Mineralocorticoid Induced BP Elevation: The characteristics of mineralocorticoid induced hypertension have been most extensively studied in experimental animals treated with deoxycorticosterone acetate (DOCA). Administration of aldosterone induced a mild form of hypertension when administered alone to intact rats, to rats drinking a 0.5% or 1% NaCl solution, or to uninephrectomized rats drinking a 1% NaCl solution [88-92]. Hypertension induced by DOCA treatment (in uninephrectomized rats drinking a 1% NaCl solution) was characterized by more pronounced vascular lesions in various organs (heart, kidney, mesentery) than in hypertensive rats treated for the same time period with aldosterone [88, 90]. Equivalent doses (100μg/day) of DOCA and aldosterone given to separate groups of uninephrectomized saline-drinking rats over a three week period induced hypertension only in the aldosterone treated group in this time period, indicating that the hypertensinogenic potency of aldosterone was far greater than that of DOCA [92]. Administration of DOCA to rats does not induce hypertension unless the rats are "sensitized" to its effects by prior uninephrectomy and dietary sodium loading [91]. Aldosterone alone will induce hypertension in rats even without prior uninephrectomy or sodium loading which suggests that the mechanisms by which these two mineralocorticoids induce hypertension differ somewhat [91].

Hypertension induced by DOCA administration is dependent on a primary increase in TPR (CO remaining normal), and it thus seems unlikely that an initial elevation in CO and subsequent autoregulation occurs [93, 94]. Hypertension induced in dogs by oral administration of metapyrone (an adrenal 11β-hydroxylase inhibitor which prevents the conversion of 11-deoxycorticosterone to corticosterone) is a model of mineralocorticoid hypertension dependent on elevated deoxycorticosterone secretion [95, 96].
This form of hypertension is resistance mediated from the very beginning and is also dependent on the level of dietary sodium intake [95, 96]. If metapyrone treated dogs were placed on a diet low in sodium, hypertension did not develop. When sodium intake was increased, MAP and TPR increased significantly more than in control dogs, but at no stage was there any rise in plasma volume or extracellular fluid volume in the metapyrone treated dogs [96]. Restoration of sodium stores rather than volume expansion played a more important role in the initiation of this resistance mediated mineralocorticoid hypertension [96]. It has been hypothesized that electrolyte active steroids alter vascular smooth muscle cell ion transport and membrane permeability leading to vasoconstriction [96].

Continuous aldosterone infusion and a high sodium diet produced hypertension in dogs which was due to augmented TPR, CO never changing (even though both blood volume and extracellular fluid volume were expanded leading to increased mean circulatory filling pressure and elevated right atrial pressure) [97]. The authors expected a rise in CO and subsequent autoregulation (the study was from Guyton's department), but this hypertension was resistance mediated from the start. One could speculate that direct effects of aldosterone on the vasculature increased TPR, while the high dietary sodium intake and increased renal effects of aldosterone caused volume expansion to occur. Reducing the sodium intake would have perhaps reduced the excess volume, while the augmented TPR would have been maintained.

Normotensive human volunteers treated with 9α-fluorocortisol (a synthetic mineralocorticoid) had increased MAP within one week, which was due to elevated CO [98]. After six weeks of treatment the hypertension was entirely the result of augmented TPR, and at this stage central venous
pressure was significantly higher than during the control period, and higher than during the initial high CO stage of the hypertension (at which time central venous pressure was normal) [98]. This apparent contradiction makes it difficult to ascertain what role volume expansion played in this form of hypertension, and perhaps direct cardiac and vascular effects of the mineralocorticoids should be considered.

A more complete study of hypertension caused by 9α-fluorocortisol was conducted in patients treated for orthostatic hypotension with this drug [99]. Recumbent hypertension is a risk associated with 9α-fluorocortisol treatment of orthostatic hypotension. Initial BP elevations were associated with sodium retention and plasma volume expansion, but with long-term treatment plasma volume decreased to control levels despite further increases in BP [99]. In the recumbent position this hypertension was entirely due to a raised TPR, since no change in CO was observed [99].

Patients with aldosterone producing adenomas (Conn's syndrome, 1° aldosteronism) have been studied hemodynamically following the withdrawal of anti-hypertensive aldosterone-antagonist medication [100, 101]. During the first two weeks following the discontinuation of spironolactone treatment, sodium retention and plasma volume expansion occurred leading to increased CO and MAP (TPR remaining unchanged) [100, 101]. Sodium loss occurred gradually following the initial sodium retention but still remained more positive than during spironolactone therapy [101]. Volume expansion appeared to be very important as the early determining factor in the initial period of BP increase since a strong correlation existed between extracellular fluid volume and MAP, and between CO and plasma volume [100]. With time however the hypertension gradually evolved into a state of high TPR (with CO and plasma volume returning to normal) [100,
TPR was inversely correlated with the ratio of plasma volume/interstitial fluid volume. Therefore TPR increased as fluid shifted from the intravascular compartment into the interstitium (when the proportion of fluid retained in the intravascular compartment was high, CO was high, and when the proportion was low TPR increased) [100]. The fact that the high flow state persisted for a long time and the fact that the rate of progression to a high resistance state varied greatly between individuals did not suggest a cause and effect relationship between flow and resistance through any "autoregulatory" mechanism [100]. A combination of increased vascular response to aldosterone excess (augmenting TPR) and pressure diuresis as well as a volume shift from the intravascular compartment into the interstitium (decreasing plasma volume and CO) probably contributed to the evolution of this hypertension from a high CO to a high resistance state.

It would appear that the short term effects of excess mineralocorticoid activity on BP involves sodium retention and volume expansion. The kidney "escapes" from the mineralocorticoid excess resulting in sodium excretion after a short time. The long term effects of excess mineralocorticoid activity on BP are not dependent on expansion of intravascular volume and hence their extra-renal effects on BP elevating mechanisms must be considered. Evidence concerning the direct cardiovascular effects of aldosterone will now be reviewed.

(v) Cardiac Effects of Aldosterone: Physiological concentrations of aldosterone have a sustained positive inotropic effect on isolated papillary muscle and whole heart preparations, indicating a direct effect of aldosterone on myocardial contractility [102,103]. Spironolactone alone also had a positive inotropic effect (partial agonist) on isolated papillary muscle,
but the effect of spironolactone and aldosterone given in combination was significantly less than when each was given alone [103]. The partial antagonism by spironolactone of aldosterone's effects suggests that the effects of aldosterone may be receptor mediated.

When rat heart-lung preparations were perfused with blood obtained from adrenalectomized rats, the left ventricular work index fell (LVWI is the product of CO and MAP) [104]. When aldosterone was added to the blood obtained from adrenalectomized rats, the LVWI of the heart-lung preparation was restored to control levels. Chloroform extracts of plasma obtained from intact rats similarly restored normal contractility to the preparation, while extracts of plasma from adrenalectomized rats had no effect [104]. Hypodynamic heart-lung preparations (i.e. perfused with blood from adrenalectomized rats) had decreased LVWI which was corrected by addition of either aldosterone, cortisol, or corticosterone to the perfusate [105]. Following adrenalectomy cardiac contractility decreased by 50% within three hours in cats. Treatment with aldosterone or cortisol at the time of adrenalectomy prevented the fall in cardiac contractility [106].

In intact open-chested whole dog preparations aldosterone infusion caused a rapid (within 15-20 seconds) increase in myocardial contractile force (by 150%), mean and phasic aortic flow (by 41% and 74% respectively), and heart rate (by 29%). The effect was sustained for as long as the infusion continued [107]. This potent positive inotropic action of aldosterone was very rapid and thus may have been due to a direct effect on myocardial Na⁺/K⁺-ATPase. On the other hand aldosterone may have affected the myocardium's responsiveness to neurotransmitters (augmented sensitivity to catecholamines, depressed reactivity to acetylcholine). Aldosterone has been shown to inhibit the action of acetylcholine on the isolated frog heart
Such an effect would alter vagal control of the heart, and would have important implications in vagotonic species such as man.

In isolated heart preparations both aldosterone and ouabain alone elicited positive inotropic actions [108]. Equimolar concentrations of ouabain were considerably more inotropic than aldosterone, and aldosterone antagonized the positive inotropism of ouabain. Addition of aldosterone 20 minutes before adding ouabain resulted in a maximal amount of antagonism. Cortisol did not interfere with the inotropism of ouabain. It appeared that aldosterone may have acted on Na\(^+\)/K\(^+\)-ATPase as a partial agonist, which resulted in the antagonism of the inhibitory (agonist) effects of ouabain on Na\(^+\)/K\(^+\)-ATPase. This strongly suggested that aldosterone affected cardiac contractility via alterations in transmembrane ion distribution. Indirect support for this also came from evidence that aldosterone induced ectopic ventricular arrhythmias in dogs following coronary artery ligation [103]. Aldosterone infusion caused the dogs to develop severe ectopic ventricular tachycardia leading to fatal ventricular fibrillation. Treatment with spironolactone prior to aldosterone administration virtually abolished the development of ectopic arrhythmias.

Within 30 minutes to 3 hours following the administration of corticosteroids (including aldosterone) to normal human subjects CO increased, this effect being related more to the mineralocorticoid than glucocorticoid properties of the corticosteroids, which possibly acted directly and independently of volume expansion [110]. Glucocorticoids are not very inotropic in normal animals or in isolated cardiac tissue under normal conditions [111]. Glucocorticoids can restore or maintain normal cardiac contractility in adrenal insufficiency but will not raise cardiac contractility
above normal values [111]. In contrast to the glucocorticoids, aldosterone can be considered as a positive inotropic agent.

Thus the cardiac effects of aldosterone must be taken into account when discussing the role of aldosterone in elevating BP.

(vi) **Effects of Aldosterone on Arterial Baroreceptors:** Application of aldosterone directly (or by cross-circulation) to the carotid-sinus region of rabbits lead to immediate increases in BP indicating that aldosterone may have had a direct resetting effect on the carotid-sinus baroreceptors (by reducing their sensitivity) so that higher arterial pressure was required to produce normal baroreceptor discharge [113]. Aldosterone may have been directly reducing vagal influence on the heart since aldosterone was shown to inhibit the action of acetylcholine on the isolated frog heart [117].

Surgical ablation of the baroreceptor reflex mechanism (sino-aortic denervation) conferred salt-sensitivity to normal rats [114]. Since alterations in aldosterone dynamics have been implicated in the etiology of salt-sensitive human EH [see section (G)] it is tempting to speculate that direct effects of aldosterone on the baroreflex are of importance in bestowing salt-sensitivity.

(vii) **Vascular Effects of Aldosterone:** In man mineralocorticoids augmented norepinephrine-induced vasoconstriction much more than did glucocorticoids [115]. The pressor effects of angiotensin II were also potentiated by combined infusion with aldosterone in man [116].

Isolated vascular smooth muscle preparations developed an increased sensitivity to angiotensin II and norepinephrine under the influence of aldosterone [118]. Aldosterone augmented responsiveness to epinephrine in
the isolated hind-quarter of the cat, but the synthetic glucocorticoid dexamethasone had no effect [119]. However the norepinephrine induced contraction of aortic strips was potentiated by both aldosterone acetate and the glucocorticoid corticosterone [120]. In the isolated rabbit hind-quarter the effects (on the changes in rate of flow and recuperation time) of perfusion with perfusate containing aldosterone or spironolactone were measured after epinephrine injection [121]. Aldosterone potentiated the actions of epinephrine on flow and greatly prolonged the recuperation time. Spironolactone had the opposite effect [121].

Following the blockade of neuronal re-uptake of norepinephrine (uptake 1), aldosterone caused dose dependent contractions of isolated rabbit vascular smooth muscle through inhibition of extra-neuronal uptake of norepinephrine (uptake 2, which decreased by 25%) [122]. The resultant increase in extracellular norepinephrine concentration produced vasoconstriction by stimulation of vascular α-adrenergic receptors (pretreatment of the tissue with prazosin or phentolamine prevented aldosterone induced contractions, and these α-blockers could relax the aldosterone contracted muscle) [122]. In the same preparation aldosterone caused further contraction of norepinephrine contracted vascular smooth muscle and increased the relaxation time by 50% [123]. Aldosterone enhanced and prolonged contraction by inhibiting removal of catecholamines from the receptor compartment of vascular smooth muscle [123].

Were these direct vascular actions of aldosterone receptor mediated?

(viii) Effects of Aldosterone on Ion Transport in Vascular Smooth Muscle: Five hours following the intravenous infusion of aldosterone in normal dogs, the sodium, K+, Ca2+, and water contents were elevated in both
mesenteric and carotid arteries and in the aorta [124]. Red cell sodium content was augmented in rabbits after two weeks of treatment with aldosterone [125]. The above data would suggest that aldosterone could increase cellular sodium and calcium content resulting in vasoconstriction by the mechanisms previously discussed [see section (D)(ii)]. In reality however, the situation is not so clear.

In both aldosterone and DOCA induced hypertension vascular smooth muscle water content was increased by about 30% over controls [126]. No major alterations in the cellular concentrations of Na\(^+\) or K\(^+\) were detected, but because of the increased water content the cellular content of Na\(^+\) was elevated. Aldosterone infusion in the rat over a three week period induced hypertension [127, 128]. Plasma levels of aldosterone in the hypertensive rats were within the "stressed" physiological range, (i.e. levels no higher than those that can be reached endogenously in response to stress) [127]. Isotope flux studies of the isolated arteries (aorta, femoral arteries) of the hypertensive rats demonstrated an increase in the turnover of K\(^+\) and Cl\(^-\) as compared to arteries from control rats [127,128]. These changes in K\(^+\) and Cl\(^-\) turnover were evident before any BP increase occurred. K\(^+\) turnover was stimulated by norepinephrine in a dose dependent fashion [128]. Arteries from aldosterone treated rats were supersensitive to the effects of norepinephrine on K\(^+\) turnover, and this supersensitivity preceeded the onset of hypertension. Enhanced sodium transport was also noted in the arteries of aldosterone hypertensive rats, probably compensating for the increased passive membrane permeability to Na\(^+\) seen in these rats, thus maintaining normal cell sodium concentration [128]. Alterations in vascular electrolyte metabolism may thus be important in the pathogenesis of mineralocorticoid induced hypertension. The precise mechanisms whereby
these changes in electrolyte metabolism could increase vascular reactivity and TPR remain to be elucidated. However, as the authors of the above studies pointed out, the prolonged in-vitro incubation of the artery strips would have washed out any circulating humoral substances. If a natriuretic hormone (Na⁺/K⁺-ATPase inhibitor) were released in greater quantities during the development of aldosterone induced hypertension, then the increased passive membrane permeability to Na⁺ induced by aldosterone and the sodium pump inhibition caused by the natriuretic hormone would lead to an accumulation of cell Na⁺. This would result in a decrease in the transmembrane Na⁺ gradient and hence a decrease in Ca²⁺ extrusion from the cell resulting in vasoconstriction as previously discussed. [see section (D)(ii)].

In isolated porcine carotid arteries exposed to aldosterone it was shown that aldosterone induced a translocation of Na⁺ from the intracellular space to the extracellular space [129]. This effect could be reproduced in dog and rat arteries but not in those of the rabbit [130]. This extra-renal action of aldosterone could be of importance in the pathogenesis of mineralocorticoid hypertension since pigs, rats, and dogs do respond to the hypertensive effects of mineralocorticoids while the rabbit is relatively resistant to salt, aldosterone, and DOCA induced hypertension [130].

Aldosterone in physiological concentrations significantly augmented the Na⁺/K⁺-ATPase activity of isolated human erythrocytes [131]. A direct action of aldosterone on Na⁺ efflux from vascular smooth muscle appeared to be due to a specific action on mineralocorticoid receptors [132]. This effect was blocked by aldosterone antagonists, while the specific glucocorticoid receptor agonist RU 26988 did not affect Na⁺ efflux [132]. In physiological concentrations aldosterone increased both passive and sodium pump
dependent Na\textsuperscript{+} efflux from the rat tail artery by both rapid and delayed actions. The delayed stimulation of Na\textsuperscript{+} efflux was completely blocked by actinomycin D, implying that transcription of genomic information occurred. The rapid effects of aldosterone were not affected by actinomycin D and therefore did not depend on the production of some aldosterone induced protein [132].

Aldosterone definitely plays a role in the regulation of free intracellular Na\textsuperscript{+}, but it is unclear how it affects vascular smooth muscle tone. In physiological concentrations aldosterone reduced free cell Na\textsuperscript{+} and thus increased the transmembrane Na\textsuperscript{+} gradient in vascular smooth muscle (rat tail artery) in vitro [133]. This effect of aldosterone is confusing since an increased transmembrane Na\textsuperscript{+} gradient would imply decreased vascular tone. However, Friedman has suggested that arteries in this state are much more reactive to vasoconstrictors [133]. This is supported by the in-vitro observation that the responsiveness of the isolated rat tail artery to exogenous norepinephrine rose progressively as the transmembrane Na\textsuperscript{+} gradient increased [134]. Increasing the transmembrane Na\textsuperscript{+} gradient in adrenergic nerve terminals decreased the re-uptake of norepinephrine [134]. This effect coupled with increased vascular reactivity may play a pivotal role in the genesis of increased TPR in mineralocorticoid induced hypertension.

Though aldosterone has profound effects on cellular ion metabolism in-vitro, more evidence is required in-vivo before its means of increasing TPR can be fully understood.

(ix) **Aldosterone Receptors and Hypertension:** Arterial smooth muscle was shown to be an aldosterone target tissue since vascular smooth muscle cells of rabbit and rat contained cytoplasmic receptors which bound
aldosterone (reversibly, with high affinity and low capacity) and differed in binding characteristics from renal mineralocorticoid receptors [135-137]. In rabbit aorta cytosol three distinct receptor subtypes were identified which had high affinity for either mineralocorticoids (11-deoxycorticosterone and aldosterone), cortisol, or dexamethasone [135]. However in rat cytosol two specific receptor subtypes were characterized as either having a higher affinity for aldosterone (Type I) or a higher affinity for dexamethasone (Type II) [136]. These two receptor subtypes were physicochemically different and could be physically separated, demonstrating that vascular smooth muscle contained distinct mineralocorticoid (Type I) and glucocorticoid (Type II) receptors [138]. Both high affinity binders (Types I and II) translocated to the cell nucleus as complexes with their respective steroids (mineralocorticoids or glucocorticoids) and bound to specific acceptor sites on nuclear chromatin and thus exhibited a fundamental property of steroid receptors [139]. The affinity of the mineralocorticoid binder was higher in smaller arteries (carotid and femoral) than in the aorta and evidence existed that receptor affinity in arterioles was even higher [137]. This implied that the smaller the artery, the greater the sensitivity to the action of mineralocorticoids [137]. Thus in the arterial wall a molecular mechanism exists for the in-situ action of aldosterone which may be involved in augmenting TPR in mineralocorticoid induced hypertension and could be involved in the pathogenesis of EH. The presence of aldosterone receptors in a non-epithelial tissue such as vascular smooth muscle requires redefinition of the term "mineralocorticoid" [see section (E)(i)] and may represent a new class of "hypertensinogenic" receptors [140, 141].

Rats that were highly resistant to salt and mineralocorticoid induced hypertension (Fisher 344 strain) had arterial smooth muscle aldosterone
receptors (Type I) of lower affinity than controls, which may have protected them from the hypertensive vascular effects of mineralocorticoids [142]. This observation indirectly supports the theory that mineralocorticoids play a role in BP regulation partly through direct effects on vascular smooth muscle.

Mineralocorticoid type I receptors, having a very high affinity for aldosterone, are present throughout the brain [142-144]. These receptors are found in highest concentration in the hippocampus, but are also present in the medulla oblongata and hypothalamus [142, 143]. This suggests that aldosterone may play a role in salt appetite and may modulate central nervous system control of the cardiovascular system. Evidence in support of this is scarce, but specific lesions in the hypothalamus and especially lesions of the anteroventral third ventricle (AV3V) region prevent the development of mineralocorticoid and salt induced hypertension [145, 146]. Whether or not specific mineralocorticoid receptors in the hypothalamus are necessary in order for mineralocorticoids to increase BP is unknown. The circulating Na⁺/K⁺-ATPase inhibitor(s) appear to be secreted from the hypothalamus [201]. Could the stimulation of hypothalamic mineralocorticoid receptors stimulate the release of Na⁺/K⁺-ATPase inhibitors in mineralocorticoid induced hypertension? Early work comparing the distribution of mineralocorticoid receptors in various brain regions in the Sprague-Dawley rat and the Long-Evans rat revealed that significantly fewer such receptors were present in the hypothalamus of the Long-Evans rat [147]. The Long-Evans rat is resistant to DOCA-salt hypertension, and it has been suggested that the decreased number of hypothalamic mineralocorticoid receptors protected this rat strain from the hypertensinogenic effects of mineralocorticoids [147].
Though aldosterone receptors exist in brain structures involved in cardiovascular regulation as well as in vascular smooth muscle, more information is needed before the role of these receptors in the pathogenesis of various forms of hypertension can be ascertained.

(F) SPIRONOLACTONES AND BLOOD PRESSURE:

(i) General Aspects: Spironolactones are synthetic steroidal lactones which compete for aldosterone binding sites in aldosterone target tissues and inhibit aldosterone stimulated Na\textsuperscript{+} transport [148]. Spironolactones are true competitive inhibitors of mineralocorticoids in-vivo since they block the effects of mineralocorticoids on electrolyte excretion, are effective only in the presence of mineralocorticoids, and their inhibitory effects can be surmounted by increasing dosages of mineralocorticoids [149].

When normal healthy male volunteers were treated with a synthetic mineralocorticoid (9α-fluorocortisol), BP increased. The effects of 9α-fluorocortisol on BP were completely reversed by concomitant spironolactone administration, but spironolactones did not lower BP below initial control levels [150]. This illustrated a fundamental property of spironolactones: they only lower BP when the BP increase is caused by mineralocorticoids [151]. Thus the existence of spironolactone responsive hypertension implies that mineralocorticoids play a pathogenic role.

Spironolactones did not only block the effects of aldosterone at the receptor level. They directly affected the adrenal cortex by inhibiting aldosterone biosynthesis as well as diminishing both ACTH and angiotensin II stimulated aldosterone secretion [152,153]. Spironolactones stimulated the hepatic metabolism and conjugation of aldosterone and increased the
bilary excretion of aldosterone metabolites in the rat [154]. Thus mineralocorticoid receptor antagonism, decreased biosynthesis, and augmented metabolism of aldosterone are all factors to be considered in the action of spironolactones on BP (in at least the short-term). However, with long term spironolactone treatment, inhibition of adrenal cortical function appeared to be overcome since the sensitivity of the adrenal cortex to the stimulatory effects of ACTH on aldosterone, cortisol, and 18-OH-deoxycorticosterone secretion were augmented (this may have been due to compensatory up-regulation of ACTH receptors) [156]. Thus with long term spironolactone therapy there did not appear to be any inhibition of adrenocortical function (which in fact was stimulated).

Preliminary evidence exists that man produces an endogenous natural spironolactone since a spironolactone has been isolated from the urines of normal healthy humans [155]. It is not known what role this compound plays in normal BP regulation or if a deficiency in its production leads to hypertension.

(ii) *Spironolactones in the Management of EH:* In a multicenter study of the effectiveness of spironolactone monotherapy in EH it was found that spironolactone was effective in lowering BP [157]. The effectiveness of spironolactone in lowering BP was not dependent on pretreatment BP levels, and satisfactory control of hypertension was achieved in more than half of the patients [157]. When patients with EH were sodium loaded and aldosterone secretory rates were measured, it was found that the BP lowering action of spironolactone was greatest in those with the least suppressible aldosterone secretory rates [158]. The blood pressure lowering effect of spironolactone correlated with the aldosterone secretory rate, which
strongly suggested that aldosterone played a role in the development or maintenance of hypertension in some subjects with EH [158]. Following sodium loading, aldosterone excretion in a group of patients with EH was bimodally distributed, being non-suppressible in a subgroup of 36% of the total study group [159,160]. No difference in PRA or the excretory rates of ACTH dependent steroids (cortisol, DOC) existed between the two groups, which suggested that control of aldosterone secretion (via the renin-angiotensin system and ACTH) did not differ between the two groups [160]. However the group with non-suppressible aldosterone excretion responded with a far greater fall in BP following spironolactone therapy, suggesting that aldosterone played a greater role in maintaining high BP in this group [159, 160].

The existence of spironolactone responsive hypertension in a sub-group of patients with EH (36% to over 50%) implies that mineralocorticoids play a pathogenic role, and aldosterone may be a key factor in the pathogenesis of EH in these patients.
(G) ALDOSTERONE IN ESSENTIAL HYPERTENSION:

(i) **Interactions Between Aldosterone and BP:** In an age and race homogeneous group of men encompassing the entire BP spectrum, plasma aldosterone was the only component of the renin-angiotensin-aldosterone axis to correlate with MAP. MAP did not change much in relation to aldosterone at lower BP but increased with increasing aldosterone at higher BP, indicating that aldosterone was the most important component of the renin-angiotensin-aldosterone system for sustaining high BP and may have been crucial for the development and maintenance of EH [161]. Administration of aldosterone to human subjects increased vascular reactivity to norepinephrine, this effect being far more pronounced in individuals with EH (in whom vascular reactivity was already higher) than in normotensive controls [162]. Half of subjects with EH had both increased plasma aldosterone and increased vascular reactivity, these two parameters being positively correlated only in the EH patients with increased plasma aldosterone, which suggested that aldosterone played a pathogenic role in some subjects with EH by increasing vascular reactivity [163]. During high sodium intake, salt-sensitive patients with EH (in whom BP increased by more than 10% following Na\(^+\) loading) did not suppress plasma aldosterone levels (compared to non salt-sensitive patients) which were also elevated compared to the level of PRA [71]. These salt-sensitive patients also had augmented pressor responsiveness to norepinephrine both before and after Na\(^+\) loading) which may have been linked to the non-suppressibility of plasma aldosterone [71]. Thus aldosterone may have directly [see section (E)(vii)] or indirectly affected vascular reactivity in some individuals (approximately half) with EH.
Sodium loading augmented BP in subjects with EH, while no effect was observed in age matched controls [164]. Suppression of plasma aldosterone and aldosterone excretion was less pronounced in the group with EH (even though PRA was suppressed to an equal extent in both the hypertensive and normotensive groups of patients) which may have been attributable to a blunted sensitivity of the adrenal cortex to changes in circulating levels of angiotensin II in the hypertensive patients [164]. Of importance was the finding that the changes in plasma aldosterone concentration during salt loading, were inversely (and highly significantly) correlated to changes in BP (i.e. the less suppressible plasma aldosterone was, the more BP increased in response to Na\(^+\) loading) [164]. The secretion rate of aldosterone following Na\(^+\) loading was directly correlated (p<0.001) to the level of MAP in subjects with EH [165].

It is abundantly clear that the "turn-off" of aldosterone by Na\(^+\) loading is defective in EH, and this may directly contribute to the sensitivity of BP to the effects of a high dietary salt intake.

Plasma aldosterone concentrations were found to be elevated in 57% of adolescents with EH. Treatment with β-blockers (anaprilin, obsidan) was only effective in lowering BP in those subjects with initially high plasma aldosterone levels, while those with initially normal aldosterone levels failed to respond with any change in BP [189]. Adults with EH could also be divided into responders (fall in DBP>10%) and non-responders to β-blocker (propranolol) or diuretic (chlorthaladone) therapy [190]. Renin changes during treatment did not differ between responders and non-responders but aldosterone levels fell considerably more in the responders than in the non-responders [190]. The patients responding best to treatment had greater aldosterone decrements, and the changes in aldosterone were more closely
linked to treatment response than were either control levels of PRA or treatment induced changes in PRA. The non-suppressible levels of aldosterone in non-responders did not influence BP through any changes in fluid or electrolyte balance and thus the pressor effects of aldosterone could have been mediated through direct actions on the circulatory system [190].

Altered aldosterone dynamics, either through derangements in the metabolism or control of aldosterone secretion, may lead to enhanced aldosterone mediated BP increases.

(ii) **Aldosterone Dynamics in Essential Hypertension**: The plasma levels and distribution of aldosterone appear to be altered in EH. The concentration of aldosterone in crystalline lenses (obtained during cataract surgery) of patients with EH was significantly higher than in normotensive controls. (The lens epithelium contained high affinity aldosterone receptors, which differed from glucocorticoid receptors, and thus the eye appears to be an aldosterone target tissue) [166]. The significance of this finding is difficult to ascertain but it does suggest that aldosterone distribution and target tissue uptake may be altered in EH.

A high sodium intake (400 mEq/day) failed to suppress aldosterone secretion or plasma aldosterone in patients with EH in whom aldosterone levels remained within or above the limits seen in normotensive controls on a normal Na⁺ intake (120 mEq/day) [167]. During sodium depletion, PRA, aldosterone secretion rate, and plasma aldosterone rose in both the hypertensive and normotensive individuals, and the augmented aldosterone parameters correlated with the elevated PRA [167]. The authors were unable to explain the lack of suppressibility of aldosterone following sodium loading in EH through any derangement in regulation by the renin-
angiotensin system. On a normal Na⁺ intake plasma aldosterone levels correlated well with PRA in normotensive subjects, but in patients with EH the correlation was weak or absent [168]. The dissociation between PRA and aldosterone in EH suggested that either the regulation through the renin-angiotensin system was disturbed, or that a portion of aldosterone is not in the feedback loop (i.e. bound, sequestered). Further evidence for this came from the fact that in subjects with EH (on normal Na⁺ intakes) there was an excess of plasma aldosterone relative to the levels of extracellular fluid volume and PRA (which were both in the normal range) [169]. The excess of aldosterone relative to PRA correlated positively with the excess of aldosterone relative to the extracellular fluid volume which suggested that a portion of aldosterone was indeed outside of the renin-angiotensin-volume feedback loop.

In patients with mild EH plasma aldosterone levels were mildly elevated (PRA being normal), the secretion rate of aldosterone was normal or low, while the metabolic clearance rate (MCR) of aldosterone was decreased by 46% (which thus accounted for the mild but significant hyperaldosteronism) [84, 170, 171]. The urinary excretion of the 18-oxo-conjugate of aldosterone (renal metabolite) was increased in patients with EH while the excretion of tetrahydroaldosterone (hepatic metabolite) was low which suggested decreased hepatic catabolism of aldosterone in EH [171, 172]. Splanchnic blood flow was found to be decreased by only 10% in patients with EH, and this decrease in splanchnic blood flow thus could only account for a small portion of the 46% decrease in the MCR of aldosterone observed in EH [173]. The augmented plasma levels of aldosterone in subjects with EH were linked to a decreased MCR of aldosterone which in turn was correlated with an increased plasma binding capacity for
aldosterone [due to the presence of a specific high-affinity low-capacity aldosterone binding globulin (ABG) clearly distinct from the corticosteroid binding globulin (CBG)] [174,175,176]. Plasma aldosterone concentrations declined with age in normotensive control subjects, but increased with age in subjects with EH this being related to increased plasma ABG binding capacity for aldosterone and hence lowered aldosterone metabolism (the difference in age related changes in plasma aldosterone levels could not be attributed to PRA, since PRA declined similarly with age in both subjects with EH and in normotensive controls) [174]. The failure of aldosterone to decrease with age in EH could be contributing to the hypertensive process by increasing the bioavailability of this hypertensinogenic steroid.

The effect of specific high affinity plasma steroid binding proteins on the MCR of their ligands has been studied directly: Intravenous infusion of pure sex hormone binding globulin (SHBG) decreased the MCR of testosterone in rhesus monkeys [177]. Infusion of antibodies to SHBG greatly decreased the SHBG binding capacity and concomitantly increased the MCR of testosterone [177]. The MCR of testosterone increased as the percentage of non SHBG bound testosterone rose in plasma following the intravenous infusion of testosterone [178]. Inducing antibodies against corticosterone or cortisol decreased the MCR of these steroids and increased their plasma concentrations [179,180]. The MCR of a given steroid is also dependent on the affinity of its plasma binding protein for it. The binding affinity of CBG increased progressively for corticosterone, cortisone and cortisol, and the MCR of these three steroids were inversely related to the binding affinity of CBG for them [179].

Acute ACTH infusions in normal subjects induced a marked fall in the plasma ABG binding capacity for aldosterone while augmenting the
aldosterone MCR by up to 50% without any changes in hepatic blood flow [171,181]. A significant negative correlation between the aldosterone MCR and the plasma ABG binding capacity for aldosterone was observed, and a decrease in the ratio of urinary oxoconjugate:tetrahydroaldosterone was observed in normal subjects [172]. The plasma protein binding of aldosterone thus appeared to have an effect on the MCR and hepatic metabolism of aldosterone.

(iii) Plasma Protein Binding of Aldosterone: Early work on the plasma protein binding of aldosterone clearly demonstrated that aldosterone was bound to a significant extent to a protein or proteins other than albumin [186]. The studies on aldosterone metabolism in patients with EH showing that the decreased MCR of aldosterone was linked to increased plasma protein binding of aldosterone stimulated the search for the plasma protein which was responsible. Initial candidates were thought to be transcortin (CBG) or a transcortin-like plasma fraction [84,171]. Plasma cortisol concentrations however exceeded those of aldosterone by over a thousand fold, and the CBG binding capacity for cortisol (15-31 μg/dl) was far greater than the plasma concentrations of cortisol (over 90% of cortisol circulates bound to CBG) [182]. If aldosterone bound CBG with high affinity, there would be no free circulating aldosterone! Aldosterone bound CBG with low affinity, the binding affinity of CBG for aldosterone being only 1% of that for cortisol in the rabbit [179]. The presence of an 11β-OH group at position C-11 of the steroid molecule appeared to be one of the factors necessary for binding to CBG, and thus cortisol (having an 11-OH group) binds with high affinity and 11-dehydrocorticosterone (lacking an 11-OH group) binds with low affinity [179,182]. Aldosterone circulates principally in the 11-18
hemiacetal form (no free 11-OH group) which contributes to its low binding affinity in plasma for CBG [179,182]. (The introduction of 11β-OH groups in progesterone, deoxycorticosterone, and 11-deoxycortisol greatly strengthened their association with CBG [183]).

Aldosterone was found to bind reversibly to a novel specific high-affinity low-capacity aldosterone binding globulin (ABG), a glycoprotein having a molecular weight (mw) of 27,500 daltons [175, 176]. The binding of labelled aldosterone and cortisol in plasma could be clearly separated chromatographically. When the fractions binding aldosterone and cortisol were further purified, all the binding of aldosterone in these fractions was associated with ABG, and all the cortisol binding with CBG (mw 52,000) [176]. ABG was present in at least three forms in plasma: a thermolabile fraction (mw 27,500) designated ABG, a thermostable fraction (mw 27,500) designated ABG-Ts, and a dimer (mw 55,000) which appeared to be the main carrier of aldosterone in fresh plasma (all three forms of ABG were interconvertible) [176]. A larger thermolabile aldosterone binding macromolecule (mw>70,000) was also present in plasma which could be converted into ABG, and thus appeared to be a polymer of ABG [176]. Unlike CBG, all forms of ABG bound dehydroepiandrosterone sulfate (DHEA-SO₄), specifically and with high affinity [176]. There was no competition between aldosterone and DHEA-SO₄ for binding sites on ABG (thus at least two binding sites existed on ABG), and ABG failed to bind other sulfated corticosteroids or non sulfated DHEA [176, 184]. ABG did however bind K-Canrenoate (a spironolactone) which competed for the aldosterone binding site (70% cross reactivity, relative to aldosterone, in the ABG binding assay) [184]. Administration of K-Canrenoate to human subjects decreased the plasma ABG binding capacity for aldosterone, as did the addition of K-
Canrenoate to plasma in-vitro, through competitive displacement of aldosterone [191, 192]. In addition, ABG failed to bind cortisol, cortisone, corticosterone, dexamethasone, 11-deoxycorticosterone 11-deoxycorticisol, pregnenolone, progesterone, estradiol, testosterone, or DHEA and thus differed greatly in binding characteristics from any previously described plasma steroid binding proteins [184]. Most of the above steroids bound CBG (which has a single steroid binding site) with moderate to high affinity (but not aldosterone or DHEA-SO₄) which further distinguished ABG from CBG [182]. Some confusion has arisen in the literature concerning the binding of aldosterone to plasma proteins other than CBG, which may be attributable (in addition to inadequate methodology) to the presence of the ABG dimer (mw 55,000) whose molecular weight was very close to that of CBG (mw 52,000) [176, 185].

The binding affinity of total plasma for aldosterone was greater than the binding affinity of CBG for aldosterone, and the binding capacity of plasma for aldosterone was smaller than that of CBG for other corticosteroids which suggested that a protein other than CBG bound aldosterone in plasma with high-affinity and low-capacity [187]. When cortisol was added to plasma in a concentration sufficient to saturate all of the CBG binding sites (one per CBG molecule), subsequent addition of aldosterone displaced bound aldosterone (which must have been bound to some other plasma component distinct from CBG or albumin) [187]. Gel filtration of plasma incubated with ³H-aldosterone revealed that the aldosterone binding protein was eluted at a molecular weight weight greater than that of CBG (mw 52,000), and lesser than that of albumin (mw 67,000), and corresponded to a protein with a molecular weight of approximately 57,000 daltons [187]. This high-affinity
aldosterone binding protein may very well have been the dimer of ABG (mw 55,000) [176].

The specific high-affinity binding of aldosterone in plasma to ABG and the non-specific nature of the CBG binding of aldosterone has been confirmed by other investigators [188].

(iv) Modifications in the Plasma Protein Binding of Aldosterone in Essential Hypertension: The plasma ABG binding capacity for aldosterone was increased in 52% of subjects with EH and their first degree relatives [191]. In pedigree analysis of this trait (i.e. elevated ABG binding capacity for aldosterone) the cumulative frequency distribution was found to be continuous in normotensive control families (no family history of EH) and bimodally distributed in families with EH [191]. The pattern of inheritance in families with EH demonstrated that the increased ABG binding capacity for aldosterone was inherited as an autosomal dominant trait [191, 192]. Furthermore, the ABG binding capacity for aldosterone correlated between siblings, between parents and offspring, but not between mother and father which suggested that a strong genetic influence existed in determining the level of the plasma ABG binding capacity for aldosterone [192]. This inherited increase may thus have been a genetic marker of EH. This modification in ABG binding capacity was only present in EH, no alterations in ABG binding capacity were detected in secondary forms of hypertension [191]. However, in contrast to the elevated ABG binding capacity for aldosterone detected in patients with EH, the CBG binding capacity for other corticosteroids was decreased in these same subjects with EH [191].

The augmented plasma ABG binding capacity for aldosterone reported in subjects with EH has been confirmed by other investigators [193].
Interestingly these investigators found that treating EH patients with β-blockers for two weeks greatly increased the plasma ABG binding capacity for aldosterone [193]. This could explain the observed decline, in patients with EH, of the MCR of aldosterone following treatment with propranolol [194]. Whether or not the increased ABG binding capacity for aldosterone induced by propranolol therapy was related to the previously discussed resistance to propranolol's effects on BP [see section (G)(i)] in some EH patients with non-suppressible aldosterone is unknown.

Indirect evidence was however obtained showing that ABG may directly influence BP. Since administration of estrogens was known to augment hepatic glycoprotein synthesis, MAP, and the plasma binding of aldosterone [186], the effects of oral contraceptives (OC) on ABG binding capacity and MAP were studied in normal healthy women to determine whether or not elevations of plasma ABG binding capacity for aldosterone contributed to increasing BP [195]. In the OC treated group the ABG binding capacity was significantly elevated (compared to control subjects) and this correlated positively with the dose of estrogen administered. The ABG binding capacity for aldosterone correlated positively with MAP in the OC treated group only [195]. Thus it appeared that estrogens elevated the ABG binding capacity for aldosterone which in turn elevated MAP [195]. This data indirectly pointed to a role for ABG in BP regulation.

Direct proof that ABG is involved in elevating BP has been obtained by isolating the thermostable urinary homologue of ABG (designated ABG-TsU) and administering it to rats in whom it induces a hypertension with many of the characteristics of borderline EH [196,197]. These studies [196, 197] form the basis of this thesis.
THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR):

(i) **A Model of Essential Hypertension:** In 1963 Okamoto and Aoki introduced a new animal model of hypertension that did not require any surgical or pharmacological manipulations to become hypertensive [202]. Selective genetic inbreeding (brother to sister) of Wistar rats resulted in 100% of offspring developing hypertension (spontaneously) without any visible organic lesions in the initial stages of the hypertensive process [203,204]. This strain of spontaneously hypertensive rats (SHR) eventually developed (as do untreated human hypertensive subjects) various hypertensive lesions such as cerebral hemorrhage, cerebral infarction, myocardial lesions, and nephrosclerosis [203]. Strokes, heart failure and renal lesions result in a life span shortened by about one third in both SHR and untreated human hypertension [205]. The normotensive control strain for the SHR, developed at a later stage from the same colony of Wistar rats in Kyoto, was named the Wistar-Kyoto (WKY) rat [205].

Once hypertension was established in the adult SHR, high BP was maintained by increased TPR, CO being normal [33,205]. In the young SHR (<12 weeks of age) though, CO was found to be increased or normal and thus during its initial stages the hypertension in the SHR was not completely resistance mediated (as it was in the adult SHR) [205]. In the adult SHR body fluid volumes were completely normal and the normal extracellular fluid volume (ECFV) and lack of correlation with MAP excluded volume expansion as a pressor mechanism in the established phase of hypertension [206]. During the early phases of the development of high BP in the SHR, when CO was sometimes elevated, intravascular volume was normal or even contracted [205]. In the very early stages of increasing BP in the SHR (4 and
5 weeks of age) evidence of augmented plasma volume was obtained even though ECFV was lower in SHR than in aged matched WKY control rats [207]. In the pre-hypertensive SHR (at 12 days of age) total body water and ECFV were greater in the SHR than in WKY rats, but no differences in plasma volume existed [208]. However during the major part of the development of hypertension in the SHR (from 6 to 20 weeks of age) no volume expansion existed [205]. In 20 week old SHR absolute blood volume was normal, but venoconstriction existed which caused an increase in mean circulatory filling pressure (i.e. increased driving force for venous return) compared to WKY rats [243]. In younger SHR (6 and 12 weeks of age) blood volume was decreased (compared to WKY rats) but the fraction of blood contained in the cardiopulmonary area was increased [242]. This increase in central blood volume may have contributed to the development and maintenance of spontaneous hypertension. Several similarities thus exist between the SHR and human EH hemodynamically [see section (C)].

BP in the SHR responded to many of the same anti-hypertensive drugs that humans with EH responded to such as vasodilators, Ca\(^{2+}\) channel blockers, converting enzyme inhibitors, and \(\alpha\)-blockers [209]. Unlike humans with EH though, the SHR did not respond well to \(\beta\)-blockers or thiazide diuretics [209]. As in EH, dietary sodium loading and stress accelerated and augmented the development of hypertension in the SHR [203, 204, 211, 217], while dietary K\(^+\) loading decreased BP in SHR (but not in WKY rats) [229], and dietary sodium restriction retarded (but did not prevent) the development of hypertension in the SHR and resulted in an attainment of a lesser maximal BP [253]. On the other hand it has been reported that unlike human EH, hypertension in the SHR did not appear to be sodium dependent since severe dietary Na\(^+\) restriction from birth did not
affect the development of hypertension [288] unless the Na\(^+\) restriction was so severe as to retard growth [289].

Though there are many similarities between the SHR and human EH, and though the SHR is the closest experimental counterpart of human EH, one must be cautious in stating that it is an excellent model of human EH. Fundamental differences exist between hypertension in man and in the SHR, and after all a rat is a rat and "the proper study of mankind is man" [210].

(ii) Transmembrane Ion Transport in the SHR: Vascular smooth muscle cells of SHR contained significantly more free intracellular Na\(^+\) and Ca\(^{2+}\) than those of WKY normotensive control rats [212]. This difference however disappeared with prolonged in-vitro culture and therefore was due to some humoral factor(s) [212]. Whether or not increased levels of a circulating Na\(^+\)/K\(^+\)-ATPase transport inhibitor was involved in increasing intracellular Na\(^+\) and consequently Ca\(^{2+}\) was unknown. The lack of any exaggerated natriuresis following the dietary sodium loading of SHR argued against the presence of increased circulating levels of a Na\(^+\)/K\(^+\)-ATPase inhibitor [218]. Other investigators however reported that exaggerated natriuresis in the young hypertensive SHR existed, and this was shown to be caused by a relative lack of aldosterone-mediated distal tubular Na\(^+\) reabsorption [222]. The fractional excretion of sodium was decreased in SHR from 4-7 weeks of age (at which time BP was rising) compared to WKY rats, but this difference disappeared by 8 weeks of age [253]. This sodium retention also argued against the presence of increased circulating levels of a Na\(^+\)/K\(^+\)-ATPase inhibitor in the young SHR (however this could have acted as a stimulus for the release of such an inhibitor). In addition, in the SHR no evidence of sodium pump suppression existed, and in fact the activity of
vascular Na+/K+-ATPase was increased in SHR resistance vessels (possibly in order to compensate for increased passive sodium permeability which may have been a primary defect) [213,214]. The increased flux of Na+ and K+ through the erythrocyte membranes of SHR could not be accounted for by alterations in Na+/K+-ATPase activity or the presence of humoral factors, but was due to intrinsic differences in the SHR cell membranes resulting in increased Na+ influx and augmented K+ efflux [215]. Other investigators have reported that the only cation transport difference observed between SHR and WKY rats was that of increased Na+ permeability in SHR erythrocytes [219]. However, acetone extracts of plasma obtained from mature SHR had a greater capacity to inhibit Na+/K+-ATPase than did extracts of plasma obtained from WKY rats [290]. It was not clear if the increased Na+/K+-ATPase inhibitory activity of SHR plasma was a cause or a consequence of increased BP, since no determinations of the Na+/K+-ATPase inhibitory activity of plasma obtained during the initial phase of hypertension development in younger SHR were made [290].

Vascular smooth muscle cell permeability to Na+ was increased in SHR but the accumulation of cell Na+ was prevented by increased sodium pump activity [220]. This was similar to the observed increase in vascular smooth muscle cell membrane permeability to Na+ observed in rats whose hypertension was induced by aldosterone administration, in whom normal cell sodium concentrations were maintained by enhanced sodium transport [see section (E)(viii)] [128]. Aldosterone directly stimulated erythrocyte Na+/K+-ATPase and could cause an increase in Na+ extrusion from vascular smooth muscle cells [131, 57]. Adrenalectomy caused a far greater drop in erythrocyte Na+/K+-ATPase activity in SHR than in WKY control rats. However, aldosterone replacement restored the Na+/K+-ATPase activity to
the levels measured in intact rats [221]. In addition, as in aldosterone induced hypertension, it was found that the turnover of K⁺ and Cl⁻ was increased in SHR vascular smooth muscle cells [216]. Could the similarities in cellular ion transport abnormalities observed in aldosterone induced hypertension and in the SHR indicate a role for aldosterone in the pathogenesis of hypertension in the SHR?

(iii) **Role of Aldosterone in the Development of Hypertension in the SHR:** The development of hypertension in the SHR was prevented by bilateral adrenalectomy but not by bilateral adrenal medullectomy [223, 224]. Since the presence of the adrenal cortex was essential for the development of hypertension in the SHR, the possible role of aldosterone in the pathogenesis of hypertension in the SHR was actively investigated. Administration of supraphysiological amounts of aldosterone to adrenalectomized SHR restored hypertension starting at 7 weeks of age [225, 226] but not at 10 weeks of age [227]. The administration of physiological amounts of aldosterone to adrenalectomized SHR at 12-13 weeks of age also failed to restore high BP [228]. In WKY rats, adrenalectomy, with or without aldosterone replacement, did not result in any change in BP with time compared to sham operated WKY normotensive rats [225, 226]. The lack of BP response to adrenalectomy in WKY rats as opposed to the profound effect of adrenalectomy on BP in the same time period in the SHR clearly emphasized the importance of the adrenal cortex in the development of hypertension in the SHR. The restorative effect of aldosterone on BP in the 7 week old SHR but not in the 10 or 12-13 week old SHR indicated that aldosterone may have been important in the development but not in the maintenance of high BP in the SHR.
Unfortunately, comparisons of the secretion rates and plasma levels of aldosterone between SHR and WKY rats have confused the issue. Plasma aldosterone concentrations were higher in the newborn prehypertensive SHR than in age matched WKY control rats [230, 231]. Plasma aldosterone levels rose concomitantly with rising BP and age in SHR but declined with age in WKY rats [232]. (Likewise plasma aldosterone levels increased with age in humans with EH but declined with age in normotensive controls [174]). Plasma aldosterone levels and secretion rates were higher in SHR than in WKY rats up to 14 weeks of age [231]. At 6 weeks of age the metabolic clearance of aldosterone was decreased in SHR, and this decrease was related to increased plasma ABG binding capacity for aldosterone, which resulted in higher plasma aldosterone levels in SHR than in WKY rats [231]. (Likewise the decreased MCR of aldosterone and mild hyperaldosteronism in EH was linked to increased ABG binding capacity for aldosterone [84]). Young hypertensive SHR had higher plasma aldosterone levels than did WKY rats (at 8, 12, 16 weeks of age) [229, 232, 233].

In contrast however were the observations that plasma aldosterone concentrations and secretion rates in young hypertensive SHR were lower (at 8, 12, and 22 weeks of age) than in WKY rats [222, 234, 235, 236, 237] and that circulating aldosterone levels in SHR declined with age compared to WKY controls [238]. It was also reported that no difference in plasma aldosterone concentrations were detected between SHR and WKY rats at 4, 8, 12, 15, 16 and 20 weeks of age [236, 238]. Decreased levels of plasma aldosterone in adult SHR (as compared to WKY rats) were shown to be due to a blunted sensitivity of the adrenal cortex to angiotensin II stimulation [237].

Plasma aldosterone levels however rose far more in SHR than in either WKY or Sprague-Dawley rats in response to stress [231, 239, 240]. This
phenomenon was linked to an augmented sensitivity of the pituitary adrenal axis to stress [240] with an exaggerated increase in ACTH secretion [241].

In one of the studies presented in this thesis, the role of mineralocorticoids in the development and maintenance of hypertension in the SHR was investigated by treating SHR of different ages with spironolactone. The importance of aldosterone was examined, in the age group (7-9 weeks) whose hypertension responded to spironolactone, by administering physiological amounts of aldosterone following bilateral adrenalectomy to observe the degree of restoration of the hypertension and the effect on the renin-aldosterone and ABG-aldosterone systems.
The purpose of this thesis was to develop a new animal model of hypertension in order to investigate the importance of the Aldosterone Binding Globulin (ABG) in the pathogenesis of human essential hypertension (EH). This was accomplished by first isolating and purifying a quantity of the thermostable urinary homologue of ABG, designated ABG-TsU, and administering it to normotensive Sprague-Dawley rats to see if ABG-TsU administration could induce hypertension. The characteristics of ABG-TsU induced hypertension were further investigated to determine if this new animal model of hypertension had any characteristics in common with those of human EH. In addition, the initiating mechanisms of hypertension in this new animal model of hypertension were compared with those of an established genetic rat model of hypertension.

The experiments were divided into two main sections: Biochemical Studies, and Physiological Studies. The Biochemical Studies section details the isolation procedures used to purify ABG-TsU from human urine as well as describing the physicochemical characteristics of ABG-TsU. The presence of an ABG in urine allowed for the isolation of enough ABG-TsU to carry out
the characterizations of ABG-TsU (determination of molecular weight, isoelectric point, electrophoretic homogeneity, and binding affinity for aldosterone and dehydroepiandrosterone-sulfate) in order to compare the characteristics of the urinary homologue to the known characteristics of plasma ABG. These studies showed that ABG-TsU was most likely a homologue of plasma ABG. Urine was chosen as the starting material for the isolation of the ABG homologue because of its ready availability and its relatively simple composition as compared to plasma. For these reasons, much larger quantities of ABG could be obtained from urine than from plasma, which made it possible to isolate a large enough quantity of ABG-TsU to carry out the Physiological Studies.

The Physiological Studies section was divided into experiments on the induction of hypertension with ABG-TsU in Sprague-Dawley rats, followed by investigations concerning the role of aldosterone in the development of hypertension in the Spontaneously Hypertensive Rat (SHR).
Methods: Biochemical Studies

(A) **ISOLATION OF ABG-TsU:**

(i) **Source of Urine:** ABG-TsU was isolated from the pooled urines of a large number of both male and female hospital personnel. This ABG-TsU was designated ABG-TsU (POOL 1). ABG-TsU was also isolated from the pooled urines of a large number of male only hospital personnel. This ABG-TsU was designated ABG-TsU (POOL 2).

Pooled human urine from hospital personnel or urine from individual subjects was collected in containers in which sodium azide was present in a concentration of at least 0.1%. After overnight refrigeration at 4°C the urine was filtered through fine glass wool.

(ii) **Ultrafiltration:** The urine was then concentrated under positive nitrogen pressure in an ultrafiltration cell (Amicon Canada Ltd, Oakville, Ontario) using an Amicon UM-20 Diaflo membrane retaining globular molecules of molecular weights (mw) greater than 20,000 daltons (figure 1). This concentrate was forced through an Amicon XM-30 Diaflo membrane.
Isolation of ABG-TsU

ABG-TsU isolation procedure from human urine. Refrigerated urine was filtered through glass wool following which differential ultrafiltration, ion exchange chromatography, and gel filtration were carried out.
(retaining molecules with mw exceeding 30,000 daltons) in the same ultrafiltration system. The filtrate obtained was exhaustively dialyzed against water and lyophilized. This fraction consisted of urinary molecules in the 20,000 to 30,000 dalton mw range (figure 1).

(iii) **Ion Exchange Chromatography:** The 20-30K fraction obtained by ultrafiltration was further purified by DEAE-Cellulose (Pharmacia, Uppsala, Sweden) chromatography in a Pharmacia K-16 (16 x 500 mm) column using a stepwise pH elution at a flow rate of 0.6 ml/min. Initial elution was carried out using a Tris-HCl buffer (pH 8.4, 0.08 M) followed by elution with an acetate buffer (pH 5.0, 0.2 M). The eluate was continuously monitored at 280 nm (Dual Monitor UV-2 apparatus, Pharmacia, Uppsala, Sweden) and the elution profile plotted (using a chart recorder) prior to the collection of 2 ml fractions (using a fraction collector). Aliquots (0.1 ml) of the fractions collected were assayed for aldosterone binding activity [184, 191] by incubation with $^3$H-aldosterone for 3 hours at 37°C followed by removal of unbound $^3$H-aldosterone with dextran coated charcoal (2:1 w/w) for 10 min at 4°C, centrifuging, and counting aliquots of the supernatant for protein bound radioactivity.

The protein eluted (figure 2) with the acetate buffer from the DEAE-Cellulose column (corresponding to the pH 5 peak in the elution profile) was dialyzed against water and then lyophilized.

(iv) **Gel Filtration:** This protein fraction (pH 5 peak) was then further purified by gel filtration through Sephadex G-75 gel (Pharmacia, Uppsala, Sweden) in a Pharmacia K-16 column (16 x 500 mm) using phosphate buffer (pH 7.2, 0.07 M) for the elution at a flow rate of 0.3 ml/min. The
FIGURE 2

Chromatography of ABG-TsU

Upper Panel: The 20-30K fraction obtained by ultrafiltration was further purified by DEAE-Cellulose chromatography in a 16 x 500 mm column using a stepwise pH elution at a flow rate of 0.6 ml/min. Initial elution was carried out using a Tris-HCl buffer (pH 8.4, 0.08 M) followed by elution with an acetate buffer (pH 5.0, 0.2 M). The eluate was continuously monitored at 280 nm (absorbance in arbitrary units). Aliquots of the fractions collected were assayed for aldosterone binding activity by incubation with $^3$H-aldosterone for 3 hours at 37°C followed by removal of unbound $^3$H-aldosterone with dextran coated charcoal (2:1 w/w) for 10 min at 4°C, centrifuging, and counting aliquots of the supernatant for protein bound radioactivity.

Lower Panel: The protein fraction eluted with the acetate buffer (pH 5 peak, upper panel) was further purified by gel filtration through Sephadex G-75 gel in a 16 x 500 mm column using phosphate buffer (pH 7.2, 0.07 M) for the elution at a flow rate of 0.3 ml/min. The eluate was continuously monitored at 280 nm (absorbance in arbitrary units). The fractions corresponding to the centre of the peak eluted were pooled, dialyzed against water, and then lyophilized. This fraction was designated ABG-TsU.
eluate was continuously monitored at 280 nm and the elution profile plotted (figure 2) using a chart recorder immediately prior to the collection of 2 ml fractions. The fractions corresponding to the centre of the peak eluted were pooled, dialyzed against water, and then lyophilized. This fraction was designated ABG-TsU.

(B) CHARACTERIZATION OF ABG-TsU:

(i) Determination of Molecular Weight: The mw of ABG-TsU (POOL 1) was determined by gel filtration using a column (16 x 600 mm) of Sephadex G-75 through which known standards were eluted with phosphate buffer (pH 7.2, 0.07 M). (Bovine Serum Albumin, mw=67,000; Ovalbumin, mw=43,000; Trypsin Inhibitor, mw=28,000; Chymotrypsinogen-A, mw=25,000; Ribonuclease-A, mw=13,700). From the elution volumes determined for the known standards, a curve of log mw vs elution volume was constructed (figure 3). The elution volume of ABG-TsU was then determined (using the same column) and its mw found from the standard curve.

Elution volumes of ABG-TsU were also determined after pre-incubation with either $^3$H-aldosterone or $^{14}$C-DHEA-SO$_4$ (dehydroepiandrosterone sulfate) for 3 hours at 37°C. At the end of the incubation period the steroid and ABG-TsU solution was treated with dextran coated charcoal (2:1 w/w) for 10 minutes at 4°C (to remove free steroid) and then centrifuged. The supernatant (containing only ABG-TsU and steroid bound to it) was then run on the column and the elution volume of ABG-TsU determined (figure 3). Aliquots of the eluted fractions were counted for either $^3$H or $^{14}$C to determine where aldosterone or DHEA-SO$_4$ were eluted.
FIGURE 3
Molecular Weight Determination of ABG-TsU

Upper Panel: Determination of mw of purified ABG-TsU by gel filtration on a Sephadex G-75 column (16 x 600 mm).

Lower Panel: Elution profile (absorbance in arbitrary units) of purified ABG-TsU from the same Sephadex G-75 column either alone or after incubation with $^3$H-aldosterone or $^{14}$C-DHEA-SO$_4$. 
The elution profile of ABG-TsU was continuously monitored at 280 nm and its elution profile matched to the fractions counted for $^3$H or $^{14}$C (figure 3).

(ii) Electrophoretic Mobility: The electrophoretic mobility of ABG-TsU (POOL 1) was compared to known standards (Pharmacia Calibration Kit) on sodium dodecyl sulfate (SDS) polyacrilamide gel (gradient 10-30%) in glass tubes ($0.6 \times 10$ cm) at pH 7.0 in 0.01 M phosphate buffer (figure 4) [252]. The standards used were Phosphorylase-b, Bovine Serum Albumin, Ovalbumin, Carbonic Anhydrase, Soybean Trypsin Inhibitor, and $\alpha$-lactalbumin. The electrophoretic mobility of ABG-TsU was also compared to that of Staphylococcus Aureus V8 (mw 27,700; Miles Laboratories, Elkhart, Indiana).

(iii) Isoelectric Focussing: The determination of the isoelectric point (pI) of ABG-TsU (POOL 1) was ascertained by isoelectric focussing in polyacrylamide (30%) rods ($0.6 \times 12$ cm) having a gradient from pH 4-6 formed with Bio-Lyte 4-6 ampholytes (2%). The gels were run for 1 hour at 150 V (constant), at 300V for the next hour, and at 500 V for the subsequent 15 hours. They were then stained in 26% isopropanol and 10% acetic acid containing 0.4% Coomassie Blue R-250 and 0.05% acetic acid containing 0.4% cupric sulfate for at least 2 hours. The gels were then destained in 12% isopropanol and 7% acetic acid and stored in H$_2$O prior to being photographed. The pH of 1 cm sections of an identical though unstained gel were determined so that the pI of stained protein bands could be determined from the curve of distance (along gel) vs observed pH (figure 4).
(iv) Binding Affinity: The binding affinity of ABG-TsU (POOL 2) for aldosterone and DHEA-SO₄ was determined by equilibrium dialysis followed by Scatchard plot analysis. A series of equilibrium dialyses were carried out in 0.05 M phosphate buffer (pH 7.4) at 4°C with a constant amount of ABG-TsU and ³H-aldosterone (or ¹⁴C-DHEA-SO₄) in the presence of different amounts of unlabeled aldosterone (or DHEA-SO₄) according to the method of Muldoon and Westphal [244]. The concentrations used were: ABG-TsU 100 μg/ml in 1 ml buffer (inside bag); ³H-aldosterone (88 Ci/mmol) 5.3 x 10⁵ dpm in 15 ml buffer (outside bag); unlabeled aldosterone 2.5 to 1000 ng (outside bag). Parallel experiments were carried out using ABG-TsU 100 μg in 1 ml buffer (inside bag); ¹⁴C-DHEA-SO₄ (55 Ci/mmol) 3.5 x 10⁴ dpm in 15 ml buffer (outside bag); and unlabeled DHEA-SO₄ 2.5 to 1000 ng (outside bag). The bags (seamless dialyzer tubing, mw cut-off 12,000 daltons, Fisher Scientific, Toronto, Canada) were placed inside 10 ml vials and the solution stirred for 24 hours, this time being sufficient to reach equilibrium. Scatchard plot analysis was carried out by plotting bound/free vs. bound hormone and calculating the equation of the regression line by the method of least squares (figure 5). From the slope of the line the association constant $K_a$ was computed.

(C) PARTIAL CHARACTERIZATION OF RAT PLASMA ABG:

Plasma was obtained from control male Sprague-Dawley rats (Charles River Canada, St-Constant, Que.). The plasma (2.5 ml) was passed through a column (16 x 200 mm) of Blue Sepharose CL-6B (Pharmacia, Uppsala, Sweden) for the partial removal of albumin by affinity chromatography [176, 191]. The non-adsorbed proteins were eluted as described [176, 191]
and the total eluate pooled, dialyzed against water and lyophilized. This plasma fraction was reconstituted, incubated with $^3$H-aldosterone at 37°C for 3 hours, and then treated with dextran coated charcoal (2:1 w/w) for 10 min at 4°C and then centrifuged. The supernatant was then subjected to gel filtration in a column (16 x 600 mm) of Sephadex G-75 (Pharmacia, Uppsala, Sweden). The elution profile was continuously monitored at 280 nm and 1 ml fractions collected. Aliquots of these fractions were then counted for radioactivity to determine where the bound aldosterone was eluted (figure 6). ABG-TsU was similarly pre-incubated with $^3$H-aldosterone, treated with dextran coated charcoal, and passed through the same Sephadex G-75 column. The elution profile of ABG-TsU was continuously monitored at 280 nm (figure 6) and 1 ml fractions collected. Aliquots of eluate were then counted for radioactivity.
Results:
Biochemical Studies

(A) **ISOLATION OF ABG-TsU:**

Following differential ultrafiltration, the 20-30K fraction was further purified by DEAE-Cellulose chromatography using a stepwise pH gradient. Aldosterone binding activity was found in the eluted fractions corresponding to the peak eluted with the acetate buffer at pH 5.0 (figure 2, upper panel). This fraction was further purified by gel filtration, with aldosterone binding activity corresponding to the eluted peak seen in the lower panel of figure 2. This fraction (after dialysis and lyophilization), designated ABG-TsU, was used for further characterizations.

(B) **CHARACTERIZATION OF ABG-TsU:**

(i) **Determination of Molecular Weight:** The mw determination of ABG-TsU by gel filtration gave a value of 27,500 daltons, with $^3$H-aldosterone and $^{14}$C-DHEA-SO$_4$ binding peaks coinciding at this mw as illustrated in figure 3.
(ii) **Electrophoretic Mobility:** Following ultrafiltration, ion exchange chromatography, and gel filtration, ABG-TsU (POOL 1) was found to be electrophoretically homogeneous (figure 4). The mobility of ABG-TsU was virtually indistinguishable from that of Staphylococcus Aureus V8 (mw=27,700) in SDS-PAGE electrophoresis and very close to that of carbonic anhydrase (mw = 30,000) as shown in figure 4.

(iii) **Isoelectric Focusing:** ABG-TsU (isolated from pooled urine, i.e. POOL 1) displayed microheterogeneity in isoelectric focusing with two bands; one major (pI 4.76) and one minor (pI 4.80).

(iv) **Binding Affinity:** Purified ABG-TsU (POOL 2) displayed reversible, saturable, high-affinity low-capacity binding for aldosterone ($K_a = 3.5 \times 10^{-9}$ M) and DHEA-SO$_4$ ($K_a = 1.5 \times 10^{-9}$ M) as determined by equilibrium dialysis (figure 5). These two steroids appeared to bind ABG-TsU at different binding sites as they did not displace each other from ABG-TsU.

(C) **PARTIAL CHARACTERIZATION OF RAT PLASMA ABG:**

After partial removal of albumin and pre-incubation with $^3$H-aldosterone, rat plasma was subjected to gel filtration showing bound aldosterone to be eluted at the same mw as purified ABG-TsU (which was run separately on the same column after prior incubation with $^3$H-aldosterone). Aldosterone was thus shown to bind to a protein fraction (ABG?) in rat plasma having the same mw as ABG-TsU (27,500 daltons) (figure 6).
FIGURE 4
Electrophoresis and Isoelectric Focussing of ABG-TsU

Left: Electrophoretic mobility of ABG-TsU compared to known standards (1: Phosphorylase-b, 2: Bovine Serum Albumin, 3: Ovalbumin, 4: Carbonic Anhydrase, 5: Soybean Trypsin Inhibitor, and 6: α-lactalbumin).

Right: Isoelectric focussing of ABG-TsU demonstrating microheterogeneity.
**FIGURE 5**

**ABG-TsU Binding Affinity for Aldosterone and DHEA-SO$_4$**

Determination of binding affinity ($K_a$) of ABG-TsU for aldosterone and DHEA-SO$_4$ by equilibrium dialysis followed by Scatchard plot analysis.
**FIGURE 6**

Partial Characterization of Rat Plasma ABG

**Upper Panel:** Following partial removal of albumin, rat plasma was pre-incubated with $^3$H-aldosterone at 37°C for 3 hours, and then treated with dextran coated charcoal (2:1 w/w) for 10 min at 4°C and then centrifuged. The supernatant was then subjected to gel filtration in a column (16 x 600 mm) of Sephadex G-75. The elution profile (absorbance in arbitrary units) was continuously monitored at 280 nm and 1 ml fractions collected. Aliquots of these fractions were then counted for radioactivity to determine where the bound aldosterone was eluted.

**Lower Panel:** Purified ABG-TsU was chromatographed separately on the same column after prior incubation with $^3$H-aldosterone to standardize the mw at which rat plasma binds aldosterone.
Methods: Physiological Studies

(A) CARE AND HOUSING OF ANIMALS:

Rats were housed four per cage and Purina rat chow and tap water were made available ad libitum. Following a 5 to 7 day acclimatization period (which included handling every 1 or 2 days), control levels of SBP and body weight were obtained and experimental protocols begun. To minimize the effects of stress on the rats, all feeding, care, cleaning of cages, and handling were conducted by the author only.

(B) SURGICAL PROCEDURES:

(i) Adrenalectomy: Bilateral adrenalectomies (ADRX) were conducted either under light ether anesthesia or under sodium thiopental anesthesia (40 mg/kg intraperitoneally). After making a midline ventral incision the adrenal glands were removed whole with their surrounding fat pads. If the capsules of the adrenal glands were at all damaged during surgery the animal was not included in the experimental protocol. Control rats were
sham operated (a midline ventral incision was made in anesthetized rats and probing of the abdomen conducted without removing or damaging the adrenal glands). A 1% NaCl solution was made available ad libitum (instead of tap water) following surgery to both ADRX rats and their sham operated controls.

(ii) Implantation of Osmotic Mini-Pumps: Osmotic mini-pumps (Alzet, Palo Alto, California) were implanted either under light ether anesthesia or under sodium thiopental anesthesia (40 mg/kg intraperitoneally). Mini-pumps delivering a spironolactone (K-Canrenoate, Soldactone, Searle, Montreal, Quebec) were implanted intraperitoneally (i.p.) while those delivering aldosterone were implanted subcutaneously (to avoid hepatic first pass effect). Control rats were sham operated.

(iii) Post Operative Care: Following all surgical procedures from which animals were expected to recover, rats received one 0.15 ml subcutaneous injection of a solution of Procain Penicillin G (200,000 IU/ml) and Dihydro-Streptomycin (250 mg/ml) (Pen-Di-Strep, Rogar/STB, Montreal, Quebec).

(C) METHOD OF ABG-TsU ADMINISTRATION:

ABG-TsU was administered by i.p. injection once daily in doses per 100 g body weight. The ABG-TsU was dissolved in physiological saline such that the given dose of ABG-TsU administered per 100 g of body weight was contained in a volume of 0.1 ml. Thus the volume of vehicle administered with the ABG-TsU was only 0.1 ml/100 g body weight. Control rats were
similarly handled and received vehicle only once daily by i.p. injection (0.1 ml saline/100 g body weight).

(D) DETERMINATION OF BLOOD PRESSURE:

Two methods for measuring systolic blood pressure (SBP) indirectly in conscious rats from the proximal portion of the tail were used [245, 246].

(i) Indirect SBP Determination With Preheating: The first method [245] involved SBP determination using a cuff and pneumatic pulse transducer coupled to a programmed electro-sphygmomanometer (Model PE-300, Narco Bio-Systems, Houston, Texas). Rats were pre-warmed to 39° for 20 minutes (with a heat lamp) and maintained at this temperature during SBP measurements in a temperature controlled restraining device (Narco Bio-Systems). At least three readings were averaged per rat per measurement session.

(ii) Indirect SBP Determination Without Preheating: The second improved method of measuring SBP did not involve preheating the rats and thus eliminated increases in BP induced by the stress of heating [246]. The SBP of conscious unstressed non-preheated rats was measured in the proximal portion of the tail using a tail-cuff incorporating a photo-electric sensor coupled to a pre-amplifier (Model 59 Pulse Amplifier, IITC, Landing, NJ) which was automatically inflated and deflated by a programmed electro-sphygmomanometer (Model PE-300, Narco Bio-Systems, Houston, Texas). Cuff pressure and arterial pulsations were recorded on a physiograph (Model
MK IV, Narco Bio-Systems) and a minimum of three readings were averaged to obtain SBP.

(iii) Direct BP Determination: Direct measurements of BP were made in thiobutabarbitral (Inactin BYK, 100 mg/kg i.p.) anesthetized rats from a carotid artery cannulated with PE-50 polyethylene tubing connected to a pressure transducer (Model RP 1500i, Narco Bio-Systems). Systolic, diastolic and mean BP (SBP, DBP, MAP) were obtained from the arterial pressure wave (recorded on one physiograph channel). Heart rate (HR) was monitored on a second physiograph channel using the signal from the BP channel fed through a rate meter (Biotachometer Coupler, Model 7302, Narco Bio-Systems).

(E) MEASUREMENT OF CARDIAC OUTPUT:

Cardiac output (CO) was measured in thiobutabarbitral (Inactin BYK, 100 mg/kg i.p.) anesthetized rats using the reference sample microsphere method [247, 250]. Radioactive microspheres (15 μm, 57Co, New England Nuclear, Boston, Massachusetts) were injected over 20 seconds in the left ventricle (cannulated via the right carotid artery with PE-50 polyethylene tubing). Arterial blood was sampled (to obtain the Reference Sample) at a constant rate (0.9 ml/min beginning 5 seconds prior to microsphere injection) for 50 seconds from a femoral artery cannulated with PE-50 polyethylene tubing and connected to an infusion/withdrawl pump (Model 940, Harvard Apparatus, South Natick, Massachusetts). The lost blood volume was simultaneously replaced with blood from a donor rat injected at the same rate into a femoral vein (cannulated with PE-50 polyethylene
tubing and connected to the infusion/withdrawal pump). At the time CO was determined, SBP, DBP, and MAP were measured directly. CO was calculated according to the following formula [250]:

\[
CO = \frac{\text{Counts injected} \times \text{Reference sample withdrawal rate}}{\text{Reference sample counts}}
\]

(F) DETERMINATION OF PRESSOR RESPONSIVENESS TO PHENYLEPHRINE (PD25):

The pressor responsiveness to phenylephrine (an \(\alpha\) agonist) was used as an index of vascular reactivity in Sprague-Dawley rats. To validate the method a total of 19 untreated rats weighing 306 ± 17 g were anesthetized with thiobutabarbitral (Inactin BYK, 100 mg/kg i.p.), the right femoral artery was cannulated (for direct measurement of SBP, DBP, and HR), and the cannulated right femoral vein was connected to an infusion pump (Model 940, Harvard Apparatus, South Natick, Massachusetts). Four doses of phenylephrine (1.04 ± 0.06, 2.09 ± 0.11, 4.18 ± 0.22, and 10.37 ± 0.54 \(\mu\)g/kg/min) were infused continuously at rates of 0.0051, 0.0103, 0.0206, and 0.051 ml/min until a new steady level of DBP was reached. The increase in DBP (\(\Delta\) DBP) was determined by subtracting the infusion maximum from the pre-infusion value. A waiting period of 5 to 10 minutes was observed between infusions in order for BP to normalize. The logarithm of the dose of phenylephrine and the \(\Delta\) DBP were used to calculate the equation of the regression line (by the method of least squares) as well as the correlation coefficient (r) between the two variables. From the equation of the regression line the dose of phenylephrine needed to increase DBP by
Log dose response curve of phenylephrine on DBP in untreated Sprague-Dawley rats. The arrows indicate the dose of phenylephrine required to elevate DBP by 25 mmHg (PD$_{25}$).
25 mmHg was calculated (the "pressor dose" or PD$_{25}$, figure 7).

(G) PLASMA ASSAYS:

In all experiments involving the determination of plasma variables, blood was obtained from the cannulated femoral or carotid artery (after determination of BP, PD$_{25}$ etc.) and collected either in tubes containing disodium edetate (5.5 mg) immersed in an ice bath (4°C for Plasma Renin Activity, PRA) or in tubes containing lithium heparin (143 USP units). Blood was immediately centrifuged at 4°C and plasma stored at -20°C until used for determination of electrolytes by flame photometry, PRA by radioimmunoassay of angiotensin I generated following incubation of plasma (precision within an assay for determination of angiotensin I was ±4% and the detection limit was 100 pg of angiotensin I formed during the incubation period) [248], total aldosterone by radioimmunoassay (detection limit 15 pg/sample, variation within assay ±4.5%) [249], and the plasma ABG binding capacity for aldosterone [171, 184].

A specific binding assay for the estimation of plasma aldosterone binding capacity was employed [184]. Plasma binding capacity for aldosterone was related to the total concentration of aldosterone (measured by radioimmunoassay [249]) in the plasma sample. The binding assay [184] was able to distinguish between heat-stable (ABG-Ts) and heat-labile (ABG) aldosterone binding, the former (ABG-Ts) being determined following the heating of plasma for 25 minutes at 60°C. Two aliquots of the same plasma sample (one aliquot was preheated as described above, the other aliquot was not preheated) were incubated with a known amount of $^3$H-aldosterone for 2.75 hours at 37°C. At the end of the incubation period, free aldosterone was
separated from bound aldosterone by the adsorption of free aldosterone to dextran coated charcoal (dextran coated charcoal strips aldosterone from low affinity binders such as albumin) [184]. Following centrifugation, the supernatant (containing only bound aldosterone) was counted for radioactivity. The binding capacity of unheated plasma for aldosterone and the thermostable binding capacity (ABG-Ts binding capacity) of heated plasma for aldosterone were thus measured and the thermolabile binding capacity (ABG binding capacity) obtained from the difference [184]. The binding capacities of the various fractions were measured as percentages of the amount of aldosterone bound relative to the total amount of aldosterone present in the sample. The measured binding capacities reflect the concentrations of ABG and ABG-Ts. The binding capacities are independent of variations in total plasma aldosterone concentration unless aldosterone levels increase sufficiently to exceed the total binding capacities of ABG and ABG-Ts. The amount of aldosterone bound was calculated from the various percentages (ABG bound + ABG-Ts bound = total bound aldosterone) once the concentration of total aldosterone was known.

**H) Statistics:**

The significance of differences between two means was assessed by Student's unpaired two-tailed t-test. Correlations were determined using Pearson's correlation coefficient (r). A p of 0.05 or less was considered significant. Following one way analysis of variance (ANOVA), multiple comparisons between means (when three means were involved) were made using Tukey's modified t-test [251]. A p of 0.05 or less was considered significant.
Methods: Experimental Protocols

(A) ABG-TsU INDUCED HYPERTENSION IN THE SPRAGUE-DAWLEY RAT:

Male Sprague-Dawley rats (n=42, Charles River Breeding Laboratories) weighing initially between 175 and 205 g were divided into two groups (figure 8). Group A (n=28) received ABG-TsU (POOL1) (2 μg/100 g/day) for 12 days, and Group B (n=14) received vehicle only for 12 days. During the 12 day injection period SBP was measured every one or two days in both groups of preheated conscious rats using the indirect tail-cuff method previously described. Following the final determination of SBP (on the twelfth day of treatment) individual blood samples and organs for histological examination were collected. After anesthesia was induced by sodium pentothal (50 mg/rat, i.p.), blood was rapidly drawn by aortic puncture with a large gauge needle (18 G) for the determination of plasma variables (PRA, electrolytes, aldosterone, ABG) as previously described. The hearts were excised, blotted, and weighed in all rats following exsanguination. From five of the control rats (chosen at random) and 13 of
Figure 8
Protocol for ABG-TsU Induced Hypertension

Male Sprague-Dawley rats were either treated with one daily injection (GROUP A) of ABG-TsU (POOL 1) or vehicle only for 12 days (GROUP B). SBP was measured every one or two days in conscious rats and on day 12 blood and organs were obtained.
the ABG-TsU treated rats (with the highest SBP) adrenal glands, brains, kidneys and hearts were removed for histological examination (by a pathologist, Dr. W. Boyko) to assess the degree of hypertensive end organ damage. The organs were fixed in buffered formalin, embedded in paraffin, and 6 μm sections mounted on glass slides and stained with hematoxilin and eosin. The slides were coded to remove observer bias during scrutiny. Adrenal glands were embedded whole and serial sections were obtained. Six random measurements of zona glomerulosa width (taken from the inner surface of the fibrous capsule) were made from each adrenal gland with an eyepiece microscopic micrometer at 400X from sections containing the largest cross-sectional area of adrenal medulla.

**(B) ADRENAL DEPENDENCE OF ABG-TsU INDUCED HYPERTENSION IN THE SPRAGUE-DAWLEY RAT:**

Male Sprague-Dawley rats (n=8), initially weighing between 175 and 205 g, underwent bilateral adrenalectomy (ADRX) (group C, figure 9). These rats received a daily injection of ABG-TsU (POOL 1) (2 μg/100 g/day) for 32 days beginning on the day following ADRX (at which time drinking water was replaced with 0.9% NaCl). From the 14th day of ABG-TsU administration physiological amounts of aldosterone (2 μg/rat/day) were administered continuously by osmotic mini-pump concomitantly with ABG-TsU (figure 9). ABG-TsU was discontinued on Day 32 while aldosterone administration continued until Day 36. Throughout the treatment period SBP was monitored every one or two days by the tail-cuff method (with preheating) previously described.
FIGURE 9
Protocol for Determining the Adrenal Dependence of ABG-TsU Induced Hypertension in Sprague-Dawley Rats

Male Sprague-Dawley rats were bilaterally adrenalectomized, given 0.9% NaCl to drink, and treated with ABG-TsU (POOL 1) alone for 14 days (GROUP C) after which time physiological amounts of aldosterone (2 µg/rat/day) were administered concomitantly with ABG-TsU through to Day 32 at which time ABG-TsU treatment was discontinued while aldosterone alone was administered to Day 36.

Intact rats received ABG-TsU concomitantly with a spironolactone (Soldactone) for 14 days (GROUP D) after which time the Soldactone was withdrawn and ABG-TsU treatment continued to Day 32. SBP was measured in conscious rats (GROUPS C, D) every few days.

---

175-205g SD RATS
SBP measured
(n=15)

GROUP C (n=8)

DAY = 0:
ADRX,
ABG-TsU BEGUN

DAY = 14:
ALDOSTERONE BEGUN

DAY = 32:
ABG-TsU WITHDRAWN

SACRIFICED DAY = 36

GROUP D (n=7)

DAY = 0:
SOLDACTONE, ABG-TsU BEGUN

DAY = 14:
SOLDACTONE WITHDRAWN

DAY = 32:
ABG-TsU WITHDRAWN

△ - adrenal gland; □ - osmotic mini-pump
Intact male Sprague-Dawley rats (n=7), initially weighing between 175 and 205 g, were treated once daily with ABG-TsU (POOL 1) (2 μg/100 g/day i.p.) for 32 days while concomitantly receiving a spironolactone (Soldactone) for the first 14 days (1.5 mg/rat/day) by continuous infusion using osmotic minipumps (Group D, figure 9). ABG-TsU treatment was discontinued on Day 32. SBP was measured up to Day 36 (using the tail-cuff method with preheating as previously described) following which the rats were sacrificed.

(C) HEMODYNAMICS OF ABG-TsU INDUCED HYPERTENSION:

Male Sprague-Dawley rats (n=14) initially weighing approximately 225 g were randomly divided into two groups. The first group (n=7) were treated with ABG-TsU (POOL 2) (25 μg/100 g/day) for 14 days. The second group received vehicle only for 14 days. After 14 days of treatment BP was measured directly and cardiac output (CO) determined in both groups of rats as previously described.

(D) EFFECT OF ABG-TsU ISOLATED FROM INDIVIDUAL HYPERTENSIVE AND NORMOTENSIVE HUMANS ON THE BP OF THE SPRAGUE-DAWLEY RAT:

ABG-TsU was isolated from the urine of individual humans having normal (N) or high (H) plasma ABG binding capacity for aldosterone. (ABG binding capacity was 9.7%±0.2, normal range 2-17%, in 290 normotensive control subjects without any family history of EH [191]. In EH ABG binding
capacity was $23.2\% \pm 0.6$ in 95 subjects studied). ABG-TsU was isolated from a hypertensive subject with essential hypertension (EH) having a normal plasma ABG binding capacity (15.5%) for aldosterone (EHN), from two hypertensive patients with EH having increased plasma ABG binding capacities (21.9%, 20.8%) for aldosterone (EHH), and from one hypertensive individual with renovascular hypertension (RH, due to renal artery stenosis) having normal plasma ABG binding capacity (13.9%) for aldosterone (RHN).

To compare the hypertensinogenic effects of ABG-TsU isolated from different hypertensive subjects (EHN, EHH and RHN), a standard dose of ABG-TsU (10 \(\mu g/100\) g body weight) was administered i.p. once daily to Sprague-Dawley rats for 14 days (EHN n=9, controls n=9; EHH n=8, controls n=7; RHN n=7, controls n=9). Control rats (always from the same shipment as their ABG-TsU treated counterparts) were injected once daily i.p. with an equivalent volume of vehicle (0.9% NaCl, 0.1 ml/100 g body weight) for 14 days. During the course of ABG-TsU or vehicle administration SBP was measured indirectly with a tail-cuff (no preheating) and on the 14th day BP was measured directly, pressor responsiveness to phenylephrine (PD\(_{25}\)) determined, and blood obtained.

ABG-TsU isolated from two normotensive (NT) subjects without any family history of EH having normal plasma ABG binding capacity (6.7%, 10.2%) for aldosterone (NTN) was administered i.p. once daily to rats for 14 days in doses of 25 \(\mu g/100\) g body weight (n=12) and 200 \(\mu g/100\) g body weight (n=5). Control rats (n=12) received once daily an equivalent volume of vehicle only i.p. for 14 days. After 14 days of treatment BP was measured directly and blood was obtained from controls and from the group treated with 25 \(\mu g/100\) g ABG-TsU. Only indirect SBP measurements were made in the group treated with 200 \(\mu g/100\) g ABG-TsU.
(B) MINERALOCORTICOID CONTRIBUTION TO THE INITIATION OF HYPERTENSION IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR):

(i) Animals: Male SHR and Wistar-Kyoto (WKY) control rats were obtained (Taconic Farms, Germantown, New York) and all indirect measurements of blood pressure conducted without preheating.

(ii) Comparison of Baseline Variables Between 12 Week Old WKY and SHR: For the purpose of comparison, BP, PRA, and aldosterone (total, free, and bound) were measured in intact untreated 12 week old SHR (n=8) and WKY (n=9) rats. Direct measurements of BP were made from a carotid artery and then blood samples were obtained from the carotid cannula.

(iii) Spironolactone Treatment of SHR of Different Ages: SHR of different ages were treated with Soldactone by continuous administration using osmotic mini-pumps implanted immediately after initial indirect determination of SBP (no preheating). Control rats were similarly sham operated (see protocols in figures 10, 11, 12, and 13).

SHR at 7 weeks of age (Group 1, figure 10) were either sham operated (n=7) or implanted with minipumps (n=7) delivering Soldactone (1.5 mg/rat/day) for 2 weeks. At 8 weeks of age SBP was measured with a tail-cuff (no preheating) and at 9 weeks of age BP was measured directly and blood was obtained.

SHR at 7 weeks of age (Group 2, figure 11) were either sham operated (n=8) or implanted with minipumps (n=8) delivering Soldactone (5 mg/rat/day) for one week. At 8 weeks of age BP was measured directly and blood was obtained.
SHR at 10 weeks of age (Group 3, figure 12) were either sham operated (n=10) or implanted with minipumps (n=11) delivering Soldactone (1.5 mg/rat/day) for 2 weeks. At 12 weeks of age BP was measured directly and blood was obtained. No SBP values were obtained before implantation in these two groups.

SHR at 12 weeks of age (Group 4, figure 13) were either sham operated (n=7) or implanted with mini-pumps (n=8) delivering Soldactone (5 mg/rat/day) for one week. At 13 weeks of age BP was measured directly and blood was obtained.

(iv) Adrenalectomy and Aldosterone Replacement in 7 Week Old SHR: Immediately following the indirect measurement of SBP (no preheating) 7 week old SHR were either sham operated (SHAM, n=11), bilaterally adrenalectomized (ADRX, n=8), or bilaterally adrenalectomized and given physiological amounts (2 μg/rat/day) of aldosterone for two weeks (ADRX + ALDO, n=8) by osmotic minipumps (figure 14). Rats in all three of the above groups were given 0.9 % NaCl to drink following surgery at 7 weeks of age and at 9 weeks of age BP was measured directly in a carotid artery and blood samples were obtained (figure 14).
FIGURE 10

Soldactone Treatment of SHR from 7 to 9 Weeks of Age

Following the measurement of SBP, 7 week old SHR (GROUP 1) were either sham operated, or implanted i.p. with osmotic minipumps delivering Soldactone (1.5 mg/rat/day). At 9 weeks of age BP was measured directly and blood was obtained.

---

**GROUP 1:**

7 WEEK OLD SHR
SBP measured
(n=14)

OPERATED at 7 WEEKS

SHAM IMPLANTED CONTROLS
(n=7)

MINI-PUMPS IMPLANTED DELIVERING SOLDACTONE 1.5 mg/day
(n=7)

9 WEEKS of AGE SBP/DBP MEASURED and BLOOD COLLECTED

△ - adrenal gland; □ - osmotic mini-pump
Following the measurement of SBP, 7 week old SHR (GROUP 2) were either sham operated, or implanted i.p. with osmotic minipumps delivering Soldactone (5 mg/rat/day). At 8 weeks of age BP was measured directly and blood was obtained.

**FIGURE 11**

**Soldactone Treatment of SHR from 7 to 8 Weeks of Age**

Following the measurement of SBP, 7 week old SHR (GROUP 2) were either sham operated, or implanted i.p. with osmotic minipumps delivering Soldactone (5 mg/rat/day). At 8 weeks of age BP was measured directly and blood was obtained.
Soldactone Treatment of SHR from 10 to 12 Weeks of Age

Ten week old SHR (GROUP 3) were either sham operated, or implanted i.p. with osmotic minipumps delivering Soldactone (1.5 mg/rat/day). At 12 weeks of age BP was measured directly and blood was obtained.

**FIGURE 12**

**GROUP 3:**
10 WEEK OLD SHR (n=21)

OPERATED at 10 WEEKS

- **SHAM IMPLANTED CONTROLS** (n=10)
- **MINI-PUMPS IMPLANTED DELIVERING SOLDACTONE 1.5 mg/day** (n=11)

12 WEEKS of AGE SBP/DBP MEASURED and BLOOD COLLECTED

▲ - adrenal gland; ◀ - osmotic mini-pump
FIGURE 13

Soldactone Treatment of SHR from 12 to 13 Weeks of Age

Following the measurement of SBP, 12 week old SHR (GROUP 4) were either sham operated, or implanted i.p. with osmotic minipumps delivering Soldactone (5 mg/rat/day). At 13 weeks of age BP was measured directly and blood was obtained.

\[\text{GROUP 4:} \]
12 WEEK OLD SHR
SBP measured
\(n=15\)

OPERATED at 12 WEEKS

SHAM
IMPLANTED CONTROLS
\(n=7\)

MINI-PUMPS
IMPLANTED DELIVERING SODACTONE 5.0 mg/day
\(n=8\)

13 WEEKS of AGE SBP/DBP MEASURED and BLOOD COLLECTED

\[\text{\textbullet - adrenal gland; } \text{- osmotic mini-pump}\]
FIGURE 14

Protocol for Determining the Adrenal Dependence of Hypertension in the SHR

After measuring SBP in 7 week old SHR, rats were either sham operated (SHAM), bilaterally adrenalectomized (ADRX), or bilaterally adrenalectomized and given physiological amounts of aldosterone (2 μg/rat/day) continuously by osmotic minipumps implanted i.p. (ADRX+ALDO). Following surgery at 7 weeks of age all rats were given 0.9% NaCl to drink through to 9 weeks of age at which time BP was measured directly and blood was obtained.
Results:
Physiological Studies & Experimental Protocols

(A) **ABG-TsU INDUCED HYPERTENSION IN THE SPRAGUE-DAWLEY RAT:**

Sustained hypertension developed in ABG-TsU (POOL 1) treated rats (Group A, figure 8) after 5 to 8 days (figure 15), SBP being already significantly higher (p<0.01) than in control rats (Group B, figure 8) after only 4 days of treatment.

After 12 days of ABG-TsU administration, the treated rats (Group A) had significantly (p<0.001) higher SBP (figures 15, 16) and heart weights (corrected for body weight) than controls (table 1). No significant differences in the biochemical variables were observed (no suppression of PRA, aldosterone, or hypokalemia; figure 16) between the two groups with the exception of lower total bound aldosterone, ABG bound aldosterone, and total, ABG-Ts, and ABG binding capacities in the ABG-TsU treated rats as compared to controls (table 1).
FIGURE 15

Effect of ABG-TsU (POOL 1) on SBP in Sprague-Dawley Rats

Effect of ABG-TsU (POOL 1) (GROUP A, closed circles) or vehicle only treatment (GROUP B, open circles) on SBP in conscious Sprague-Dawley rats over a 12 day administration period (starting on Day=0).
**TABLE 1**

*Effect of ABG-TsU (POOL 1) or Vehicle on Various Variables (x ± SD) after 12 Days of Treatment*

<table>
<thead>
<tr>
<th>Variable</th>
<th>CONTROLS (n=14)</th>
<th>ABG-TsU TREATED (n=28)</th>
<th>p* (df=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>120 ± 9</td>
<td>151 ± 15</td>
<td>0.001</td>
</tr>
<tr>
<td>Body Weight</td>
<td>300 ± 20</td>
<td>288 ± 20</td>
<td>NS</td>
</tr>
<tr>
<td>Heart Weight/Body Weight (mg/g)</td>
<td>3.14± 0.30</td>
<td>3.45± 0.17</td>
<td>0.001</td>
</tr>
<tr>
<td>Zona Glomerulosa Width (μm)</td>
<td>19 ± 3</td>
<td>18 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>10.9 ± 3</td>
<td>11.8 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Total Aldosterone (ng/100 ml)</td>
<td>14.9 ±1.1</td>
<td>11.1 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Free Aldosterone (ng/100 ml)</td>
<td>12.7 ± 9</td>
<td>9.9 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Total Bound Aldosterone (ng/100 ml)</td>
<td>2.18± 1.6</td>
<td>1.20± 0.8</td>
<td>0.02</td>
</tr>
<tr>
<td>ABG-Ts Bound Aldosterone (ng/100 ml)</td>
<td>1.35± 0.9</td>
<td>0.85± 0.6</td>
<td>0.05</td>
</tr>
<tr>
<td>ABG Bound Aldosterone (ng/100 ml)</td>
<td>0.83± 0.7</td>
<td>0.41± 0.4</td>
<td>0.02</td>
</tr>
<tr>
<td>% Total Bound Aldosterone</td>
<td>15.0 ± 3.4</td>
<td>11.3 ± 3.3</td>
<td>0.01</td>
</tr>
<tr>
<td>% ABG-Ts Bound Aldosterone</td>
<td>9.1 ± 1.4</td>
<td>7.5 ± 1.8</td>
<td>0.01</td>
</tr>
<tr>
<td>% ABG Bound Aldosterone</td>
<td>5.9 ± 2.9</td>
<td>3.8 ± 2.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Plasma Na⁺ (mEq/L)</td>
<td>140.2 ± 1.5</td>
<td>139.8 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma K⁺ (mEq/L)</td>
<td>4.0 ± 0.2</td>
<td>4.2 ± 0.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Students two tailed t-test for independent means

The binding capacity of unheated plasma for aldosterone (% Total Bound Aldosterone) and the thermostable binding capacity (% ABG-Ts Bound Aldosterone) of heated plasma for aldosterone were measured and the thermolabile binding capacity (% ABG Bound Aldosterone) obtained from the difference. The binding capacities of the various fractions were measured as percentages of the amount of aldosterone bound relative to the total amount of aldosterone present in the sample. The measured binding capacities reflect the concentrations of ABG and ABG-Ts. The amount of aldosterone bound was calculated from the various percentages (ABG bound + ABG-Ts bound = total bound aldosterone) once the concentration of total aldosterone was known.
FIGURE 16

Plasma Aldosterone, K⁺, and PRA following 12 Days of ABG-TsU (POOL 1) or Vehicle Administration

PRA, plasma aldosterone, and plasma K⁺ in Sprague-Dawley rats after 12 days of treatment with either ABG-TsU (POOL 1) (GROUP A) or vehicle (GROUP B).
No histological abnormalities (i.e. no hypertensive end organ damage) could be detected in ABG-TsU treated rats. There was no evidence of renal abnormalities, arteriolar hyalinization, fibrointimal hyperplasia, fibrinoid necrosis, or glomerular injury. The cerebral microvasculature showed no evidence of haemorrhage or perivascular hemosiderin deposition. No hypertrophy or hyperplasia of the zona glomerulosa was detected (table 1).

(B) ADRENAL DEPENDENCE OF ABG-TsU INDUCED HYPERTENSION IN THE SPRAGUE–DAWLEY RAT:

The rats which were bilaterally adrenalectomized (ADRX) and treated with ABG-TsU (POOL 1) (Group C, figure 9) did not show any increase in SBP during the first 14 days of ABG-TsU treatment (figure 17, Group C, upper panel). Their SBP on day 14 was only $107 \pm 3$ mmHg. Following this determination of SBP on the 14th day, the rats received physiological amounts of aldosterone continuously (2 μg/rat/day) (figure 9). After 18 days of concomitant ABG-TsU and aldosterone administration (during which time SBP rose steadily) these ADRX rats (Group C) were hypertensive (Day 32) with a SBP of $157 \pm 13$ mmHg (figure 17 upper panel). When ABG-TsU treatment was withdrawn (while aldosterone administration was continued), SBP normalized within 2 days to $116 \pm 7$ mmHg.

The intact rats which were treated concomitantly with ABG-TsU (POOL 1) and a spironolactone (Soldactone, Group D, figure 9) for 14 days failed to develop hypertension (Group D figure 17, lower panel). Their SBP remained normal at $122 \pm 4$ mmHg on the 14th day of treatment. The SBP in Group D rose to hypertensive levels ($153 \pm 5$ mmHg, Day 32) 18 days after
FIGURE 17

Mineralocorticoid Dependent Effect of ABG-TsU on SBP in Sprague-Dawley rats

Upper Panel: Effect of ABG-TsU (POOL 1) (GROUP A) or vehicle only (GROUP B) treatment for 12 days on SBP (see figure 8 for protocol). Effect of ABG-TsU on SBP in adrenalectomized rats (GROUP C) either treated with ABG-TsU alone or in combination with physiological amounts of aldosterone (see figure 9 for protocol).

Lower Panel: Effect on SBP of ABG-TsU treatment alone (GROUP A) or ABG-TsU administered in combination with a spironolactone (GROUP D) (see figure 9 for protocol).
discontinuation of the spironolactone (during which time ABG-TsU administration was continued) (figure 17 lower panel). At this time ABG-TsU treatment was withdrawn and SBP declined to normal levels (120 ± 4 mmHg) within two days.

(C) HEMODYNAMICS OF ABG-TsU INDUCED HYPERTENSION:

Following 14 days of ABG-TsU (POOL 2) or vehicle administration, SBP, DBP, MAP, and CO were significantly higher in ABG-TsU (POOL 2) treated rats (n=7) than in controls (n=7) (figure 18, table 2). No significant difference in TPR was detected between the two groups. Pulse pressure tended to be higher in the ABG-TsU (POOL 2) treated rats though not significantly so (p<0.1) (figure 18, table 2). The hypertension was therefore due to an increase in CO, TPR remaining inappropriately normal.
FIGURE 18

Hemodynamics of ABG-TsU Induced Hypertension

Hemodynamic variables in rats treated for 14 days with either ABG-TsU (POOL 2) (25 μg/100g) or vehicle.
TABLE 2

Effect of ABG-TsU (POOL 2) or Vehicle on Various Variables (x ± SD) 
after 14 Days of Treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>CONTROLS (n=7)</th>
<th>ABG-TsU TREATED (n=7)</th>
<th>p* (df=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>122 ± 18</td>
<td>168 ± 9</td>
<td>0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>90 ± 18</td>
<td>127 ± 8</td>
<td>0.001</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>101 ± 17</td>
<td>141 ± 7</td>
<td>0.001</td>
</tr>
<tr>
<td>Pulse Pressure (mmHg)</td>
<td>32 ± 9</td>
<td>41 ± 8</td>
<td>0.1</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>289 ± 13</td>
<td>314 ± 13</td>
<td>0.01</td>
</tr>
<tr>
<td>CO (ml/min)</td>
<td>71 ± 19</td>
<td>104 ± 23</td>
<td>0.02</td>
</tr>
<tr>
<td>TPR (mmHg/ml/min)</td>
<td>1.60 ± 0.5</td>
<td>1.41 ± 0.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Student's two tailed t-test for independent means
(D) Effect of ABG-TsU Isolated from Individual Hypertensive and NNormotensive Humans on BP in the Rat:

(i) Effect of ABG-TsU Isolated from a Renovascular Hypertensive Subject Having Normal ABG Binding Capacity (RHN): Treatment of rats with ABG-TsU isolated from RHN (10 μg/100 g/day for 14 days) failed to increase either SBP or DBP (figure 19).

However, total plasma binding capacity for aldosterone was significantly decreased in ABG-TsU (RHN) treated rats compared to controls as was ABG-Ts binding capacity (table 3). ABG binding capacity tended (p<0.1) to be lower in ABG-TsU (RHN) treated rats.

(ii) Effect of ABG-TsU Isolated From Subject With EH Having Normal ABG Binding Capacity (EHN): Treatment of rats with ABG-TsU (10 μg/100 g/day for 14 days) isolated from EHN failed to increase either SBP or DBP compared to controls (figure 19) but it did cause a significant decrease in heart rate (table 4). No significant differences in any other variables were observed, with the exception that ABG bound aldosterone tended (p<0.1) to be lower in ABG-TsU (EHN) treated rats than in controls, and the total, ABG-Ts, and ABG binding capacities for aldosterone were significantly lower in the ABG-TsU (EHN) treated rats (table 4).

(iii) Effect of ABG-TsU Isolated from Subjects With EH Having Increased ABG Binding Capacity (EHH): Administration of ABG-TsU (10 μg/100 g/day for 14 days) isolated from EHH caused SBP to gradually increase compared to controls after 8 days of administration (figure 20), reaching significantly higher SBP after 12 days of treatment. After 14 days
of administration the ABG-TsU (EHH) treated rats had both systolic and
diastolic hypertension (figure 19). No suppression of PRA, aldosterone, or
hypokalemia was observed in the ABG-TsU (EHH) treated group (figure 21,
table 5). ABG bound aldosterone tended (p<0.1) to be lower in the ABG-TsU
treated rats, as was the ABG binding capacity (table 5).

(iv) **Effect of ABG-TsU Isolated from Normotensives Having Normal
ABG Binding Capacity (NTN):** Treatment of rats with ABG-TsU (25 µg/100
gram/day for 14 days) isolated from NTN failed to increase either SBP or DBP
(figure 19). The only effect of treatment was a significant decrease in ABG-
Ts binding capacity for aldosterone in ABG-TsU (NTN) treated rats as
compared to controls (table 6).

Treatment of rats with a much higher dose (200 µg/100 g/day for 14
days) of the same ABG-TsU (NTN) also failed to modify SBP (table 7).
FIGURE 19

Hypertensinogenicity of ABG-TsU Isolated from Individual Normotensive or Hypertensive Humans

SBP (top of bar) and DBP (bottom of bar) in Sprague-Dawley rats following 14 days of ABG-TsU administration. ABG-TsU was isolated from normotensive individuals having normal plasma ABG binding capacity for aldosterone (NTN), from a renovascular hypertensive patient having normal ABG binding capacity for aldosterone (RHN), from a subject with EH having normal ABG binding capacity for aldosterone (EHN), and from individuals with EH having increased ABG binding capacity for aldosterone (EHH). (*) represents a significant difference in DBP while (**) represents a significant difference in SBP.
### TABLE 3

Effect of ABG-TsU (RHN) or Vehicle on Various Variables (x ± SD) after 14 Days of Treatment

<table>
<thead>
<tr>
<th></th>
<th>CONTROLS (n=9)</th>
<th>ABG-TsU TREATED (n=7)</th>
<th>p* (df=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>136 ± 18</td>
<td>142 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>99 ± 14</td>
<td>104 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td>111 ± 15</td>
<td>117 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td><strong>HR (min⁻¹)</strong></td>
<td>341 ± 13</td>
<td>347 ± 32</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Weight (g)</strong></td>
<td>380 ± 13</td>
<td>383 ± 18</td>
<td>NS</td>
</tr>
<tr>
<td><strong>PRA (ng/ml/hr)</strong></td>
<td>12.0 ± 5</td>
<td>12.0 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Total Aldosterone (ng/100 ml)</strong></td>
<td>9.6 ± 4</td>
<td>10.7 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Free Aldosterone (ng/100 ml)</strong></td>
<td>8.4 ± 4</td>
<td>9.8 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Total Bound Aldosterone (ng/100 ml)</strong></td>
<td>1.22 ± 0.8</td>
<td>0.92 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td><strong>ABG-Ts Bound Aldosterone (ng/100 ml)</strong></td>
<td>0.53 ± 0.4</td>
<td>0.57 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td><strong>ABG Bound Aldosterone (ng/100 ml)</strong></td>
<td>0.59 ± 0.5</td>
<td>0.35 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td><strong>% Total Bound Aldosterone</strong></td>
<td>11.8 ± 4</td>
<td>8.2 ± 3</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>% ABG-Ts Bound Aldosterone</strong></td>
<td>6.3 ± 1</td>
<td>5.2 ± 1</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>% ABG Bound Aldosterone</strong></td>
<td>5.5 ± 3</td>
<td>3.0 ± 3</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Plasma Na⁺ (mEq/L)</strong></td>
<td>141.3 ± 4.2</td>
<td>141.4 ± 1.7</td>
<td>NS</td>
</tr>
<tr>
<td><strong>PD₂₅ (µg/kg/min)</strong></td>
<td>4.01 ± 1.4</td>
<td>5.94 ± 2.5</td>
<td>NS</td>
</tr>
<tr>
<td><strong>r</strong></td>
<td>0.96 ± 0.03</td>
<td>0.97 ± 0.02</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Student's two tailed t-test for independent means

The binding capacity of unheated plasma for aldosterone (% Total Bound Aldosterone) and the thermostable binding capacity (% ABG-Ts Bound Aldosterone) of heated plasma for aldosterone were measured and the thermolabile binding capacity (% ABG Bound Aldosterone) obtained from the difference. The binding capacities of the various fractions were measured as percentages of the amount of aldosterone bound relative to the total amount of aldosterone present in the sample. The measured binding capacities reflect the concentrations of ABG and ABG-Ts. The amount of aldosterone bound was calculated from the various percentages (ABG bound + ABG-Ts bound = total bound aldosterone) once the concentration of total aldosterone was known.
**TABLE 4**

Effect of ABG-TsU (EHN) or Vehicle on Various Variables (x ± SD) after 14 Days of Treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>CONTROLS (n=9)</th>
<th>ABG-TsU TREATED (n=9)</th>
<th>p* (df=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>136 ± 18</td>
<td>126 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>99 ± 14</td>
<td>89 ± 16</td>
<td>NS</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>111 ± 15</td>
<td>102 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>HR (min⁻¹)</td>
<td>341 ± 13</td>
<td>319 ± 28</td>
<td>0.05</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>380 ± 13</td>
<td>372 ± 20</td>
<td>NS</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>12.0 ± 5</td>
<td>14.0 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>Total Aldosterone (ng/100 ml)</td>
<td>9.6 ± 4</td>
<td>8.0 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Free Aldosterone (ng/100 ml)</td>
<td>8.4 ± 4</td>
<td>7.3 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Total Bound Aldosterone (ng/100 ml)</td>
<td>1.22 ± 0.8</td>
<td>0.68 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>ABG-Ts Bound Aldosterone (ng/100 ml)</td>
<td>0.53 ± 0.4</td>
<td>0.42 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>ABG Bound Aldosterone (ng/100 ml)</td>
<td>0.59 ± 0.5</td>
<td>0.26 ± 0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>% Total Bound Aldosterone</td>
<td>11.8 ± 4</td>
<td>7.9 ± 4</td>
<td>0.05</td>
</tr>
<tr>
<td>% ABG-Ts Bound Aldosterone</td>
<td>6.3 ± 1</td>
<td>4.8 ± 2</td>
<td>0.05</td>
</tr>
<tr>
<td>% ABG Bound Aldosterone</td>
<td>5.5 ± 3</td>
<td>3.1 ± 2</td>
<td>0.05</td>
</tr>
<tr>
<td>Plasma Na⁺ (mEq/L)</td>
<td>141.3 ± 4.2</td>
<td>141.0 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>PD²₅ (µg/kg/min)</td>
<td>4.01 ± 1.4</td>
<td>4.04 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>t</td>
<td>0.96 ± 0.03</td>
<td>0.97 ± 0.02</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Student's two tailed t-test for independent means

The binding capacity of unheated plasma for aldosterone (% Total Bound Aldosterone) and the thermostable binding capacity (% ABG-Ts Bound Aldosterone) of heated plasma for aldosterone were measured and the thermolabile binding capacity (% ABG Bound Aldosterone) obtained from the difference. The binding capacities of the various fractions were measured as percentages of the amount of aldosterone bound relative to the total amount of aldosterone present in the sample. The measured binding capacities reflect the concentrations of ABG and ABG-Ts. The amount of aldosterone bound was calculated from the various percentages (ABG bound + ABG-Ts bound = total bound aldosterone) once the concentration of total aldosterone was known.
FIGURE 20

Time Course of SBP Changes in ABG-TsU (EHH) Treated Rats

Time course of SBP changes in ABG-TsU (EHH) (closed circles, n=9) or vehicle only treated (open circles, n=7) Sprague-Dawley rats. ABG-TsU was isolated from individuals with EH having increased ABG binding capacity for aldosterone (EHH).
FIGURE 21

Plasma Aldosterone, K⁺, and PRA following 14 Days ABG-TsU (EHH) or Vehicle Administration

PRA, plasma aldosterone, and plasma K⁺ in Sprague-Dawley rats after 14 days of treatment with either ABG-TsU (EHH) or vehicle.
### TABLE 5

**Effect of ABG-TsU (EHH) or Vehicle on Various Variables (x ± SD) after 14 Days of Treatment**

<table>
<thead>
<tr>
<th>Variable</th>
<th>CONTROLS (n=7)</th>
<th>ABG-TsU TREATED (n=9)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>139 ± 10</td>
<td>160 ± 7</td>
<td>0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>106 ± 7</td>
<td>118 ± 5</td>
<td>0.01</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>117 ± 8</td>
<td>132 ± 6</td>
<td>0.001</td>
</tr>
<tr>
<td>HR (min⁻¹)</td>
<td>360 ± 24</td>
<td>377 ± 17</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>351 ± 23</td>
<td>348 ± 17</td>
<td>NS</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>8.1 ± 4</td>
<td>5.9 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>Total Aldosterone (ng/100 ml)</td>
<td>12.5 ± 9</td>
<td>15.5 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>Free Aldosterone (ng/100 ml)</td>
<td>11.1 ± 7</td>
<td>14.4 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>Total Bound Aldosterone (ng/100 ml)</td>
<td>1.4 ± 0.9</td>
<td>1.1 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>ABG-Ts Bound Aldosterone (ng/100 ml)</td>
<td>0.9 ± 0.6</td>
<td>1.0 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>ABG Bound Aldosterone (ng/100 ml)</td>
<td>0.5 ± 0.5</td>
<td>0.1 ± 0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>% Total Bound Aldosterone</td>
<td>10.7 ± 5</td>
<td>7.2 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>% ABG-Ts Bound Aldosterone</td>
<td>7.0 ± 1.0</td>
<td>6.6 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>% ABG Bound Aldosterone</td>
<td>3.7 ± 4</td>
<td>0.8 ± 0.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Plasma Na⁺ (mEq/L)</td>
<td>139 ± 1</td>
<td>140 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma K⁺ (mEq/L)</td>
<td>4.5 ± 0.5</td>
<td>4.8 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>PD25 (µg/kg/min)</td>
<td>4.41 ± 1.3</td>
<td>4.75 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>r</td>
<td>0.97 ± 0.03</td>
<td>0.97 ± 0.02</td>
<td>NS</td>
</tr>
</tbody>
</table>

*p*Student's two tailed t-test for independent means

The binding capacity of unheated plasma for aldosterone (% Total Bound Aldosterone) and the thermostable binding capacity (% ABG-Ts Bound Aldosterone) of heated plasma for aldosterone were measured and the thermolabile binding capacity (% ABG Bound Aldosterone) obtained from the difference. The binding capacities of the various fractions were measured as percentages of the amount of aldosterone bound relative to the total amount of aldosterone present in the sample. The measured binding capacities reflect the concentrations of ABG and ABG-Ts. The amount of aldosterone bound was calculated from the various percentages (ABG bound + ABG-Ts bound = total bound aldosterone) once the concentration of total aldosterone was known.
### TABLE 6

**Effect of ABG-TsU (NTN) or Vehicle on Various Variables (x ± SD) after 14 Days of Treatment**

<table>
<thead>
<tr>
<th></th>
<th>CONTROLS (n=12)</th>
<th>ABG-TsU TREATED (n=12)</th>
<th>p* (df=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>130 ± 16</td>
<td>129 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>93 ± 14</td>
<td>95 ± 16</td>
<td>NS</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>107 ± 15</td>
<td>108 ± 16</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>349 ± 40</td>
<td>328 ± 49</td>
<td>NS</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>16.0 ± 5</td>
<td>16.1 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Total Aldosterone (ng/100 ml)</td>
<td>11.7 ± 5</td>
<td>10.5 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>Free Aldosterone (ng/100 ml)</td>
<td>10.3 ± 4</td>
<td>9.2 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>Total Bound Aldosterone (ng/100 ml)</td>
<td>1.43 ± 0.7</td>
<td>1.25 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>ABG-Ts Bound Aldosterone (ng/100 ml)</td>
<td>0.88 ± 0.4</td>
<td>0.66 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>ABC Bound Aldosterone (ng/100 ml)</td>
<td>0.56 ± 0.4</td>
<td>0.59 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>% Total Bound Aldosterone</td>
<td>11.9 ± 2</td>
<td>11.6 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>% ABC-Ts Bound Aldosterone</td>
<td>7.4 ± 1</td>
<td>6.2 ± 0.6</td>
<td>0.05</td>
</tr>
<tr>
<td>% ABC Bound Aldosterone</td>
<td>4.5 ± 2</td>
<td>5.4 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma Na⁺ (mEq/L)</td>
<td>144 ± 2</td>
<td>145 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma K⁺ (mEq/L)</td>
<td>5.3 ± 0.6</td>
<td>5.3 ± 0.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Student's two tailed t-test for independent means*

The binding capacity of unheated plasma for aldosterone (% Total Bound Aldosterone) and the thermostable binding capacity (% ABG-Ts Bound Aldosterone) of heated plasma for aldosterone were measured and the thermolabile binding capacity (% ABG Bound Aldosterone) obtained from the difference. The binding capacities of the various fractions were measured as percentages of the amount of aldosterone bound relative to the total amount of aldosterone present in the sample. The measured binding capacities reflect the concentrations of ABG and ABG-Ts. The amount of aldosterone bound was calculated from the various percentages (ABG bound + ABG-Ts bound = total bound aldosterone) once the concentration of total aldosterone was known.
### TABLE 7

**SBP (x ± SD) in Rats Treated With Vehicle for Two Weeks or with ABG-TsU Isolated From Various Sources**

<table>
<thead>
<tr>
<th>SOURCE OF ABG-TsU</th>
<th>DAILY DOSE OF ABG-TsU (µg/100 g)</th>
<th>CONTROLS n</th>
<th>ABG-TsU TREATED n</th>
<th>p*</th>
<th>BP DETERMINATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABG-TsU (Pool 1)</td>
<td>2</td>
<td>120 ± 9</td>
<td>151 ± 15</td>
<td>28</td>
<td>0.001</td>
</tr>
<tr>
<td>ABG-TsU (Pool 2)</td>
<td>25</td>
<td>122 ± 18</td>
<td>168 ± 9</td>
<td>7</td>
<td>0.001</td>
</tr>
<tr>
<td>ABG-TsU (NTN)</td>
<td>25</td>
<td>130 ± 16</td>
<td>129 ± 14</td>
<td>12</td>
<td>NS</td>
</tr>
<tr>
<td>ABG-TsU (NTN)</td>
<td>200</td>
<td>130 ± 16</td>
<td>126 ± 2</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>ABG-TsU (RHN)</td>
<td>10</td>
<td>136 ± 18</td>
<td>147 ± 9</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>ABG-TsU (EHN)</td>
<td>10</td>
<td>136 ± 18</td>
<td>126 ± 14</td>
<td>9</td>
<td>NS</td>
</tr>
<tr>
<td>ABG-TsU (EHH)</td>
<td>10</td>
<td>139 ± 10</td>
<td>160 ± 7</td>
<td>9</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Student's two tailed t-test for independent means*
Pressor Responsiveness to Phenylephrine in ABG-TsU Treated Rats

Dose of phenylephrine required to elevate DBP by 25 mmHg (PD$_{25}$) in rats following 14 days of ABG-TsU or vehicle treatment. ABG-TsU was isolated from a renovascular hypertensive patient having normal ABG binding capacity for aldosterone (RHN), a subject with EH having normal ABG binding capacity for aldosterone (EHN), and individuals with EH having increased ABG binding capacity for aldosterone (EHH).
**E** DETERMINATION OF PRESSOR RESPONSIVENESS TO PHENYLEPHRINE (PD$_{25}$):  

The dose of phenylephrine required to elevate DBP by 25 mmHg (PD$_{25}$) in 19 untreated rats was 4.49 ± 1.9 µg/kg/min. A very strong correlation existed between the increase in DBP and the log dose of infused phenylephrine (figure 7).

**F** PRESSOR RESPONSIVENESS TO PHENYLEPHRINE (PD$_{25}$) IN ABG-TsU TREATED RATS:  

No difference in PD$_{25}$ was detected between ABG-TsU (RHN, EHN, EHH) treated rats and their respective controls (figure 22).

In all groups of rats a correlation of 0.96 ± 0.03 (p<0.001) or better existed between the increase in DBP and the log dose of infused phenylephrine (tables 3, 4, 5).

**G** MINERALOCORTICOID CONTRIBUTION TO THE INITIATION OF HYPERTENSION IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR):  

(i) **Comparison of Baseline Variables Between 12 Week Old WKY and SHR:** Comparison of baseline variables between 12 week old untreated intact SHR and WKY rats (table 8) showed SHR to have much higher SBP, DBP, and MAP than WKY rats while no difference in heart rate existed. SHR were slightly but significantly lighter than the WKY rats. PRA was lower in SHR
### TABLE 8

**Baseline Variables (x ± SD) in Twelve Week Old WKY and SHR**

<table>
<thead>
<tr>
<th></th>
<th>WKY CONTROLS (n=9)</th>
<th>SHR (n=8)</th>
<th>p* (df=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (Weeks)</strong></td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>127 ± 8</td>
<td>219 ± 17</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>97 ± 4</td>
<td>152 ± 10</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td>111 ± 5</td>
<td>179 ± 13</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>HR (min⁻¹)</strong></td>
<td>350 ± 17</td>
<td>362 ± 34</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Weight (g)</strong></td>
<td>285 ± 11</td>
<td>272 ± 8</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>PRA (ng/ml/hr)</strong></td>
<td>11.9 ± 6</td>
<td>5.2 ± 3</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Total Aldosterone (ng/100 ml)</strong></td>
<td>31.1 ± 6</td>
<td>14.7 ± 6</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Total Aldosterone/PRA</strong></td>
<td>4.15 ± 4</td>
<td>3.52 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Free Aldosterone (ng/100 ml)</strong></td>
<td>28.9 ± 6</td>
<td>14.0 ± 5</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Total Bound Aldosterone (ng/100 ml)</strong></td>
<td>2.2 ± 0.7</td>
<td>0.7 ± 0.3</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>ABG-Ts Bound Aldosterone (ng/100 ml)</strong></td>
<td>1.8 ± 0.5</td>
<td>0.7 ± 0.3</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>ABG Bound Aldosterone (ng/100 ml)</strong></td>
<td>0.40 ± 0.3</td>
<td>0.05 ± 0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>% Total Bound Aldosterone</td>
<td>6.7 ± 2</td>
<td>5.3 ± 2</td>
<td>0.1</td>
</tr>
<tr>
<td>% ABG-Ts Bound Aldosterone</td>
<td>5.5 ± 1</td>
<td>4.9 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>% ABG Bound Aldosterone</td>
<td>1.2 ± 1</td>
<td>0.4 ± 0.2</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*p*Student's two tailed t-test for independent means

The binding capacity of unheated plasma for aldosterone (% Total Bound Aldosterone) and the thermostable binding capacity (% ABG-Ts Bound Aldosterone) of heated plasma for aldosterone were measured and the thermolabile binding capacity (% ABG Bound Aldosterone) obtained from the difference. The binding capacities of the various fractions were measured as percentages of the amount of aldosterone bound relative to the total amount of aldosterone present in the sample. The measured binding capacities reflect the concentrations of ABG and ABG-Ts. The amount of aldosterone bound was calculated from the various percentages (ABG bound + ABG-Ts bound = total bound aldosterone) once the concentration of total aldosterone was known.
than in WKY rats as was total and free plasma aldosterone. The ratio of total aldosterone to PRA however did not differ between SHR and WKY rats. ABG binding capacity was significantly lower in SHR compared to WKY rats. Because of the significantly lower plasma aldosterone concentrations in the SHR, the total amount of aldosterone bound was lower as were the amounts of ABG-Ts and ABG bound aldosterone compared to WKY rats (table 8).

(ii) **Spironolactone Treatment of SHR of Different Ages:** Treatment of 7 week old SHR (Group 1, figure 10) for two weeks with Soldactone (1.5 mg/rat/day) resulted in significantly lower SBP (figures 23 and 24), DBP, and MAP (table 9) when compared to sham operated controls. The decrease in BP was accompanied by a significant reduction in heart rate and an increase in plasma aldosterone when compared to controls (table 9).

A higher dose of Soldactone (5 mg/rat/day), administered for one week only to 7 week old SHR (Group 2, figure 11), lowered SBP (figure 23) somewhat (p<0.1) and resulted in a significant reduction in body weight and a significant increase in PRA when compared to sham operated controls (table 10).

Treatment of 10 week old SHR (Group 3, figure 12) for two weeks with Soldactone (1.5 mg/rat/day) failed to significantly lower BP compared to sham operated controls (figure 23, table 11) and had no significant effect on any other variables (table 11).

Treatment of 12 week old SHR (Group 4, figure 13) with a higher dose of Soldactone (5 mg/rat/day) for one week failed to lower BP compared to sham operated controls (figure 23). Body weight was significantly lower in Soldactone treated rats compared to controls (table 12).
FIGURE 23

Effect of Spironolactone on BP of SHR at Different ages

Upper Panel: SBP in 7 and 12 week old SHR (GROUPS 2 and 4) immediately prior to i.p. implantation of osmotic minipumps delivering Soldactone (5 mg/rat/day) for one week (closed circles). Control rats (open circles) were sham operated (see figures 11 and 13 for protocols).

Lower Panel: Effect of Soldactone (1.5 mg/rat/day, closed circles) similarly administered for two weeks on SBP in 7 to 9 week old SHR (GROUP 1) and 10 to 12 week old SHR (GROUP 3). Controls (open circles) were sham operated (see figures 10 and 12 for protocols). SBP was not measured in GROUP 3 prior to the initiation of Soldactone or control treatment.
TABLE 9

Effect of Spirotonolactone (1.5 mg/rat/day) on Various Variables (x ± SD) in SHR (Group 1) Treated from 7 to 9 Weeks of Age

<table>
<thead>
<tr>
<th></th>
<th>CONTROLS (n=8)</th>
<th>SOLDACTONE TREATED (n=8)</th>
<th>p* (df=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Age (weeks)</td>
<td>7</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Dose of Soldactone (mg/rat/day)</td>
<td>-</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>Duration of Treatment (weeks)</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>198 ± 11</td>
<td>169 ± 22</td>
<td>0.01</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>139 ± 7</td>
<td>121 ± 17</td>
<td>0.02</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>164 ± 8</td>
<td>146 ± 18</td>
<td>0.05</td>
</tr>
<tr>
<td>HR (min⁻¹)</td>
<td>393 ± 28</td>
<td>361 ± 28</td>
<td>0.05</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>220 ± 10</td>
<td>219 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>5.7 ± 7</td>
<td>10.9 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma Aldosterone (ng/100 ml)</td>
<td>7.9 ± 4</td>
<td>15.1 ± 5</td>
<td>0.01</td>
</tr>
<tr>
<td>Plasma Na⁺ (mEq/L)</td>
<td>138.0 ± 2</td>
<td>139.9 ± 2</td>
<td>0.1</td>
</tr>
<tr>
<td>Plasma K⁺ (mEq/L)</td>
<td>4.4 ± 0.5</td>
<td>4.7 ± 0.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Student’s two tailed t-test for independent means
## TABLE 10

**Effect of Spironolactone (5 mg/rat/day) on Various Variables (± SD) in SHR (Group 2) Treated from 7 to 8 Weeks of Age**

<table>
<thead>
<tr>
<th></th>
<th>CONTROLS (n=8)</th>
<th>SOLDACTONE TREATED (n=8)</th>
<th>p* (df=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial Age (weeks)</strong></td>
<td>7</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td><strong>Dose of Soldactone (mg/rat/day)</strong></td>
<td>-</td>
<td>5.0</td>
<td>-</td>
</tr>
<tr>
<td><strong>Duration of Treatment (weeks)</strong></td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>194 ± 11</td>
<td>179 ± 19</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>127 ± 7</td>
<td>123 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td>158 ± 8</td>
<td>147 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td><strong>HR (min⁻¹)</strong></td>
<td>367 ± 28</td>
<td>371 ± 47</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Weight (g)</strong></td>
<td>186 ± 10</td>
<td>170 ± 14</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>PRA (ng/ml/hr)</strong></td>
<td>9.8 ± 2</td>
<td>14.3 ± 6</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Plasma Aldosterone (ng/100 ml)</strong></td>
<td>8.3 ± 4</td>
<td>9.4 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Plasma Na⁺ (mEq/L)</strong></td>
<td>139.1 ± 2</td>
<td>138.2 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Plasma K⁺ (mEq/L)</strong></td>
<td>4.6 ± 1</td>
<td>4.9 ± 1</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Student’s two tailed t-test for independent means*
**TABLE 11**

Effect of Spironolactone (1.5 mg/rat/day) on Various Variables (x ± SD) in SHR (Group 3) Treated from 10 to 12 Weeks of Age

<table>
<thead>
<tr>
<th></th>
<th>CONTROLS (n=10)</th>
<th>SOLDACTONE TREATED (n=11)</th>
<th>p* (df=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial Age (weeks)</strong></td>
<td>10</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td><strong>Dose of Soldactone (mg/rat/day)</strong></td>
<td>-</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td><strong>Duration of Treatment (weeks)</strong></td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>220 ± 18</td>
<td>210 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>147 ± 9</td>
<td>142 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td>178 ± 12</td>
<td>170 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td><strong>HR (min⁻¹)</strong></td>
<td>352 ± 30</td>
<td>365 ± 48</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Weight (g)</strong></td>
<td>268 ± 20</td>
<td>260 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td><strong>PRA (ng/ml/hr)</strong></td>
<td>7.5 ± 3</td>
<td>6.4 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Plasma Aldosterone (ng/100 ml)</strong></td>
<td>10.1 ± 4</td>
<td>10.2 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Plasma Na⁺ (mEq/L)</strong></td>
<td>139.8 ± 2</td>
<td>140.0 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Plasma K⁺ (mEq/L)</strong></td>
<td>4.4 ± 0.4</td>
<td>4.5 ± 0.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Student's two tailed t-test for independent means*
TABLE 12

Effect of Spironolactone (5 mg/rat/day) on Various Variables (\(\bar{x} \pm SD\)) in SHR (Group 4) Treated from 12 to 13 Weeks of Age

<table>
<thead>
<tr>
<th>Initial Age (weeks)</th>
<th>Controls Treated</th>
<th>p* (df=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose of Soldactone (mg/rat/day)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Duration of treatment (weeks)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>222 ± 19</td>
<td>220 ± 23</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>146 ± 11</td>
<td>147 ± 19</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>177 ± 13</td>
<td>180 ± 20</td>
</tr>
<tr>
<td>HR (min⁻¹)</td>
<td>365 ± 34</td>
<td>375 ± 22</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>277 ± 5</td>
<td>253 ± 13</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>10.5 ± 5</td>
<td>10.3 ± 2</td>
</tr>
<tr>
<td>Plasma Aldosterone (ng/100 ml)</td>
<td>6.0 ± 5</td>
<td>7.8 ± 6</td>
</tr>
<tr>
<td>Plasma Na⁺ (mEq/L)</td>
<td>141.4 ± 3</td>
<td>144.6 ± 4</td>
</tr>
<tr>
<td>Plasma K⁺ (mEq/L)</td>
<td>4.3 ± 0.3</td>
<td>4.4 ± 0.4</td>
</tr>
</tbody>
</table>

*Student's two tailed t-test for independent means
Adrenalectomy and Aldosterone Replacement: BP was not affected by sodium loading SHR from 7 to 9 weeks of age (SHAM, table 13, figure 14) at which time SBP/DBP was not significantly greater than in 9 week old SHR (table 9, Group 1 controls-figure 10) drinking water (206 ± 16/141 ± 8 vs 198 ± 11/139 ± 7 mmHg, p NS). However, total plasma aldosterone was significantly decreased in SHR that were sodium loaded from 7 to 9 weeks of age (SHAM, table 13, figure 14) compared to 9 week old SHR (table 9, Group 1 controls figure 10) drinking water (3.7 ± 1.3 vs. 7.9 ± 3.8 ng/100 ml, p<0.01).

Adrenalectomy (ADRX, figure 14) resulted in a large drop in SBP (51 mmHg) in SHR from 7 to 9 weeks of age while sham operated SHR (SHAM, figure 14) showed a significant rise in SBP (40 mmHg) over the same time period (figure 24, upper panel). Treatment of 7 week old adrenalectomized SHR with aldosterone for two weeks (ADRX+ALDO, figure 14) prevented the large decrease in SBP seen with ADRX alone (figure 24). Direct determinations of SBP/DBP at 9 weeks of age (figure 25) showed the SHAM group to be very hypertensive while the ADRX group was normotensive. Treatment of adrenalectomized SHR with aldosterone (ADRX + ALDO) restored BP to hypertensive values (167/116 mmHg) which were half way back to SHAM levels (figure 25). ADRX rats had significantly reduced heart rates and body weights (compared to SHAM rats) but these were corrected by ADRX+ALDO treatment (table 13). ADRX significantly increased PRA (compared to SHAM). PRA, however, did not differ significantly between the ADRX+ALDO and SHAM rats (figure 26). The marked hyperkalemia and slight hyponatremia observed in the ADRX rats (figure 27) were completely corrected by aldosterone administration as was the decrease in the plasma Na+/K+ ratio (table 13). ADRX resulted in a significant drop in total and free
plasma aldosterone when compared to SHAM (figure 28, table 13). Total plasma binding capacity for aldosterone was significantly increased by adrenalectomy, this increase being entirely accounted for by an increase in ABG binding capacity (table 13). Even though total aldosterone was greatly reduced by ADRX, the total amount of aldosterone bound did not differ between the SHAM and ADRX rats, however, in ADRX rats the largest bound fraction of aldosterone was that bound to ABG, thus the amount of ABG bound aldosterone was greatly increased (figure 28).

Compared to ADRX rats the ADRX+ALDO rats had significantly higher total, free, total bound, ABG bound, and ABG-T's bound aldosterone (figure 28).

Free plasma aldosterone concentrations did not differ between the SHAM and ADRX+ALDO rats (figure 28). However, total bound, ABG bound, and ABG-T's bound aldosterone were greatly increased in the ADRX+ALDO group (compared to SHAM) as were the total plasma binding capacity for aldosterone and the ABG binding capacity for aldosterone, which resulted in a small but significant increase in total aldosterone (table 13). Total aldosterone, however, did not differ significantly between 9 week old ADRX+ALDO (drinking saline) and intact 9 week old SHR (table 9, Group 1, figure 10) drinking water (5.6 ± 1.8 vs 7.9 ± 3.8 ng/100 ml, p NS).
### TABLE 1.3

**Effect of Sham Operation, Adrenalectomy, or Adrenalectomy with Aldosterone Replacement at 7 Weeks of Age on Various Variables (x ± SD) in 9 Week Old SHR**

<table>
<thead>
<tr>
<th></th>
<th>SHAM</th>
<th>ADRX</th>
<th>ADRX + ALDO</th>
<th>*p</th>
<th>SHAM vs ADRX</th>
<th>SHAM vs ADRX + ALDO</th>
<th>ADRX vs ADRX + ALDO</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>206 ± 16</td>
<td>118 ± 17</td>
<td>167 ± 24</td>
<td>0.001</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>141 ± 8</td>
<td>89 ± 17</td>
<td>116 ± 20</td>
<td>0.001</td>
<td>0.005</td>
<td>0.01</td>
<td>0.005</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>167 ± 14</td>
<td>103 ± 19</td>
<td>135 ± 20</td>
<td>0.001</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>HE (min⁻¹)</td>
<td>390 ± 24</td>
<td>351 ± 39</td>
<td>379 ± 21</td>
<td>0.05</td>
<td>0.05</td>
<td>NS</td>
<td>0.005</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>226 ± 10</td>
<td>192 ± 18</td>
<td>225 ± 13</td>
<td>0.001</td>
<td>0.005</td>
<td>NS</td>
<td>0.005</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>6.8 ± 4</td>
<td>13.1 ± 2</td>
<td>3.8 ± 3</td>
<td>0.001</td>
<td>0.01</td>
<td>NS</td>
<td>0.005</td>
</tr>
<tr>
<td>Total Aldosterone (ng/100 ml)</td>
<td>3.7 ± 1.3</td>
<td>1.6 ± 0.7</td>
<td>5.6 ± 1.8</td>
<td>0.001</td>
<td>0.01</td>
<td>0.05</td>
<td>0.005</td>
</tr>
<tr>
<td>Total Aldosterone/PRA</td>
<td>0.59 ± 0.2</td>
<td>0.15 ± 0.1</td>
<td>2.18 ± 1.6</td>
<td>0.005</td>
<td>NS</td>
<td>0.05</td>
<td>0.005</td>
</tr>
<tr>
<td>Free Aldosterone (ng/100 ml)</td>
<td>3.5 ± 1.2</td>
<td>1.3 ± 0.6</td>
<td>4.5 ± 1.5</td>
<td>0.001</td>
<td>0.005</td>
<td>NS</td>
<td>0.005</td>
</tr>
<tr>
<td>Total Bound Aldosterone (ng/100 ml)</td>
<td>0.25 ± 0.08</td>
<td>0.37 ± 0.1</td>
<td>1.1 ± 0.6</td>
<td>0.001</td>
<td>NS</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>ABG-Ts Bound Aldosterone (ng/100 ml)</td>
<td>0.23 ± 0.06</td>
<td>0.1 ± 0.04</td>
<td>0.37 ± 0.1</td>
<td>0.001</td>
<td>0.05</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>ABG Bound Aldosterone (ng/100 ml)</td>
<td>0.03 ± 0.03</td>
<td>0.25 ± 0.08</td>
<td>0.73 ± 0.2</td>
<td>0.001</td>
<td>0.01</td>
<td>0.05</td>
<td>0.005</td>
</tr>
<tr>
<td>% Total Bound Aldosterone</td>
<td>6.9 ± 1.4</td>
<td>22.8 ± 2.6</td>
<td>19.5 ± 1.4</td>
<td>0.001</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>% ABG-Ts Bound Aldosterone</td>
<td>6.4 ± 0.7</td>
<td>7.0 ± 0.7</td>
<td>6.7 ± 0.3</td>
<td>0.005</td>
<td>NS</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>% ABG Bound Aldosterone</td>
<td>0.74 ± 0.2</td>
<td>15.8 ± 2.2</td>
<td>12.9 ± 1.4</td>
<td>0.001</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>Plasma Na⁺ (mEq/L)</td>
<td>138.9 ± 2.7</td>
<td>136.0 ± 4.7</td>
<td>142.3 ± 4.5</td>
<td>0.025</td>
<td>NS</td>
<td>NS</td>
<td>0.05</td>
</tr>
<tr>
<td>Plasma K⁺ (mEq/L)</td>
<td>4.9 ± 0.4</td>
<td>6.9 ± 1.3</td>
<td>5.0 ± 0.9</td>
<td>0.001</td>
<td>0.005</td>
<td>NS</td>
<td>0.005</td>
</tr>
<tr>
<td>Plasma Na⁺/K⁺</td>
<td>28.8 ± 2.6</td>
<td>20.6 ± 4.7</td>
<td>29.2 ± 5.7</td>
<td>0.001</td>
<td>0.005</td>
<td>NS</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*One way analysis of variance (df = 2, 24)

**Tukey’s Modified t-test for Multiple Comparisons between means (df = 3, 24)

The binding capacity of unheated plasma for aldosterone (% Total Bound Aldosterone) and the thermostable binding capacity (% ABG-Ts Bound Aldosterone) of heated plasma for aldosterone were measured and the thermolabile binding capacity (% ABG Bound Aldosterone) obtained from the difference. The binding capacities of the various fractions were measured as percentages of the amount of aldosterone bound relative to the total amount of aldosterone present in the sample. The measured binding capacities reflect the concentrations of ABG and ABG-Ts. The amount of aldosterone bound was calculated from the various percentages (ABG bound + ABG-Ts bound = total bound aldosterone) once the concentration of total aldosterone was known.
FIGURE 24
Effect of Adrenalectomy, Aldosterone Replacement, and Spironolactone on the Development of Hypertension in SHR

Upper Panel: SBP in 7 week old SHR immediately prior to and two weeks following either sham operation (SHAM), bilateral adrenalectomy (ADRX), or bilateral adrenalectomy and administration of physiological amounts of aldosterone (2 μg/rat/day from 7 to 9 weeks, ADRX+ALDO): (see figure 14 for protocol).

Lower Panel: SBP in 7 week old SHR (GROUP 1) immediately prior to implantation of osmotic minipumps delivering Soldactone, and following one and two weeks of treatment (see figure 10 for protocol).
FIGURE 25

Systolic and Diastolic BP Two Weeks following Sham Operation or Adrenalectomy with and without Aldosterone Replacement

SBP/DBP measured directly in 9 week old SHR which at 7 weeks of age were either sham operated (SHAM), bilaterally adrenalectomized (ADRX), or bilaterally adrenalectomized and given physiological amounts (2μg/rat/day) of aldosterone (ADRX+ALDO) through to 9 weeks of age (see figure 14 for protocol).
FIGURE 26

Plasma Renin Activity Two Weeks following Sham Operation or Adrenalectomy with and without Aldosterone Replacement

PRA in 9 week old SHR which at 7 weeks of age were either sham operated (SHAM), bilaterally adrenalectomized (ADRX), or bilaterally adrenalectomized and given physiological amounts (2 µg/rat/day) of aldosterone (ADRX+ALDO) through to 9 weeks of age (see figure 14 for protocol).
Plasma Potassium Two Weeks following Sham Operation or Adrenalectomy with and without Aldosterone Replacement

Plasma potassium in 9 week old SHR which at 7 weeks of age were either sham operated (SHAM), bilaterally adrenalectomized (ADRX), or bilaterally adrenalectomized and given physiological amounts (2μg/rat/day) of aldosterone (ADRX+ALDO) through to 9 weeks of age (see figure 14 for protocol).
FIGURE 28

Free and Bound Plasma Aldosterone Two Weeks following Sham Operation or Adrenalectomy with and without Aldosterone Replacement

Free plasma aldosterone, ABG bound aldosterone (to the thermolabile Aldosterone Binding Globulin), and ABG-Ts bound aldosterone (to the thermostable Aldosterone Binding Globulin) in 9 week old SHR which at 7 weeks of age were either sham operated (SHAM), bilaterally adrenalectomized (ADRX), or bilaterally adrenalectomized and given physiological amounts (2 μg/rat/day) of aldosterone (ADRX+ALDO) through to 9 weeks of age (see figure 14 for protocol). Throughout the two week experiment rats were given a 0.9% NaCl solution to drink.
Discussion

(A) CHARACTERIZATION OF ABG-TsU:

The urinary protein designated ABG-TsU does indeed appear to be the urinary homologue of plasma ABG. ABG-TsU isolated from pooled human urine has the same molecular weight (27,500 daltons) and isoelectric points (pI=4.76, 4.80) as does plasma ABG (and its thermostable plasma counterpart, ABG-Ts), and both plasma ABG and ABG-TsU bind aldosterone and dehydroepiandrosterone sulfate (DHEA-SO₄) reversibly, with high-affinity, and low capacity [176, 197]. ABG-TsU is thermostable and thus shares the same physicochemical characteristics as its plasma homologue, ABG-Ts [176]. A salivary homologue of ABG, designated ABG-S, had the same molecular weight, isoelectric points, and high-affinity low-capacity binding for both aldosterone and DHEA-SO₄ as did ABG-TsU and its plasma homologues [254]. In addition, administration of the same doses of either ABG-S or ABG-TsU to Sprague-Dawley rats induced hypertension [254]. It thus seems highly likely that the urinary, salivary, and plasma aldosterone
binding globulins are all homologues of the same protein (ABG), which is clearly distinct from any previously described steroid binding proteins.

ABG-TsU was unrelated to another hypertensinogenic human urinary glycoprotein with similar physicochemical characteristics, namely the Aldosterone Stimulating Factor (ASF) [256, 257]. Some puzzling facts concerning ASF emerged: it had a molecular weight of 26,000 daltons [259] (ABG-TsU having a mw of 27,500 daltons), and when further purified by DEAE-Cellulose ion-exchange chromatography using a stepwise pH gradient (barbiturate buffer pH 8.6 followed by acetate buffer pH 5.0), it was eluted from the DEAE-Cellulose column with the acetate buffer at pH 5.0 [257]. Thus it must have had an isoelectric point (pI) very close to pI=5, which was very close to that of ABG-TsU (pI=4.76, 4.80) which was likewise eluted from a DEAE-Cellulose column with acetate buffer at pH 5.0. Since the molecular weight and pI of ASF were almost identical to those of ABG-TsU, our isolation procedure should have resulted in the isolation of a considerable quantity of ASF along with ABG-TsU. In addition to being physicochemically similar, both materials induced mild hypertension when given in the same doses for the same period of time to Sprague-Dawley rats [197, 257]. However, unlike ABG-TsU, ASF was a potent stimulator of aldosterone production both in-vivo and in-vitro and was apparently unrelated to ACTH, angiotensin II, or β-lipotropin [258, 259]. ASF immunoreactivity was found to be localized in the anterior lobe of the pituitary gland, from which it may be secreted, suggesting that it might play a role in aldosterone homeostasis [258].

Our initial isolation procedure for ABG-TsU from total urine was very mild (differential ultrafiltration) and differed considerably from the initial step used in the isolation of ASF. ASF was precipitated from total urine by adsorption on benzoic acid (in an ethanolic solution). Subsequently, the
adsorbed material was suspended in a (Tris)-ethanol solution (1 part of a 0.5 M Tris solution in 9 parts of 95% ethanol) until fully dissolved and then allowed to stand for 3 hours at -23°C. The precipitate was then dissolved in cold absolute ethanol and kept at -23°C for 3 hours. The precipitate formed was then further purified by ion exchange chromatography and gel filtration to yield pure ASF [257]. These procedures used to precipitate ASF (especially the use of ethanol) are quite drastic. It is likely that the use of differential ultrafiltration (a very mild procedure) as the initial step in the isolation of ABG-TsU from total urine should have resulted in the isolation of ASF as well. Since this mild procedure failed to include any ASF in the protein fraction that was obtained (no stimulation of plasma aldosterone in ABG-TsU treated rats), perhaps ASF actually existed as a molecule with a molecular weight other than 26,000 daltons prior to the exposure to absolute ethanol. It is possible that ASF might be a fragment of some known stimulator of aldosterone secretion (α-MSH, β-MSH, β-lipotropin) contained in the pro-opiomelanocortin sequence.

(B) PARTIAL CHARACTERIZATION OF RAT PLASMA ABG:

Aldosterone binding proteins have been shown (by gel filtration techniques) to be present in the plasma of both intact and adrenalectomized rats [85]. In addition to binding aldosterone, these proteins also bind its reduced metabolite, 3α, 5β-tetrahydroaldosterone, as well as other unknown polar metabolites of aldosterone [85]. Preliminary identification and characterization of aldosterone binding proteins in rat plasma and comparison with purified ABG-TsU were carried out by gel filtration. After
partial removal of albumin and incubation with $^3$H-aldosterone, the rat plasma was chromatographed on a Sephadex G-75 column and the molecular weight at which the bound aldosterone was eluted was determined. This corresponded to the same molecular weight as that at which ABG-TsU was eluted when run separately on the same column. It thus appears that in the rat aldosterone binds to a plasma fraction with a molecular weight of 27,500 daltons. Since this is the same molecular weight as ABG, it is tempting to speculate that in rat plasma aldosterone binds to ABG.

Two other laboratories have reported that the specific high affinity binding of aldosterone in rat plasma can be accounted for by the presence of ABG [188, 231].

(C) **ABG-TsU INDUCED HYPERTENSION IN THE SPRAGUE-DAWLEY RAT:**

Administration of ABG-TsU (POOL 1) to Sprague-Dawley rats produced a sustained hypertension within 5-8 days of starting treatment. After 14 days of ABG-TsU administration, rats had a mild hypertension and normal levels of plasma aldosterone, PRA, Na$^+$, and K$^+$. This hormonal and electrolyte profile in plasma resembles that seen in EH patients more closely than that observed in any secondary or experimental forms of hypertension [255]. ABG-TsU induced hypertension was clearly adrenal dependent since it was prevented by adrenalectomy or administration of an aldosterone antagonist, but not by adrenalectomy when physiological amounts of aldosterone were concomitantly administered with ABG-TsU. It thus appears that the hypertension was caused by an interaction between
aldosterone and ABG-TsU. Because a control series of adrenalectomized rats receiving only ABG-TsU for 32 days was not included, one cannot be absolutely certain that the BP would not have risen in the absence of aldosterone in the latter part of the course of ABG-TsU treatment. However, since ABG-TsU alone did not increase BP even slightly in adrenalectomized rats during the initial 14 day treatment period, this seems unlikely. When aldosterone was given concomitantly with ABG-TsU to adrenalectomized rats, BP rose to hypertensive levels. When ABG-TsU treatment was withdrawn, BP dropped to normotensive levels despite the continuation of aldosterone administration. Thus the dose of aldosterone administered was insufficient to cause hypertension. The hypertension was therefore due to the interaction between aldosterone and ABG-TsU. This raised the intriguing point that ABG-TsU-bound (but not free) aldosterone was hypertensinogenic.

Spironolactone displaces aldosterone from human plasma ABG in vivo and in vitro by apparently competing with aldosterone for binding sites, thereby increasing aldosterone turnover [184, 191, 192]. Spironolactone probably prevented ABG-TsU from interacting with sufficient aldosterone to mediate a rise in BP by displacing aldosterone from ABG-TsU binding sites. (In addition, spironolactone may have reduced BP by interfering with the action of aldosterone on target tissues). If the interaction between aldosterone and ABG-TsU was prevented, either by adrenalectomy or by spironolactone, no increase in BP occurred. The aldosterone dependence and spironolactone responsiveness of ABG-TsU induced hypertension is comparable to what has been observed in at least a subgroup of patients with EH [see Introduction (F), (G)], suggesting that the etiology of ABG-TsU induced hypertension may relate to that of some cases of EH.
The biological half-life of ABG-TsU appeared to be short since the BP of ABG-TsU hypertensive rats normalized within two days following the discontinuation of ABG-TsU treatment. The lowered levels of bound aldosterone and decreased ABG binding capacity for aldosterone in ABG-TsU treated rats may have been due to metabolism of the exogenously administered ABG-TsU, as well as suppression of endogenous ABG production (possibly in response to the presence of exogenous ABG-TsU).

Although ABG-TsU induced hypertension was aldosterone dependent, it clearly differed from mineralocorticoid hypertension due to the lack of hypokalemia, absence of suppression of PRA, the presence of normal levels of plasma aldosterone (which would be elevated in aldosterone dependent hypertension, or suppressed if the hypertension were caused by another mineralocorticoid), and the lack of hyperplasia or hypertrophy of the adrenal zona glomerulosa (or the rest of the adrenal cortex). This also clearly distinguished ABG-TsU induced hypertension (after 12 days of administration) from the aldosterone dependent hypertension caused by the administration of ASF to Sprague-Dawley rats [256, 257]. ASF treatment resulted in a rise in BP after only 5 days, sustained hypertension developing by day 10 of the treatment period [257]. After 5 or 10 days of ASF administration, plasma aldosterone levels were increased 100-fold over those measured in control rats, resulting in sodium retention, volume expansion, and suppression of PRA by day 21 of the ASF treatment period. The marked hyperaldosteronism in ASF induced hypertension was accompanied by increased adrenal gland weight and hypertrophy of the adrenal cortex [257]. However, ABG-TsU hypertensive rats had no signs of stimulation of plasma aldosterone or hypertrophy of the adrenal cortex. Thus no ASF was present in our batches of electrophoretically pure ABG-TsU.
Increased CO and inappropriately normal TPR are characteristics of borderline EH [see section (C) of the Introduction]. The hypertension induced in the Sprague-Dawley rat by the administration of ABG-TsU for two weeks had similar hemodynamic characteristics in that MAP and CO were elevated, while TPR remained inappropriately normal indicating an inability to vasodilate in response to increased flow. These hemodynamic findings resembled those observed in borderline EH more closely than those reported in any secondary or experimental forms of hypertension [197, 331]. The increased CO may have been due to direct positive inotropic effects of aldosterone on myocardial contractility [see Introduction (E)(v)], to resetting effects of aldosterone on cardiovascular control mechanisms resulting in decreased baroreceptor sensitivity [see Introduction (E)(vi)], or to increased venous tone leading to increased venous return, mean circulatory filling pressure, and hence augmented CO due to the rise in central blood volume. All of these mechanisms have also been proposed to be involved in the genesis of the elevated CO in borderline EH [see Introduction (C)]. Longer duration studies are needed to determine whether or not ABG-TsU induced hypertension evolves into a state of normal CO and increased TPR as may be the case in human EH [see Introduction (C)].

Although blood volume was not measured in ABG-TsU induced hypertension, the lack of suppression of PRA suggested that it was normal and therefore that the hypertension was not due to volume expansion. The increased CO could have reflected an increase in cardiopulmonary blood volume relative to total blood volume (even if total blood volume remained unchanged). No increase in heart rate was observed in subsequently studied ABG-TsU hypertensive rats, suggesting that the observed increase of CO was due to increased stroke volume. The hemodynamic picture in ABG-TsU
induced hypertension may have been very similar to that of borderline EH. In borderline EH CO was increased (heart rate was normal, stroke volume was elevated) as was cardiopulmonary blood volume (even though total blood volume was decreased) compared to age matched control subjects and TPR was inappropriately normal in the borderline EH group [41]. The subjects with borderline EH had increased pressor responsiveness to norepinephrine which correlated with basal levels of CO and thus indicated that they had enhanced venous sympathetic tone [41]. The increased cardiopulmonary blood volume (and hence CO) could have been the result of translocation of blood from the venous vasculature as a consequence of augmented venous sympathetic tone (which in turn may have been due to a greater venous sensitivity to vasoconstrictors).

If the change in DBP (ΔDBP) following graded norepinephrine infusions is measured, and a curve of the ΔDBP vs. dose of norepinephrine is constructed, then the dose of norepinephrine required to elevate DBP by a given amount can be determined. This "pressor dose" (PD) is commonly used in human subjects as a measure of vascular reactivity [41]. Therefore, in order to be able to make direct comparisons with studies conducted in humans with borderline EH, a similar technique was used in rats (i.e. determining the PD_{25} of phenylephrine). Since phenylephrine is predominantly an α agonist and lacks any significant β₁ stimulatory activity, it was considered to be better suited than norepinephrine for the determination of an index of vascular reactivity (PD_{25}). The dose response curves did not show any difference in pressor responsiveness to phenylephrine between ABG-TsU hypertensive rats and their control group. "Normal" PD_{25} in ABG-TsU hypertensive rats was inappropriate (as was "normal" TPR) since in response to elevated BP there should have been a
compensatory fall in sympathetic tone as well as down-regulation of α receptor numbers leading to diminished pressor responsiveness. ABG-TsU hypertensive rats were originally healthy normotensive Sprague-Dawley rats which presumably had inherited normal compensatory cardiovascular reflex mechanisms for buffering elevations in BP. Aldosterone (in physiological concentrations) has been shown to increase vascular reactivity [see Introduction (E)(vii)], decrease baroreceptor sensitivity [see Introduction (E)(vi)], and decrease the extraneuronal uptake (uptake 2) of norepinephrine [see Introduction (E)(vii)]. Since ABG-TsU induced hypertension was aldosterone dependent, perhaps these effects of aldosterone participated in the hypertensive process, in addition to the inotropic actions of aldosterone leading to increased stroke volume and hence CO [Introduction (E)(v)]. The lack of any change in TPR or PD$_{25}$ in ABG-TsU hypertensive rats is puzzling. One could speculate that during the course of ABG-TsU administration some compensatory vasodilation and down-regulation of α receptors occurred whose effects on TPR and PD$_{25}$ were however countered by a decrease in baroreceptor sensitivity, and diminished extraneuronal uptake (uptake 2) of norepinephrine.

A new animal model of hypertension, which resembles borderline EH both hormonally and hemodynamically, has thus been developed with the administration of a homologue of ABG. In the light of previous studies [see Introduction (G)(vii)] which show that ABG has an increased binding capacity for aldosterone in 52% of the population with EH, an increase that is inherited as an autosomal dominant trait, the data on ABG-TsU induced hypertension point to a possible etiological role for ABG in a subgroup of subjects with EH.
Apart from the obvious species difference, ABG-TsU induced hypertension differs from human EH in one other very important respect: Sprague-Dawley rats are genotypically normotensive whereas humans predisposed to developing EH are probably genotypically hypertensive and therefore several underlying genetic abnormalities in humans (but not in Sprague-Dawley rats) may have been inherited (one of which is the augmented ABG binding capacity for aldosterone) which are all conspiring to elevate BP. Do humans predisposed to developing EH have optimally functioning mechanisms for buffering BP elevations? Or do abnormalities in these compensatory mechanisms give any hypertensive stimuli unchecked opportunities to induce hypertension? It is obvious that the Sprague-Dawley rat is in full possession of intact compensatory systems which act to buffer the effects of hypertensive stimuli. Part of the effect of ABG-TsU on BP may however be due to effects which result in blunted compensatory responses to the hypertension induced by ABG-TsU (i.e. decreased baroreceptor sensitivity, diminished release of hypotensive factors such as kinins and prostaglandins).

The inherited increase in ABG binding capacity for aldosterone in EH did not appear to simply be a quantitative change in ABG. ABG-TsU isolated from EH patients having augmented ABG binding capacity for aldosterone appeared to differ qualitatively from ABG-TsU isolated from normotensive or hypertensive subjects having normal ABG binding capacity. The first experiments in which hypertension was induced in Sprague-Dawley rats by ABG-TsU administration were conducted using ABG-TsU (POOL 1, POOL 2) isolated from the pooled urines of a large number of subjects [196, 197]. Doses of ABG-TsU of only 2μg/100g (POOL 1) and 25μg/100g (POOL 2) induced hypertension within two weeks [196, 197]. In subsequent studies
ABG-TsU was isolated from individual normotensive (NTN), renovascular hypertensive (RHN), or essentially hypertensive (EHN) subjects having either normal (NTN, RHN, EHN), or high (EHH) plasma ABG binding capacities for aldosterone. ABG-TsU isolated from NTN failed to increase BP within a two week treatment period in doses from 25 µg/100g to as high as 200 µg/100g [i.e. 100 times greater than the dose of ABG-TsU(POOL 1) previously used to induce hypertension]. Since all of the isolation procedures as well as the protocols carried out in rats were identical, it must be concluded that ABG-TsU (NTN) lacked hypertensinogenicity, and therefore ABG-TsU(POOL 1) was infinitely more potent than ABG-TsU (NTN). ABG-TsU isolated from RHN and EHN, administered for two weeks at a dose of 10 µg/100g, failed to affect BP and was therefore devoid of hypertensinogenicity. However, ABG-TsU isolated from EHH induced hypertension within two weeks when an equivalent dose (10 µg/100g) was administered. The significant difference between NTN, RHN, EHN, and EHH was that the EHH subjects had elevated plasma ABG binding capacity for aldosterone. It is possible that increased ABG binding capacity for aldosterone represent a qualitative difference (and not simply a quantitative one) conferring hypertensinogenicity to ABG-TsU through a greater affinity for aldosterone. The subjects in the EHH group may be genotypically different in respect to the expression of a hypertensinogenic ABG phenotype.

A human CBG variant with decreased affinity for cortisol has been isolated [260]. Though physicochemically similar to normal CBG, this variant showed microheterogeneity in isoelectric focussing giving two bands (pI=5.48, 5.53), unlike normal CBG which gave only one band (pI=5.48). Such a variant with lower affinity could have resulted from a genetic mutation resulting in a single amino acid substitution or a variable amount
of sialic acid residues [260]. Hypertensinogenic ABG-TsU isolated from pooled human urine displayed microheterogeneity in isoelectric focusing with two bands; one major (pI=4.76) and one minor (pI=4.80). The possibility exists that one of these bands (the minor one?) represents a hypertensinogenic ABG-TsU variant with increased binding affinity for aldosterone originating from subjects with increased plasma ABG binding capacity for aldosterone (EHH). However it is unknown if one or both isoelectric forms of ABG-TsU were present in the individuals (NTN, RHN, EHN, EHH) studied and therefore, until this question is addressed experimentally, one can only speculate as to whether or not one of the isoelectric variants of ABG-TsU is only found in some subjects with EH.

If, as was found in the Framingham Study, 40% of men and 48% of women aged 32-64 years are hypertensive [5], and if 90% of hypertensives have EH [3], and if 52% of subjects with EH have increased ABG binding capacity for aldosterone [191], then 19-23% of the population aged 32-64 years should have inherited augmented ABG binding capacity for aldosterone as part of a predisposition to developing EH (EHH). (This figure may even be slightly higher since not all individuals who were predisposed to developing EH would have presented with high BP until later than the age of 32 years, and thus would not have been included in the hypertensive group [5]). Urine pooled from apparently healthy hospital personnel (several regular contributors were known to have EH) was bound to have included some subjects (19-23% of total) with increased ABG binding capacity for aldosterone and thus would explain why ABG-TsU(POOL 1, POOL 2) was hypertensinogenic. Containers for the collection of urine were left in washrooms along with a notice urging people to donate urine for a study on hypertension. It is very possible that individuals that had hypertension (or
a family history of hypertension) were more motivated donors, and therefore pooled urine contained a greater than expected proportion of urine from donors with increased ABG binding capacity for aldosterone.

The hypertension induced by ABG-TsU was aldosterone dependent, but the bound aldosterone (and not free aldosterone) appeared to be hypertensinogenic. This raised the possibility that protein bound steroids can be physiologically active, and therefore the importance of circulating steroid binding proteins must be examined. The presence of the plasma steroid binding proteins appears to be necessary for life. Absence of CBG could not be found after screening over 10,000 individuals [182] and the lack of CBG was a lethal mutation [260]. Likewise, deletion of the specific high affinity plasma vitamin D binding protein was a lethal mutation [261]. The physiological implications of the binding of steroids to specific high affinity plasma proteins remains controversial. The classically accepted role of steroid binding proteins was considered to be that of circulating reserves of hormone since only free protein unbound hormone was thought to be physiologically active (i.e. interaction of a physiologically active steroid with serum proteins resulted in inhibition of hormone bioactivity) [183]. Rosner suggested several possibilities for the CBG binding of cortisol: (1) only unbound cortisol was available to target tissue cells, (2) only bound cortisol was available to target tissue cells, (3) whether bound or not all cortisol was equally available to target tissue cells, and (4) tissues responded to cortisol, bound or unbound, or both, depending on the given tissue [182]. Albumin bound thyroid or steroid hormones were selectively transported into peripheral tissues such as brain, and the greater the capillary transit time, the greater the amount of hormone which was dissociated from the plasma
protein and entered the target tissue cell [262, 263]. However, sex hormone binding globulin (SHBG) and CBG selectively delivered hormones to liver cells and restricted entry of hormones into peripheral tissues such as brain [262, 263].

Steroid binding proteins however can enter the target tissues of their ligands. SHBG has been found to enter androgen responsive cells [264], and CBG has been found in certain cells of the rat anterior pituitary (but not in the intermediate lobe or posterior pituitary) [265]. It was postulated that the CBG molecules participated in the cellular uptake of corticosterone, and thus modulated the feedback signal of corticosterone on the pituitary release of ACTH [265]. Rat tissues were shown to take-up $^{125}$I-labeled CBG, and uptake was enhanced by coadministration of corticosterone which suggested that cellular uptake of corticosterone-CBG complexes occurred in vivo [266]. In vitro uptake of CBG by rat GH$_3$ cells was also enhanced by the addition of corticosterone [266]. The dosages of cortisol or prednisolone (both bind CBG) required to treat chronic inflammatory conditions in humans could be reduced 3 to 20 fold by concomitant administration of estrogen which had induced a 2 to 3 fold increase in CBG levels. It was suggested that this was due to the increased biological activity of bound cortisol (or prednisolone) since free steroid levels were considerably reduced [266].

Siiteri has proposed an alternative model of steroid hormone action based partly on the above data [266]. According to the proposed model, steroid hormones bound to high affinity plasma binding proteins (such as CBG and SHBG) are recognized (as steroid-protein complexes) by target tissue cells and bound to the outer surfaces of their cell membranes. The bound complexes are then internalized and distributed to subcellular compartments, including the nucleus [266]. It is tempting to speculate that
certain tissues which respond to plasma protein bound steroid are affected very differently than when stimulated by the free fraction of the steroid. A free circulating steroid acts at the cellular level by first binding to a cytoplasmic receptor, following which the steroid-receptor complex translocates to the nucleus eventually resulting in the production of a steroid induced protein. If, following entry into the cell, a steroid bound to its high affinity plasma binding protein translocates to the nucleus as a complex and binds to a different site on the nuclear chromatin than the steroid-cytoplasmic-receptor complexes do, then a different steroid induced protein would be produced. In this manner a steroid could have dual but distinct actions (in either the same or separate target tissues) through effects mediated either via circulating receptors (steroid binding proteins) or through cytoplasmic steroid receptors. Perhaps only certain tissues would respond to the plasma protein bound hormone, whereas others respond to free hormone. One could postulate, for example, that epithelial tissues respond best to free aldosterone, whereas vascular smooth muscle cells and myocardial cells respond better to ABG bound aldosterone.

Aldosterone receptors, clearly unrelated to renal mineralocorticoid receptors, have been identified in non-epithelial aldosterone target tissues such as vascular smooth muscle and brain [Introduction (E)(ix)]. It is not known if these receptors bear any resemblance to ABG. However ABG (or ABG-TsU) could be increasing BP by effects of aldosterone-ABG complex uptake into vascular smooth muscle, the myocardium, or arterial baroreceptors [all of these tissues respond to aldosterone by increasing BP, see Introduction (E)(v, vi, vii, viii)]. Aldosterone-ABG complexes could also increase BP by actions on the central nervous system [aldosterone receptors in the CNS have been implicated in the etiology of mineralocorticoid
dependent hypertension, see Introduction (E)(ix)]. The uptake of ABG-aldosterone complexes into veins and venules could provide the mechanism whereby venous tone and hence central blood volume could be elevated in ABG-TsU induced hypertension.

How does ABG-TsU (or ABG) cause BP to increase? The fact that ABG (and ABG-TsU) bind dehydroepiandrosterone-sulfate (DHEA-SO₄) reversibly and with high affinity in addition to binding aldosterone (at different binding sites) may be an important clue. DHEA-SO₄ is a major secretory product of the adrenal gland whose plasma concentrations in adults exceed those of any other steroid, yet its biological function remains a mystery [267]. Vasdev has recently reported that in the plasma of healthy normotensive volunteers, DHEA-SO₄ accounted for 62-100% of circulating digitalis like factors (DLF) [267, 268]. In a dose dependent fashion, both DHEA-SO₄ and human plasma DLF displaced digitalis from digitalis antibody, inhibited hog brain Na⁺/K⁺-ATPase, and displaced ouabain from Na⁺/K⁺-ATPase [267, 268]. Upon further purification of plasma DLF extracts, it was found that 80% of the material with DLF activity contained in these extracts was DHEA-SO₄ [267,268]. In human EH the secretion rate of DHEA-SO₄ was shown to be increased six fold (compared to normotensive control subjects) resulting in increased circulating levels of DHEA-SO₄ [267, 269, 270]. The endogenous digoxin-immunoactive factor in human subjects was found to exist tightly but reversibly bound to protein(s) in plasma [271]. Could this be ABG bound DHEA-SO₄? The activity of an endogenous Na⁺/K⁺-ATPase inhibitor has been measured directly in human plasma [273]. The activity of this Na⁺/K⁺-ATPase inhibitor was higher in subjects with EH (than in normotensive controls lacking any family history of hypertension), and was
also significantly higher in the normotensive offspring of EH patients than in controls [273].

It is possible that aldosterone-ABG-DHEA-SO₄ complexes are taken up into certain tissues (DHEA-SO₄ concentrations in rat brain were 20 times greater than in plasma, and digoxin-like immunoreactivity was 70 times greater in heart than in plasma [267]). It is also possible that in subjects with EH, whose ABG binding capacity for aldosterone is elevated, augmented target tissue uptake of ABG-steroid complexes occurs. Since aldosterone and DHEA-SO₄ bind to different sites on ABG [184], an equal amount of both steroids would be presented to target tissue cells. Upon entry into either vascular smooth muscle or myocardial cells, the ABG bound aldosterone could cause increased passive sodium permeability [aldosterone has been shown to increase cellular Na⁺ permeability as well as to stimulate Na⁺/K⁺-ATPase to maintain normal cell [Na⁺], see Introduction (E)(viii)]. The concomitant entry of bound DHEA-SO₄ into these cells could result in DHEA-SO₄ mediated inhibition of Na⁺/K⁺-ATPase (offseting compensatory aldosterone mediated stimulation of Na⁺/K⁺-ATPase). The combined effects of increased Na⁺ permeability and inhibition of Na⁺/K⁺-ATPase would result in an elevation in cellular Na⁺ content which could result in increased intracellular [Ca²⁺] and hence increased contractile activity in arterial, venous, and cardiac muscle [see Introduction (D)(ii)]. This proposed mechanism whereby ABG and ABG-TsU elevate blood pressure is of course highly speculative.

Daniel has proposed criteria for the establishment of etiology in hypertension [272]:

"To be established as a causal mechanism for hypertension, a putative functional change should: (a) precede the hypertension; (b) not be reversed by reversal of
hypertension unless the procedure for reversal operates directly on the causal mechanism; (c) provide a plausible and (optimistically) testable basis for initiation of hypertension; (d) produce hypertension when, if possible, applied experimentally; and (e) lead to hypertension reversal or avoidance whenever withdrawn or prevented. As yet, no proposed mechanism goes far toward satisfying such criteria.

Augmented ABG binding capacity for aldosterone may be a factor in the etiology of essential hypertension whose role may meet many of the above criteria: (a) Increased ABG binding capacity for aldosterone is inherited (in 52% of subjects with EH) as an autosomal dominant trait in families with a history of EH. The trait is expressed already in early childhood, long before hypertension develops [191, 192]. (b) Neither dietary sodium restriction nor antihypertensive drugs suppress the plasma ABG binding capacity for aldosterone, except in the case of spironolactone which competitively displaces aldosterone from ABG and thus lowers the ABG binding capacity for aldosterone [191, 192, 193]. (c) The mechanism whereby ABG increases BP is unknown, but it is known that the interaction of ABG with aldosterone is an essential component [196, 197]. Increased ABG binding capacity for aldosterone is found only in a subgroup of subjects with EH (52%), and is not modified in secondary forms of hypertension [191]. Estrogen induced increases in ABG binding capacity for aldosterone were linked to augmented MAP which indirectly pointed to a role ABG in BP regulation and hypertension [195]. (d) The urinary homologue of ABG (ABG-TsU) induces hypertension when administered to Sprague-Dawley rats, this hypertension resembling borderline essential hypertension both hormonally and hemodynamically after two weeks of ABG-TsU administration [196, 197]. It appears that only ABG-TsU isolated from subjects with essential hypertension having increased ABG binding capacity for aldosterone is
hypertensinogenic. (e) When the interaction between ABG-TsU and aldosterone is prevented (either by adrenalectomy or spironolactone treatment) hypertension is prevented, and BP normalizes following the withdrawal of ABG-TsU.

ABG may therefore be of etiological importance in that subgroup of essentially hypertensive subjects having increased ABG binding capacity for aldosterone.

(D) MINERALOCORTICOID CONTRIBUTION TO THE INITIATION OF HYPERTENSION IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR):

At twelve weeks of age, SHR were very hypertensive compared to 12 week old WKY control rats. Both PRA and total plasma aldosterone concentrations were significantly lower in SHR than in WKY rats, as others have also reported [222]. These values for plasma aldosterone in the WKY rat were considerably higher than those we have measured in Sprague-Dawley rats [197] in whom levels of aldosterone did not differ from those we and others [239] have observed in 12 week old SHR. Several authors have reported that, in relation to age matched WKY rats, SHR at 6, 12, and 22 weeks of age had decreased aldosterone responses to angiotensin II [237, 274] resulting in a compensatory increase in the activity of the renin-angiotensin system [274]. If this had also been the case in our study comparing 12 week old SHR and WKY rats, we should have observed a decrease in aldosterone concentrations relative to the level of PRA (i.e. a decrease in the ratio of total aldosterone/PRA). This was not the case, since no significant difference in the ratio of total aldosterone/PRA existed between SHR and WKY rats. A significant decrease in the plasma ABG
binding capacity for aldosterone was observed in 12 week old SHR (compared to WKY rats of the same age). These results contrasted with those of Markov, who demonstrated that in young SHR (6 weeks of age) the plasma ABG binding capacity for aldosterone was significantly greater than in control rats, and that this difference disappeared by six months of age [231]. Our observation of lower ABG binding capacity in SHR than in WKY rats (at 12 weeks of age) could have been due to the age difference between our SHR and those of Markov, or possibly due to a substrain difference between our SHR and those of Markov.

The appropriateness of the WKY rat as a control for the SHR has been questioned, and it has been suggested that the Sprague-Dawley rat is a better control for the SHR [239]. BP in WKY rats was on average 10 mmHg higher than BP in Sprague-Dawley rats from birth to four months of age [239]. Adrenocorticoid hormone levels, organ weights, and body weights varied considerably in WKY rats from shipment to shipment, season to season, and depending from which commercial breeder they were obtained [239]. These inconsistancies made it difficult to obtain reproducible and therefore meaningful results. As the authors pointed out [239]:

"The passage of time and global disbursement of SHR have given rise to many substrains of SHR to compound the confusion. Our research into the pathophysiology of SHR hypertension is better served by comparison with extensive well established, and highly reproducible data of all kinds gathered from normotensive Sprague-Dawley rats. Data gathered from normotensive WKY have been fraught with inconsistency. The appropriateness of WKY rats as the exclusive normotensive counterpart to SHR has become a moot question"

The issue is further complicated by the fact that the SHR and its control strain, the WKY rat, were not developed simultaneously (though they did originate from the same colony of Wistar rats in Kyoto, Japan) [205].
The role of mineralocorticoids in the development and maintenance of hypertension in the SHR was investigated at 7, 10, and 12 weeks of age by continuously administering an aldosterone antagonist (a spironolactone) for one or two weeks in doses of either 1.5 mg/rat/day or 5.0 mg/rat/day. The rate of rise of BP in the SHR was greatest from 7 to 9 weeks of age, reaching near maximal levels by 12 weeks of age (SBP rose by 50 mmHg from 4 to 8 weeks of age, went up a further 20 mmHg from 8 to 12 weeks of age, and climbed by only 15 mmHg from 12 to 20 weeks of age [238]). Treatment with a spironolactone (Soldactone, 1.5 mg/rat/day for two weeks) starting at 7 weeks of age completely halted the rise of BP with age in SHR. During the same time period (7 to 9 weeks of age) BP rose by 30 mmHg in untreated SHR (sham implanted controls). Treatment of SHR with Soldactone, in doses as high as 5.0 mg/rat/day, starting at 10 or 12 weeks of age failed to have any effect whatsoever on BP (compared to age matched untreated SHR). The fact that spironolactone interfered with the development of hypertension but did not lower BP once the hypertension was more fully established indicated that mineralocorticoids contributed to the initiation but not to the maintenance of hypertension in the SHR. By definition, the existence of spironolactone responsive hypertension indicated that mineralocorticoids played a pathogenic role [Introduction (F)], since spironolactones only lowered BP when the BP increase was caused by mineralocorticoids [151].

Adrenalectomy (ADRX) at 7 weeks of age in SHR resulted in BP being reduced to normotensive levels (118/89 mmHg) by 9 weeks of age. Administration of physiological amounts of aldosterone to ADRX SHR, from 7 to 9 weeks of age, (ADRX+ALDO) restored BP to hypertensive levels (167/116 mmHg) which were considerably higher than those similarly measured in WKY rats (127/97 mmHg) but were, however, lower than those
observed in 9 week old sham operated (SHAM) SHR (206/141 mmHg). No signs of mineralocorticoid lack or excess were apparent in ADRX+ALDO SHR at 9 weeks of age since free plasma aldosterone, plasma K⁺, and PRA did not differ from the values obtained in the SHAM rats. Therefore, the amount of aldosterone administered closely approximated what the adrenal gland would have been secreting. At least half of the adrenal component of spontaneous hypertension during its initiation appeared to be due to the effects of aldosterone. That aldosterone did not maintain hypertension in the SHR supports the observations made by other authors that aldosterone substitution (even in pharmacological doses) in adrenalectomized SHR starting at 10 weeks of age had no significant effect on BP [227], and that administration of physiological amounts of aldosterone to adrenalectomized SHR starting at 12-13 weeks of age also failed to increase BP [228].

Interestingly, in the experiments presented in this thesis spironolactone treatment begun at these ages (10 and 12 weeks of age) failed to affect BP.

The BP normalizing effect of adrenalectomy in the SHR was entirely due to the removal of the adrenal cortex, since it has been shown that bilateral adrenal medullectomy failed to prevent the development of hypertension in the SHR [224]. The BP lowering effect of adrenalectomy in SHR was not a phenomenon common to other rat species. In both WKY and Sprague-Dawley rats, adrenalectomy or adrenalectomy with aldosterone replacement did not result in any change in BP with time (from approximately 7 to 9 weeks of age) compared to sham operated rats [225, 226]. This strongly supports the conclusion that adrenalectomy lowers BP in young SHR through direct interference with pathophysiological mechanisms responsible for the development of hypertension.
Unlike the cat and dog (and man), the rat does not need corticosteroid replacement therapy to recover following adrenalectomy [275]. However, replacing drinking water with a physiological saline solution is necessary to ensure the survival of adrenalectomized rats [275]. As long as saline was given to drink, BP did not drop in Wistar rats following adrenalectomy, and one week after the operation no difference in BP could be detected between the adrenalectomized rats (drinking saline) and sham operated controls [275]. If, however, rats were given only water to drink, then one week following adrenalectomy rats showed signs of adrenocortical insufficiency including low BP [275]. In the studies conducted by this author as well as in those described above [225, 226], all adrenalectomized rats were given a physiological saline solution to drink post-operatively. Thus any drop in BP in SHR drinking saline post-adrenalectomy should not be due to global adrenocortical insufficiency, but probably due to the withdrawal of some specific hypertensinogenic adrenocortical factor.

The results reported in this thesis agree with those of others [225, 226] that administration of aldosterone to 7 week old adrenalectomized SHR restores hypertension. The amount of aldosterone administered in the present study to adrenalectomized SHR (2μg/rat/day) did not cause suppression of PRA when compared to SHAM SHR, as did the supraphysiological amounts of aldosterone used in previous studies (10μg/rat/day) [225, 226]. Morris et. al. [276] and Nowaczynski, M et. al. [277] have both reported (at the same meeting) that administration of physiological amounts of aldosterone to young adrenalectomized SHR (ADRX+ALDO) restored the development of hypertension. In addition, Morris reported that (in both SHR and normotensive rats) the administration of normally circulating hepatically and renally reduced metabolites of
aldosterone (5α-dihydroaldosterone; 3α, 5α-tetrahydroaldosterone; 3α, 5β-
tetrahydroaldosterone) also restored hypertension when administered to
adrenalectomized SHR starting at 6 weeks of age [276]. The
hypertensinogenic potencies of these reduced metabolites of aldosterone
were greater than their renal mineralocorticoid potencies [276] which
suggested that these metabolites increased BP through extra-renal
mechanisms.

As expected, SHR that were sodium loaded from 7 to 9 weeks of age
(SHAM) had significantly suppressed total plasma aldosterone levels at 9
weeks of age when compared to age matched SHR drinking water (Group 1
controls). BP, however, was not significantly higher in the sodium loaded
SHR (SHAM) than in those drinking water (Group 1 controls). Total plasma
aldosterone levels in ADRX+ALDO rats were slightly but not significantly
lower than those in intact age-matched SHR drinking water (Group 1
controls), which further indicated that the amount of aldosterone
administered to the ADRX+ALDO SHR were indeed physiological since no
signs of hyperaldosteronism were present.

The still detectable levels of plasma aldosterone (the half life of which
may have increased significantly as a consequence of the very large
elevation in ABG binding capacity for aldosterone) present in SHR two weeks
following adrenalectomy (ADRX group) may have possibly been partly due to
the presence of extra-adrenal cortical islands scattered around the
periadrenal and perirenal areas which have been previously described in
rats [227, 278, 279]. Extra-adrenal cortical islands were shown to secrete
copious quantities of corticosterone in SHR that had been adrenalectomized
at 10 weeks of age [227]. SHR without signs of extra-adrenal cortical islands
did not develop hypertension and remained normotensive [227]. A
significant correlation between BP and plasma corticosterone concentrations following ether stress existed in SHR possessing extra-adrenal cortical islands. In addition, betamethasone (a synthetic glucocorticoid) administration in adrenalectomized SHR resulted in a large increase in BP, while aldosterone substitution starting at the same age (10 weeks) in adrenalectomized SHR failed to affect BP [227]. Corticosterone is a potent glucocorticoid and a weak mineralocorticoid (having 1/200th of the mineralocorticoid potency of aldosterone) but the glucocorticoid betamethasone is devoid of mineralocorticoid activity [280]. It thus appeared that part of the adrenocortical component of hypertension in the SHR (starting at 10 weeks of age) was due to the permissive role of glucocorticoids in the development of hypertension [227]. However, during the initiation of hypertension in the SHR (from 3 to 15 weeks of age) plasma corticosterone levels were significantly lower than in age matched WKY control rats [232, 234, 236]. Neither did corticosterone appear to be important in the maintenance of hypertension in the adult SHR, since at 6 months of age no significant differences in plasma corticosterone levels (at rest) nor differences in the diurnal variation of corticosterone could be detected between SHR and normotensive control rats [281]. The response in plasma corticosterone levels to stress in SHR was not increased, and in some cases was even blunted compared to that observed in normotensive control rats [281].

Circulating aldosterone concentrations rose with age in SHR (from 3 to 16 weeks of age) while it declined progressively in WKY rats during the same time period [232]. By 8 weeks of age, plasma aldosterone levels in SHR were twice those measured in WKY rats [232]. This hyperaldosteronism occurred during the progressive development of hypertension in the SHR, suggesting that aldosterone was important in the genesis of the hypertension
Other investigators were however unable to demonstrate that hyperaldosteronism occurred in young SHR during the developmental phase of the hypertension [see Introduction (H)(iii)] [234, 236]. Other mineralocorticoids, such as 18-OH-deoxycorticosterone (18-OH-DOC) and deoxycorticosterone (DOC), were not found to be elevated in the plasma of SHR from 8 to 20 weeks of age (compared to age matched WKY rats) [234, 236]. The search for other possible mineralocorticoid hyperfunction revealed that in prehypertensive SHR (up to 6 weeks of age) the urinary excretion of 19-nor-DOC was considerably higher than in WKY rats of the same age [282]. After 6 or 7 weeks of age (after which time the development of hypertension began in earnest) no difference in 19-nor-DOC excretion could be detected between SHR and WKY rats [282]. It would appear therefore that aldosterone is the best candidate as the most important hypertensinogenic adrenocortical hormone involved in the development of hypertension in the SHR [Introduction (H)(iii)].

In 12 day old prehypertensive SHR the circulating levels of aldosterone were increased (compared to 12 day old WKY rats) as were total body water and extracellular fluid volume [208]. This increase in extracellular fluid volume was apparently due to a selective expansion of the interstitial fluid volume since plasma volume was normal. Spironolactone administration corrected this apparently aldosterone mediated increase in interstitial fluid volume [208]. In normotensive adult Wistar rats, aldosterone was shown to redistribute extracellular fluid between the intravascular and interstitial compartments of nephrectomized rats (and of adrenalectomized nephrectomized rats) so as to maintain intravascular volume, which strongly suggested that the actions of aldosterone on interstitial fluid volume were extra-renal [284, 285].
During the development of hypertension in the SHR aldosterone may have contributed to the hypertensive process through extra-renal actions. Several perturbations in the mechanisms affecting BP have been described in the SHR. For example, the activity of of vascular Na⁺/K⁺-ATPase was increased in SHR resistance vessels (possibly in order to compensate for the increased vascular smooth muscle cell passive Na⁺ permeability which was observed) [213, 214, 219, 220, 283]. Vascular smooth muscle cell permeability to Na⁺ was increased in SHR but the accumulation of cell Na⁺ was prevented by increased sodium pump activity [220]. This was similar to the observed increase in vascular smooth muscle cell membrane permeability to Na⁺ observed in rats whose hypertension was induced by aldosterone administration, in whom normal cell sodium concentrations were maintained by enhanced sodium transport [see Introduction (E)(viii)] [128].

Aldosterone directly stimulated erythrocyte Na⁺/K⁺-ATPase and could cause an increase in Na⁺ extrusion from vascular smooth muscle cells [131, 57]. Adrenalectomy caused a far greater drop in erythrocyte Na⁺/K⁺-ATPase activity in SHR than in WKY control rats. However, aldosterone replacement restored the Na⁺/K⁺-ATPase activity to levels measured in intact rats [221]. In addition, as in aldosterone induced hypertension, it was found that the turnover of K⁺ and Cl⁻ was increased in SHR vascular smooth muscle cells [216]. It is possible that the similarities in cellular ion transport abnormalities observed in aldosterone induced hypertension and those seen in the SHR indicate a role for aldosterone in the pathogenesis of hypertension in the SHR.

The vascular effects of aldosterone may be receptor mediated since cultured SHR aortic cells contained cytoplasmic receptors which bound aldosterone reversibly and with high affinity [283] and were apparently
similar to previously described vascular aldosterone receptors [see Introduction (E)(ix)] [135-139]. Aldosterone increased the sensitivity of vascular smooth muscle to the vasoconstrictor effects of norepinephrine [see Introduction (E)(vii)] and aldosterone (in physiological concentrations) decreased the extra-neuronal uptake of norepinephrine (uptake 2) by 25% (resulting in vasoconstriction) and greatly augmented vascular relaxation time [122, 123]. In vascular smooth muscle cells obtained from the resistance vessels of SHR (6 and 12 weeks of age), the sensitivity to norepinephrine was greater than that of WKY rat vessels [286]. The overflow of norepinephrine from mesenteric arterial beds (following electrical nerve stimulation) was greater in 7 to 8 week old SHR than in age matched WKY rats, and greater than in adult SHR (20-22 week old) [287]. Both increased release of norepinephrine from sympathetic nerve endings and decreased neuronal and extra-neuronal uptake of norepinephrine may have contributed to the augmented overflow of norepinephrine observed in 7-8 week old SHR [287].

The restoration of BP to hypertensive levels in ADRX+ALDO SHR may have partly been due to the very large increase in ABG bound aldosterone. The ABG binding capacity for aldosterone was greatly augmented in ADRX+ALDO SHR (compared to SHAM) while the ABG-Ts binding capacity for aldosterone was not affected. The physiological significance of relative changes in thermolabile (ABG) versus thermostable (ABG-Ts) bound aldosterone are unknown. The small increase in total plasma aldosterone (but not in free aldosterone) and the elevated plasma ABG binding capacity for aldosterone observed in ADRX+ALDO SHR has also been observed in EH (and in the 6 week old SHR) [174, 231].
Since the hypertension of the young (7-9 week old) SHR responded to the same interventions that prevented ABG-TsU induced hypertension (spironolactone, ADRX, but not ADRX+ALDO) it is possible that similar mechanisms may have been partly responsible for elevating BP in both forms of hypertension. Aldosterone (and ABG) may be a common link in the development of EH, the spontaneous hypertension of the SHR, and ABG-TsU induced hypertension.
References


[101] Wenting GJ, Man In 't Veld AJ, Verhoeven RP, Derkx FHM, Schalekamp MADH: Volume-Pressure Relationships During Development of


Hypertension: Mechanisms and Management, Philipp Th. Dister A (editors), Springer-Verlag, Berlin, 1980, pg 207.


[267] Vasdev S, Longerich L, Johnson E, Brent D, Gault MH: Dehydroepiandrosterone Sulfate as a Digitalis Like Factor in


PUBLICATIONS:


ABSTRACTS:


Lioy F, Nowaczynski M, Nowaczynski WJ: Hemodynamic characterization of aldosterone binding globulin (ABG) induced hypertension in the rat. (29th International Congress of the Union of Physiological Sciences, Sydney, Australia, August 1983).
Lioy F, Nowaczynski M, Nowaczynski WJ: Hemodynamic characterization of aldosterone binding globulin (ABG) induced hypertension in the rat. (Research poster at B.C. Heart Foundation Annual Cardiac Symposium, Vancouver, 1984)

Nowaczynski M, Lioy F, Nowaczynski WJ: Mineralocorticoids initiate hypertension in the spontaneously hypertensive rat (SHR). (Sixth Scientific Meeting of the Inter-American Society of Hypertension, Cleveland, Ohio, May 1985, Abstracts pg. 57)
