In Vivo Assessment of the Contribution of α_1- or ß-Adrenoceptor Stimulation in the Development of Dietary Sodium-Induced Cardiac Hypertrophy in Rats

1. **Effects of Dietary Sodium on Hemodynamic Responses to Phenylephrine and Isoproterenol**
2. **Effects of α_1- and ß-Adrenergic Blockade on Dietary Sodium-Induced Cardiac Hypertrophy**

by

DONGJIANG SONG

Department of Pharmacology
Faculty of Medicine
University of Ottawa
Ottawa, Ontario, Canada

A thesis submitted in conformity with the
requirements of the degree of Master of Science
at the University of Ottawa

ISBN 0-612-15677-X

UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

TO MY DEAR PARENTS

and MY LOVELY WIFE AND DAUGHTER

ABSTRACT

Background: Previous studies showed that high sodium diet induces left ventricular hypertrophy (LVH) in rats, without increasing resting arterial blood pressure (BP), LV filling pressures or cardiac sympathetic activity. However, rats on high sodium diet showed more frequent and higher BP and heart rate (HR) fluctuations in the night associated with enhanced water intake, and an increase in LV adrenoceptor density or affinity, or in post-receptor pathway activity. Besides, normotensive humans on high sodium diet showed increased α_1- or ß-adrenergic responses.

Hypothesis: High sodium diet in rats may induce intermittent sympathetic activation through increasing nighttime water intake, and may enhance cardiac adrenergic responses via an increase in adrenoceptor activity, which may contribute to the development of high sodium diet-induced LVH.

Method: In conscious WKY rats, resting hemodynamics and after blocking baroreflex by hexamethonium (Hex), hemodynamic responses to α_1-, or ß-agonist (phenylephrine, Phe, or isoproterenol, Iso), and then ventriclular weights and LV dimensions were determined after 1, 2, 4 or 6 *wk* medium high or high sodium diet (2 or 8% NaCl) with or without *ig* administration of α_1- or ß-blockers (60 *mg/kg/day* terazosin, Tz, 25 or 100 *mg/kg/day* nadolol, Nd, alone or both Tz and Nd).

Results: Medium high or high sodium diets for 2 to 6 *wk* increased the relative LV dry weight and LV wall thickness to radius ratio. Except for an inconsistent HR increase, resting hemodynamic parameters, such as BP, LV peak-systolic or end-diastolic

pressures (LVPSP or LVEDP), right atrial pressure, cardiac index, stroke volume or total peripheral resistance indices were not changed by the salt exposure. Resting LVPSP was decreased by Tz with Nd only in high sodium diet group. The BP decrease by Hex was augmented by the salt exposure. 6 *wk* high sodium diet augmented the HR decrease by Phe, but attenuated the MAP decrease by Iso. Although markedly antagonizing the hemodynamic responses to Phe or Iso, chronic Tz and/or Nd did not prevent the salt-induced LVH. Instead, the LVH was aggravated by Nd alone or Nd with Tz. However, the salt-increased LV wall thickness to radius ratio was attenuated by the single blockades. The salt-increased water intake was augmented by Tz, not changed by Nd.

Discussion: The results confirm that high sodium diet for 2 to 6 *wk* induces concentric LVH, without changing most resting hemodynamics including LV filling pressures. This study showed that the vasodilatation by ß-agonist was attenuated and negative chronotropy of α_1-agonist augmented by a longer salt exposure, which may indicate a decreased vascular $ß_2$- or an increased sinoatrial node α_1-receptor responses. Effects of ganglionic and adrenergic blockades suggest an salt-augmented sympathetic control on peripheral arterial resistance. Chronic α_1- and/or ß-blockers failed to prevent the high sodium diet-induced LVH, which suggests that adrenoceptor activation does not play a primary role in evoking this LVH. However, the blockers shifted the LVH from a concentric to an eccentric form, and Tz augmented the salt-increased water intake. It is plausible that a volume overload-induced hypertrophy associated with the blockers may have masked a reduction in the high sodium diet-induced LVH. Therefore, further work needs to be done to clarify the effects of a high sodium diet on cardiac sympathetic activity, adrenoceptor responses and central hemodynamics during night.

ACKNOWLEDGEMENTS

Special thanks to Drs. B. Yuan and M. Ruzicka for their helpful instructions in hemodynamic and anatomical measurements and in data processing, To Dr. B. Huang for his encouraging and instructive discussions, and to Drs. Leenen and Harmsen for their excellent guidance throughout my studies at the University of Ottawa Heart Institute, particularly, on my research protocol, literature review and thesis writing.

Financial support was partly from Heart and Stroke Foundation of Ontario.

Terazosin was a gift from Abbott Laboratories (Abbott Park, IL, USA). Nadolol was a gift from Bristol-Myer Squibb (Montreal, QP, Canada).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACE	angiotensin converting enzyme
Ang II	angiotensin II
ANP	atrial natriuretic peptide
AT_1	angiotensin II receptor-$_1$
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
BP	arterial blood pressure (*mmHg*)
cAMP	3',5'-cyclic adenosine monophosphate
CEC	chloroethylclonidine
CI	cardiac index (*ml/min/kg*)
CO	cardiac output (*ml/min*)
c-onc	proto-oncogenes
CREB	cAMP recognition element binding proteins
DAG	diacylglycerol
DBP	diastolic blood pressure (*mmHg*)
DNA	deoxyribonucleic acid
DOCA	deoxycorticosterone acetate
EGF	epidermal growth factor
ET	endothelin
ET_A	endothelin-1 receptor$_A$
FGF	fibroblast growth factor
Gs	stimulating GTP-binding protein
GTP	guanosine triphosphate
HR	heart rate (*beat/min*)
hr	hour(s)
2K 1C	2-kidney 1-clip (renal hypertensive rat)

ig	intragastrically
ip	intraperitoneally
IP_3	inositol trisphosphate
IP_4	inositol tetrakisphosphate
iv	intravenously
LV	left ventricle
LVEDP	left ventricular end-diastolic pressure (*mmHg*)
LVH	left ventricular hypertrophy
LVPSP	left ventricular peak-systolic pressure (*mmHg*)
MAP kinase	mitogen-activated protein kinase
MAP	mean arterial pressure (*mmHg*)
α-MHC	α-myosin heavy chain
ß-MHC	ß-myosin heavy chain
min	minute(s)
Nd	nadolol
OLA	ouabain like activity
PDE	phosphodiesterase
PDGF	platelet derived growth factor
PE	polyethylene catheter
PIP_2	phosphatidylinositol 4, 5-diphosphate
PKC	protein kinase C
PKA	protein kinase A
PLA_2	phospholipase A_2
PLC	phospholipase C
PLD	phospholipase D
PRA	plasma renin activity
RA	right atrium
RAP	right atrial pressure (*mmHg*)
RNA	ribonucleic acid

mRNA	messenger ribonucleic acid
RV	right ventricle
α-SA	α-skeletal actin
SBP	systolic blood pressure (*mmHg*)
sc	subcutaneously
SEM	standard error
SHR	spontaneously hypertensive rat
SVI	stroke volume index (*ml/beat/kg*)
T_3	triiodothyronine
T_4	thyroxine
TK	tyrosine kinase
TPR(I)	total peripheral resistance (index) (*mmHg/ml/min/kg*)
Tz	terazosin
wk	week(s)
WKY	Wistar Kyoto rat

INTRODUCTION

I. General Review of Cardiac Hypertrophy

I.1. *Initiation of Cardiac Hypertrophy*

Similar to other tissues and organs throughout the body, the heart has the capacity to respond to functional demands or stimuli by increasing or decreasing cell size or mass. Lack of function causes a rapid atrophy; in contrast, an increased load induces hypertrophy (Brown, 1971). Because of this, cardiac hypertrophy has been generally regarded as an adaptive response of the heart to increased load to maintain the normal cardiac functions. However, later on cardiac hypertrophy may interfere with the contractility and the compliance of the heart, resulting in heart failure in the end. Besides an increase in the load, cardiac hypertrophy can also be a response to the challenge of some endocrine or neuronal changes.

Commonly, cardiac hypertrophy refers to an increase in the heart mass or an increase in the ratio of the heart weight to body weight. Since myocardium consists of connective tissue, nerves, endothelial cells, as well as myocytes (sometimes named cardiocytes), cardiac hypertrophy should be considered as a combined response of those various cells, instead of resulting simply from myocyte responses. Actually, myocyte growth can be promoted or regulated by the surrounding nonmuscle cells through

paracrine secretion of various factors (Long *et al.*, 1991).

Cardiac growth can be from an enlargement of the already existing cells (cell hypertrophy), and/or from an increase in the number of cells (proliferation or cellular hyperplasia). The growth during the prenatal and early postnatal period involves proliferation of both muscle and nonmuscle cells. This early postnatal period is about three to six months for humans and 30 days for rats. After this period, cardiac growth is produced mainly by muscle cell enlargement and nonmuscle cell proliferation (Brown, 1971; Morgan and Bader, 1991).

Myocyte enlargement follows organelle hyperplasia, *i.e.* an increase in organelle numbers, instead of in their size. This is the same with mitochondria, sarcoplasmic reticulum and myofibrils. However, proliferation of the myofibrils is structurally different according to the types of loads added to the heart. In pressure overload hypertrophy, the diameter of myofibrils is increased as newly synthesized myofilaments are added in parallel to the original components; in volume overload hypertrophy, the myocyte fibres are elongated and chambers enlarged because of series addition of new sarcomeres (Grossman, 1980). Both of these changes are due to an increase in the number of myofilaments, and this increase results from an enhanced protein synthesis (Brown, 1971; Schreiber *et al.*, 1971) together with an increase in the assembly of individual contractile proteins into organized sarcomeric units (Iwaki *et al.*, 1990). With these structural changes in hypertrophy evoked by either pressure or volume overload, the orderly arrangement of cardiac myocytes is still maintained in the ventricles (Ferrans, 1984). However, the isoform of myosin, one of the six major components of sarcomeric contractile proteins, can be changed in the process of certain types of hypertrophy

(Waspe *et al.*, 1990). This change is mainly from an up-regulation of either α or ß (Marban and Koretsune, 1990) isogene of myosin heavy chain (MHC). With this change, the myosin ATPase activity will be decreased with an increase in α isogene, or increased with an increase in ß isogene (Hoh *et al.*, 1977). Another component of the contractile proteins, actin, may change its isoform during cardiac hypertrophy from α-cardiac actin to α-skeletal actin (α-SA) (Marban and Koretsune, 1990).

The stimuli responsible for cardiac hypertrophy can be physiological (such as several weeks or months of strenuous physical exercise) or pathophysiological (such as hypertension, myocardial infarction, hyperthyroidism, hypoxia or pheochromocytoma). These stimuli act as extracellular initiating signals and, via activation of certain membrane ion channels or membrane bound enzymes and their coupling intracellular mechanisms, regulate gene expression of certain contractile and noncontractile proteins. These initiating signals can be divided into three classes: mechanical (from pressure or volume overload), neural (α- or ß-adrenergic activation) and humoral or endocrine (such as thyroid hormone, endothelin and renin-angiotensin system) (Morgan and Bader, 1991). There are several different intracellular pathways known to mediate between the action of the extracellular stimuli and the increase in protein synthesis. The various intracellular pathways and their correlation with the corresponding extracellular stimuli will be discussed later. Cardiac hypertrophy can involve one or more of the cardiac chambers, depending on the characteristics and the duration of the stimulus.

Among the various factors associated with the occurrence of cardiac hypertrophy, dietary sodium has drawn special attention because of its modulating effect in the development of hypertension-induced cardiac hypertrophy (Gallo *et al.*, 1990). In both

normotensive humans and rat models, it has been demonstrated that high dietary sodium causes left ventricular hypertrophy (LVH), but the mechanisms underlying this phenomenon are still unknown (Fernandez *et al.*, 1988; Yuan and Leenen, 1991).

For a better understanding of the process and also the possible mechanism of a high sodium diet-induced LVH, we need a proper review of the established initiating factors for cardiac hypertrophy and the detailed intracellular pathways coupled to these factors.

I.2. *Initiating Factors for Cardiac Hypertrophy*

Several factors have been clearly confirmed by animal models to be able to induce cardiac hypertrophy (Morgan and Bader, 1991). These include mechanical factors, neuronal factors and other factors such as angiotensin II (Ang II) and thyroid hormones.

I.2.1. *Mechanical Factors*

I.2.1.1. ***Pressure overload***, as encountered in aortic or pulmonary stenosis, induces cardiac hypertrophy through an increase in the ventricular pressure and the resultant wall tension during systole. This type of change in hemodynamics is commonly referred to as an increase of afterload (Grossman, 1980). The cardiac hypertrophy from a pressure overload is in a concentric form, *i.e.*, a hypertrophy in the form of an increase in the chamber wall thickness or in the ratio of wall thickness to radius.

Histologically, this type of hypertrophy results from a hypertrophy of myocytes and in some models a proliferation of non-myocytes in the heart (Brown, 1971). The myocyte hypertrophy occurs further from an increase in myofibril diameter through an addition of newly synthesized myofilaments parallel to the original components as well as from a proliferation of other organelles, such as mitochondria and sarcoplasmic reticulum (Grossman, 1980). The increase in myofibril diameter is due to an increase in the number of myofilaments (Brown, 1971; Schreiber *et al.*, 1971) and an increase in the assembly of individual contractile proteins into organized sarcomeric units (Iwaki *et al.*, 1990).

In pressure overload-induced hypertrophy, isogenes for the two major components of the contractile proteins, ß-myosin heavy chain (ß-MHC) and α-SA, are up-regulated (Marban and Koretsune, 1990). These two isogenes are predominant during fetal life. Parallel to the up-regulation of these two isogenes, the cardiac secretory protein, atrial natriuretic peptide (ANP) is re-expressed in the hypertrophic ventricles (Ito *et al.*, 1994). In normal ventricles of adult mammals, ANP is usually not produced or undetectable (Aardal and Helle, 1991), while ß-MHC or α-SA only take a smaller percentage of their respective isoforms in the contractile proteins. The up-regulation of ß-MHC shifts the myosin isoform in ventricles of small mammals, for instance in mouse and rat, from an adult α-myosin heavy chain (α-MHC) predominant V_1 isoform (αα) back to a fetal or neonatal ß-MHC predominant V_3 isoform (ßß). The switch from V_1 to V_3 isoform is not detectable in larger mammals, including humans, because ß-MHC is already the predominant isoform expressed in the normal adult ventricles of these species (Nadal-Ginard and Mahdavi, 1989).

The time-course and detailed mechanism of the stimulated protein synthesis has been studied in animal models. After induction of aortic stenosis in rats, the total ventricular protein synthesis rate was doubled. The maximal synthesis rate appeared between 3 and 6 days after surgery; thereafter it declined to normal levels (Moalic *et al.*, 1981). The increase in protein synthesis can be either from improving the efficiency of the existing components of the protein synthetic pathway (defined as the amount of protein synthesized per unit amount of RNA in a specific part of the myocardium) or from expanding the capacity for protein synthesis of the myocardium (expressed as a change of RNA content in the myocardium) (Morgan *et al.*, 1987; Nagai *et al.*, 1988). The expansion of protein synthesis capacity probably starts via the activation of certain proto-oncogenes (*c-onc*) (Marban and Koretsune, 1990). The *c-onc* is a component of the normal genome and therefore more properly named immediate-early gene. Its activity can be easily detected by measuring the messenger RNA (mRNA) products transcribed from a particular *c-onc*. The protein product from *c-onc* re-enters the nucleus and binds to DNA. Some of the protein products are components of transcriptional activating factor and can regulate the expression of other genes.

I.2.1.2. ***Volume overload*** refers to another type of hemodynamic change, an increase in the preload as encountered in mitral or tricuspid regurgitation or in experimental arterial-venous fistula. Volume overload is characterized by an increase in the end-diastolic pressures. Cardiac hypertrophy induced by a volume overload shows a typical eccentric appearance (Grossman, 1980), *i.e.*, a heart weight increase without a significant increase in the ratio of chamber wall thickness to radius. In this situation, the heart

undergoes a remodelling of the structure with an increase in its chamber diameter.

Ultrastructurally, this anatomical change results from an increase in myofibrilar elongation and chamber enlargement by series addition of new sarcomeres (Grossman, 1980). In a rat model for volume overload (aortic incompetence), the protein synthesis rate is twofold higher than in control rat and this increase reaches a maximum around 2 to 3 *wk* after starting the load (Moalic *et al.*, 1981). Similar to pressure overload, volume overload-enhanced protein synthesis and later on the establishment of cardiac hypertrophy may also start with an activation of certain *c-onc* (Kolbeck-Ruhmkorff *et al.*, 1993). The possible role of *c-onc* in volume overload will be discussed in the following.

I.2.1.3. *Mechanisms of Hemodynamic Overload*

Mechanically, the extracellular stimuli can be divided into three types, those acting by increasing stretch force to the myocardium during systole such as in the form of pressure overload, those acting by increasing stress or press force to the myocardium and the ventricular walls during diastole such as in the form of volume overload, and those acting by a combination of the above two forces (Grossman, 1980).

Different forms of hemodynamic overload and their mechanical forces imposed on the heart can induce cardiac hypertrophy of different types, but the cellular effect of the hemodynamic overload, a deformation of myocytes, and the subcellular process for initiating protein synthesis may be similar (Katz, 1992). As suggested, the expansion of protein synthesis capacity in pressure overload-induced cardiac hypertrophy is probably via an activation of certain *c-onc*, such as *c-fos*, *c-myc* and *c-jun* (Marban and Koretsune, 1990). This hypothesis has been confirmed in an *in vitro* rat model. Adjusting the

perfusion cannula height to increase the afterload to the LV by one-fourth will induce a threefold increase in *c-fos* and *c-myc* mRNA expression. In this particular model, the peaks of these two *c-onc* mRNA appeared in 30 *min* and 90 *min*, respectively, which showed a sequence of *c-fos* then *c-myc* mRNA rise (Kolbeck-Ruhmkorff *et al.*, 1993).

Similar to the pressure overload, in the *in vitro* model for volume overload, increasing the atrial filling pressure to the perfused rat heart to two-fold also induces an increase in *c-fos* and *c-myc* mRNA expression. The mRNA of these two *c-onc* are increased by 2 and 3-fold, respectively. The increase starts at 30 or 60 *min* after initiating the load (Kolbeck-Ruhmkorff *et al.*, 1993).

From *in vitro* studies, it is clear that increased chamber wall tension from an overload itself can induce cardiac hypertrophy, without necessitating associated neural or hormonal factors. To circumvent the compounding interferences which usually occur with *in vivo* hypertrophy models, simplified *in vitro* models, such as perfusion of isolated heart or static stretching of cardiac myocytes, have been applied in studying cellular mechanisms of hypertrophy resulting from chamber wall tension or stress, to mimic the *in vivo* situation of pressure or volume overload. Based on the *in vitro* as well as *in vivo* studies, it has been suggested that the first intracellular process in response to the increased wall tension from a pressure or volume overload could be a modification of the levels of intracellular signalling factors. These factors might include Na^+, Ca^{2+}, 3', 5'-cyclic adenosine monophosphate (cAMP), inositol phosphate and H^+. A change of their levels could be a result of activation of certain plasma membrane cation channels, or stretch-sensitive ion channels and activation of the related membrane or cytoplasmic enzymes (Kent *et al.*, 1989). Among these factors, even though an increase in the

intracellular Na^+ from Na^+ influx could be an initial signal for a wall tension-induced cell deformation leading to hypertrophy, the most potential mediator connecting the extracellular stimulus and the increase in protein synthesis, is the levels of intracellular Ca^{2+} ($[Ca^{2+}]_i$). The $[Ca^{2+}]_i$ further regulates the genes for protein synthesis via activation of Ca^{2+}-dependent protein kinases (Marban and Koretsune, 1990).

Although the increase in protein synthesis and finally the induction of cardiac hypertrophy from pressure or volume overload may proceed without the participation of hormonal factors (Morgan and Bader, 1991), several similar factors may still be involved in the hypertrophic process. For example, stretching of the cultured cardiocytes will lead to an autocrine release of Ang II, and this released Ang II has been implicated to be a major mediator for induction of protein synthesis (Sadoshima *et al.*, 1993). The evidence supporting the assumption that Ang II plays an important role in the hemodynamic overload-induced hypertrophy will be discussed later. In addition to the possible involvement of Ang II, endothelin (ET) might also be important in the development of pressure overload-induced cardiac hypertrophy. In pressure overload by aortic banding, the increase in the relative LV weight, in the diameter of the cardiomyocytes, as well as in the LV α-SA and ANP expression were all blocked by BQ 123 (Ito *et al.*, 1994). BQ 123 is a specific antagonist to type-$_A$ ET-1 receptor (ET_A) and its blockade of the hypertrophic and biochemical responses of the LV to pressure overload occurred at a dosage not affecting the increased pressure.

I.2.2. *Neuronal Factors*

I.2.2.1. ***Increased Cardiac Sympathetic Activity*** may play an important regulatory role in the induction of certain types of cardiac hypertrophy. Exogenous catecholamines by themselves can initiate myocardial protein synthesis and induce cardiac hypertrophy via the activation of cytoplasmic membrane α- and ß-adrenoceptors.

As shown from the early studies, chronic infusion of adrenergic agonists, such as norepinephrine, stimulates the dog heart growth *in vivo* and thus induces ventricular hypertrophy (Laks *et al.*, 1973; King *et al.*, 1987). The doses used for infusion were within the subhypertensive range, and therefore, the heart growth was not associated with an increase in the arterial blood pressure (BP). The same *in vivo* effects on the heart growth were also observed in rats (Stanton *et al.*, 1969). In rats, the cardiac hypertrophy induced by norepinephrine appears to be mediated by stimulation of both α- and ß-adrenoceptors on the myocardium (Zierhut and Zimmer, 1989).

The effects of catecholamines were also studied in *in vitro* models. For example, exposure to norepinephrine induced growth of cultured cardiocytes from neonatal rats (Simpson *et al.*, 1982; Simpson, 1985; Ikeda *et al.*, 1991). By applying specific antagonists for different types of adrenoceptors, it has been demonstrated that myocardial α_1-adrenoceptors are more important in mediating the hypertrophic effect of norepinephrine (Simpson, 1983; Ikeda *et al.*, 1991). This α_1-adrenoceptor-mediated myocyte growth has been shown to be dissociated from the α_1-receptor-mediated positive inotropic effects, *i.e.*, the contractile activity is not necessary for the increase in cellular size or in mRNA transcription (Simpson, 1985).

The intracellular pathways as well as the role of the various receptor subtypes in the α_1-adrenoceptor activation-induced cardiac hypertrophy has also been thoroughly

studied. In cultured cardiac myocytes from neonatal rats, a 4-fold increase in 1, 4, 5-inositol trisphosphate (IP_3) was induced by stimulation of α_1-adrenoceptors. Both the increase in IP_3 and the associated increase in protein synthesis were inhibited by WB-4101, a selective blocker for α_{1A}-receptors, but not by chloroethylclonidine (CEC), a selective blocker for α_{1B}-receptors (Simpson *et al.*, 1990). This result indicates that phosphoinositide-phospholipase C (PLC) is involved in the α_1-adrenoceptor activation-mediated hypertrophic response, and that activation of α_{1A}-receptors plays a major role in this process. In supporting the presumed role of the receptor subtype, the norepinephrine-stimulated protein synthesis was also inhibited by two other selective α_{1A}-receptor blockers, 5-methyl-urapidil and [+]-niguldipine, as well as by the non-selective α_1-receptor blockers, terazosin and prazosin (Simpson *et al.*, 1990; Knowlton *et al.*, 1993).

By using cultured cardiocytes and perfused heart models, the profile and time-course of *c-onc* mRNA expression after stimulation of α_1-adrenoceptors has been studied. This stimulation of cultured neonatal rat myocytes increased *c-myc* (Starksen *et al.*, 1986) and some other *c-onc* mRNA, such as the mRNA of *c-fos* and *c-jun* (Iwaki *et al.*, 1990). In the isolated rat heart, norepinephrine added to the perfusate increased *c-fos* mRNA by five-, three- and 3.8-fold after 30, 60 and 90 *min*, respectively, and *c-myc* mRNA two- and 3.8-fold after 60 and 90 *min*, respectively (Kolbeck-Ruhmkorff *et al.*, 1993). Following the increase in *c-onc*, protein synthesis in the myocardium is enhanced. Under these conditions, the enhanced protein synthesis also involves ß-MHC and α-SA (Marban and Koretsune, 1990), the two contractile protein isogenes normally expressed to a major percentage only in the early developmental stage of small mammals, such as the rat and

mouse. Similar to the changes in myosin isoform during hypertrophy from a pressure overload, the myosin isoform is then shifted from V_1 to V_3 (Waspe *et al.*, 1990).

Stimulation of ß-receptors by isoproterenol injection can also induce rat cardiac hypertrophy *in vivo* (Zimmer and Peffer, 1986) and increase protein synthesis in cultured myocytes isolated from adult rat heart (Dubus *et al.*, 1990). It is now clear that the intracellular mediating factor between stimulation of the ß-receptors and the increase in protein synthesis is the second messenger, cAMP (Zimmer and Peffer, 1986; Xenophontos *et al.*, 1989). The elevated cAMP further regulates protein synthesis through pathways to activate mitogen-activated protein kinase (MAP kinase) (Marx, 1993), which by a phosphorylation process regulates genes for protein synthesis. Different from the profile of the stimulated protein synthesis by α-receptor activation or by pressure overload, the proteins synthesized from ß-receptor stimulation are noncontractile proteins (Dubus *et al.*, 1990).

There are still some controversial results regarding the effects of ß-receptor stimulation on protein synthesis and on cardiac hypertrophy. Contrary to the report from Dubus *et al.* (1990), Fuller *et al.* (1990) reported that the stimulated protein synthesis in isolated myocytes or perfused rat heart by adrenaline was not influenced by ß-blockade with propranolol, and the protein synthesis rate of isolated cardiac myocytes from adult rats was not increased by ß-receptor stimulation with isoproterenol. The reasons for the discrepancy in these results is still unknown. Since Dubus *et al.* (1990) exposed the cultured myocytes to catecholamine for three days while Fuller *et al.* (1990) treated the freshly isolated myocytes for only 10 *min*, the difference in duration of treatment may be responsible for the difference in results.

Besides a direct effect, sympathetic activity may play an important regulatory role in the induction of certain types of cardiac hypertrophy. In an *in vitro* model, ventricular myocytes from WKY rats with sympathetic innervation by co-culture with neurons showed an increase in the intracellular organelle volumes and grew 38% larger than the control myocytes in cultures without innervation (Atkins *et al.*, 1992). The myocardial hypertrophy of rats induced by a daily exercise for 14 *wk* was prevented by chemical sympathectomy through chronic guanethidine treatment (Ostman-Smith, 1976). With this sympathectomy, the rats were still able to maintain a normal resting BP and to undertake strenuous exercise. Similarly, the cardiac hypertrophy in hypertensive animal models, such as in renal hypertensive rats or in spontaneously hypertensive rats (SHR), can be prevented or even regressed by pharmacologic sympathectomy with α-methyldopa (Sen *et al.*, 1974; Tomanek *et al.*, 1979) or with the ß-blocker, propranolol (Fernandes *et al.*, 1976). In contrast to the result from Fernandes *et al.* (1976), propranolol failed to prevent the increase in cardiac mass in SHR (Sen and Tarazi, 1983). With the prevention or regression of hypertrophy in both hypertensive rat models, the drug treatments did not change their elevated resting BP. α_1-Adrenoceptor blockade with bunazosin reduced hypertrophic response of the heart to aortic constriction in guinea pigs (Tamai *et al.*, 1989). These results suggest an important role of cardiac sympathetic activity and activation of cardiac adrenoceptors in the development of cardiac hypertrophy under these conditions. However, the role of sympathetic activity in overload-induced hypertrophy is still controversial and the cardiac sympathetic activity may not be essential for induction of other forms of pressure overload-induced cardiac hypertrophy. For instance, sympathetic denervation by using 6-hydroxydopamine to

block sympathetic transmission failed to prevent the increase in the ratio of ventricular weight to body weight induced by deoxycorticosterone acetate (DOCA)-salt in rats (Cohen, 1974). In contrast to the report from Tamai *et al.* (1989), in rats the cardiac hypertrophy from aortic constriction was not changed by either prazosin or propranolol (O'Rourke and Reibel, 1992). Furthermore, the right ventricular (RV) hypertrophy from pulmonary banding in cats was not prevented either by an epicardial denervation or by α- or ß-receptor blockade (Cooper *et al.*, 1985).

I.2.3. *Other Factors*

I.2.3.1. ***Ang II*** is a hypertrophic factor for the myocardium both *in vitro* and *in vivo*, as well as a potent vasoconstrictor which increases systemic BP. It is now clear that the *in vivo* hypertrophic effect of Ang II can be a direct response of myocardium to Ang II rather than depending on changes in mean arterial blood pressure (MAP) (Baker *et al.*, 1992). This hypertrophic effect results from stimulation of Ang II receptors (AT) and then an enhancement of protein synthesis. Similar to the stimulated protein synthesis by increased load or by α-adrenoceptor activation, the major proteins synthesized from activation of AT receptors are also ß-MHC and α-SA, leading to a similar shift of myocardial myosin isoform phenotype from V_1 to a V_3 (Marban and Koretsune, 1990).

Both circulating and locally produced Ang II might be involved in its cardiac hypertrophic effect (Baker *et al.*, 1992; Sadoshima *et al.*, 1993). Ang II stimulates myocardial AT receptors (Aceto and Baker, 1990), as mentioned above, but only the activation of subtype-1 Ang II receptor, the AT_1 receptor, is involved in Ang II-induced

cardiac hypertrophy (Sadoshima and Izumo, 1993a; Suzuki *et al.*, 1993; Kojima *et al.*, 1994). The intracellular mediating factors between the receptor stimulation and hypertrophic responses could involve an activation of the protein kinase C (PKC) by diacylglycerol (DAG) and Ca^{2+}. PKC might stimulate the Na^+/H^+ exchange leading to proton efflux and intracellular alkalinization. In addition, it might phosphorylate nuclear proteins. Both these two processes will enhance protein synthesis. Besides, stimulation of AT also activates phospholipase D (PLD), resulting in formation of phosphatidylethanol, and phospholipase A_2 (PLA_2), leading to production of prostaglandins and leukotrienes, which might also play a role in the development of hypertrophy (Sadoshima and Izumo, 1993c). The other potential mechanism underlying Ang II-induced cardiac hypertrophy is through its direct binding to a soluble DNA-binding protein in the cytosol. This ligand-receptor protein complex will further regulate gene transcription and afterwards protein synthesis probably by binding to a specific DNA sequence (Evans, 1988).

Besides the direct effect of Ang II on myocardial growth, Ang II might also be involved in the development of hemodynamic overload-induced increases in myocyte protein synthesis and cardiac hypertrophy. There are several lines of evidence supporting this hypothesis (Sadoshima *et al.*, 1993). First, stretch-induced *c-onc* and fetal-type gene expression, as well as an increase in protein synthesis, are completely blocked by specific AT_1-receptor antagonists. The increased [^{3}H]phenylalanine incorporation, activation of MAP kinase and *c-fos* mRNA expression in the cultured cardiomyocytes from neonatal rats in response to stretch were also inhibited by an antagonist for the AT_1-receptors, TCV-116 (Kojima *et al.*, 1994). Therefore, the involvement of Ang II in

stretch-induced myocardial hypertrophy appears to be mainly via AT_1-receptors (Sadoshima and Izumo, 1993a; Suzuki *et al.*, 1993). Second, stretching cardiac myocytes *in vitro* causes an acute release of Ang II and later on increases the expression of the angiotensinogen gene. Third, the existence of Ang II in cardiac myocytes was confirmed with immunofluorescence light microscopy and immunoelectron microscopy. Fourth, the myosin isoform of Ang II-induced cardiac hypertrophy is indistinguishable from that of stretch-induced hypertrophy (Sadoshima *et al.*, 1992; Sadoshima and Izumo 1993a). Fifth, both Ang II and stretch cause activation of the same set of multiple second messenger systems in cardiac myocytes (Sadoshima and Izumo, 1993b; 1993c). And last, in the model of aortocaval shunt-induced volume overload, the cardiac hypertrophy is associated with significant increases in plasma renin activity (PRA) and in cardiac renin activity (Ruzicka *et al.*, 1993). The hypertrophic effect of this volume overload was attenuated by losartan, an antagonist for the Ang II receptors, suggesting a potential role of cardiac Ang II in the development of volume overload-induced hypertrophy.

I.2.3.2. ***Thyroid Hormones,*** including both thyroxine (T_4) and triiodothyronine (T_3), not only regulate normal developmental growth of the heart, but can also induce cardiac hypertrophy in adult animal models. The mechanisms underlying this hypertrophic effect may include a direct effect of the hormones on the heart, an indirect effect related to making the heart hypersensitive to sympathetic stimulation, or altered LV loading conditions from a combination of the thyroid hormones' action and an enhanced activity of the sympathetic nervous system. Cardiac hypertrophy induced by thyroid hormones

is associated with an increase in the intracellular levels of signalling factors in the myocytes, such as cAMP and Ca^{2+} (Morgan and Bader, 1991). With regard to $[Ca^{2+}]_i$, which has been demonstrated to be an important mediator for overload and neurogenic hypertrophy, there are no substantial data indicating a primary role in mediating the hypertrophic response of the heart to thyroid hormones (Haneda *et al.*, 1989).

About the particular mechanism for the hypertrophic effects of thyroid hormones, the latest evidence is in favour of the direct gene action hypothesis. It has been proposed that thyroid hormones increase protein synthesis by way of stimulating gene transcription via binding directly to the nuclear thyroid hormone receptors (Morgan and Bader, 1991). According to this hypothesis, the first step for the actions of thyroid hormones is to form a ligand-receptor complex with a specific cytoplasmic receptor protein, and the second step is to bind this complex to the specific DNA sequences. The binding will activate transcription of the target genes (Morgan and Bader, 1991). The nuclear binding protein for thyroid hormones has been shown to be a homologue to one of the *c-onc*, named *c-erb-A* (Weinberger *et al.*, 1986). The terminal effect of thyroid hormones' action is up-regulation of α-MHC, which shifts myosin isoform from V_3 to V_1 in the ventricles. Therefore, it is different from the change of myosin isoform associated with hypertrophy from a pressure overload or from α_1-receptor stimulation (Marban and Koretsune, 1990).

I.3. *Cellular and Molecular Mediating Pathways of Cardiac Hypertrophy*

Cardiac hypertrophy is a result of myocardial cellular hypertrophy, which is a

result of an enhanced protein synthesis, or in some rare circumstances, a result of enhanced protein synthesis combined with a decreased protein degradation (Morgan and Bader, 1991), although protein degradation is increased in some hypertrophic process. The induction of protein synthesis has been shown to be mainly controlled at the transcriptional stage as indicated by increases in mRNA levels (Simpson *et al.*, 1989), even though control at the translational levels could not be excluded (Soltoff and Cantley, 1988).

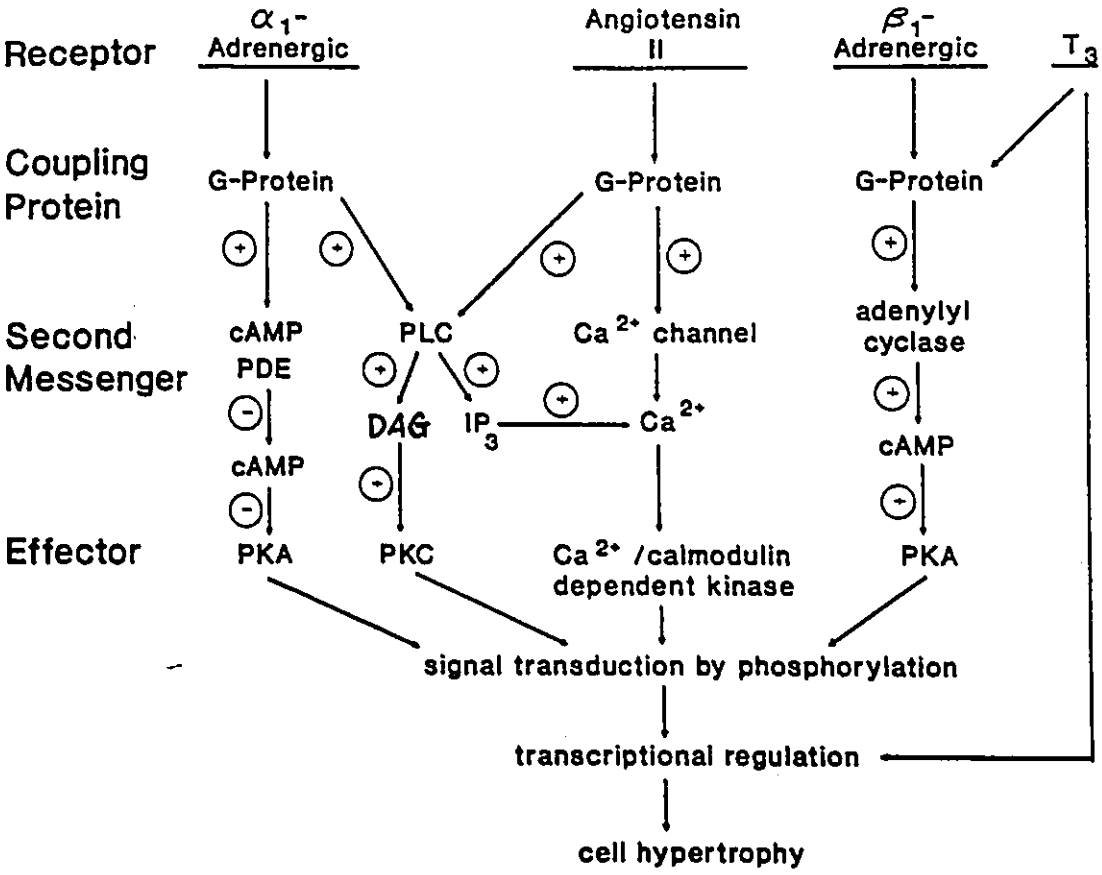
At least four different intracellular pathways, being mentioned above individually, have been shown to mediate the extracellular stimuli and the increased protein synthesis, and will be summarized here (Morgan and Bader, 1991) (Fig. 1).

I.3.1. *cAMP-Dependent Pathway*

Stimulation of ß-adrenoceptor results in an increase in the cytoplasmic cAMP levels via an activation of the membrane bound adenylate cyclase through activation of the stimulating GTP-binding protein (G_s). cAMP further activates PKA. The cytoplasmic membrane Ca^{2+} channels are activated by both G_s and PKA, with a resultant increase in $[Ca^{2+}]_i$ (Krebs, 1989). Ca^{2+} regulates many cellular processes, including protein synthesis and cell growth.

Increased levels of cAMP enhance protein synthesis in isolated cardiac myocytes (Xenophontos *et al.*, 1989). But it is not clear yet whether this increase of protein synthesis is via PKA or via Ca^{2+}-dependent phosphorylations (Marban and Koretsune, 1990). The latest studies with cultured neuron-like cells suggest that the elevated cAMP

Fig. 1. **Some of the neural and endocrine pathways with possible coupling proteins, second messengers, and effector mechanisms for transcription regulation of cardiac cell hypertrophy (from Morgan and Bader, 1991).** G-proteins, guanine nucleotide binding proteins; cAMP, cyclic AMP; PDE, phosphodiesterase; PLC, phospholipase C; DAG, diacylglycerol; IP_3 inositol trisphosphate; PKC, protein kinase C; PKA, cAMP-dependent protein kinase A. See text for details.


Receptor
α_1-Adrenergic
Angiotensin II
β_1-Adrenergic
T_3
Coupling Protein
G-Protein
G-Protein
G-Protein
Second Messenger
cAMP
PDE
cAMP
PLC
DAG
IP_3
Ca^{2+} channel
Ca^{2+}
adenylyl cyclase
cAMP
Effector
PKA
PKC
Ca^{2+} /calmodulin dependent kinase
PKA
signal transduction by phosphorylation
transcriptional regulation
cell hypertrophy

interferes with the activation of MAP kinase, which controls the activity of other enzymes through phosphorylation of the target protein enzymes and brings about the cellular responses (Marx, 1993). PKA phosphorylation of the cAMP recognition element binding protein (CREB) forms the final step of this pathway. CREB is also phosphorylated and then regulated by Ca^{2+}-dependent phosphorylation (Spaulding, 1993).

I.3.2. *Inositol Phosphate-Diacylglycerol Pathway*

Stimulation of α_1-adrenoceptors, Ang II or endothelin receptors induces the hydrolysis of membrane phosphatidylinositol 4,5-diphosphate (PIP_2) by activation of a membrane-bound PLC and from this hydrolysis the formation of IP_3 and DAG (Berridge and Irvine, 1989). The activation of PLC, at least following α_1-adrenoceptors activation, is via an unidentified G-protein. IP_3 exists in cytosol and can be further phosphorylated to inositol tetrakisphosphate (IP_4). Both IP_3 and IP_4 can increase $[Ca^{2+}]_i$, which could augment protein synthesis via phosphorylation of variety of proteins by Ca^{2+} dependent kinases (Marban and Koretsune, 1990). DAG, the other product from PIP_2 hydrolysis, is a membrane soluble product and its elevated level is sustained much longer than IP_3. DAG and the elevated $[Ca^{2+}]_i$ stimulate PKC, which can phosphorylate a variety of proteins, of which some might act as transcription factors and thus enhance or modify protein synthesis by stimulating *c-onc* expression (Simpson *et al.*, 1989).

PKC also stimulates the Na^+/H^+ exchange by increasing the affinity of this exchange system for intracellular protons (Otani *et al.*, 1990). Stimulation of Na^+/H^+

exchange leads to an increased H^+ efflux and Na^+ influx, and therefore a decrease in $[H^+]_i$ and an increase in $[Na^+]_i$ and intracellular pH (pH_i). This cellular alkalinization might subsequently increase protein synthesis, probably via an increased $[Ca^{2+}]_i$ secondary to the increase in Na^+ influx (Morgan and Bader, 1991). Stimulation of the inositol phosphate-DAG pathway causes a shift of the myosin isoform from adult V_1 to prenatal V_3 (Waspe *et al.*, 1990).

I.3.3. *Tyrosine Kinase-Dependent Processes*

Tyrosine kinase (TK)-coupled receptors have intrinsic protein-tyrosine phosphorylation activity (Yonezawa *et al.*, 1990). This type of receptor includes the receptors for insulin, heparin and several other growth factors, such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF).

After binding with the ligand, the receptors can activate several other kinases as well as autophosphorylate a number of specific tyrosine residues within the receptor. The activated kinases will stimulate and regulate protein synthesis directly, through activation of MAP kinase, and also indirectly by enhancing IP_3 and DAG formation via a modulation of PLC activity (Simpson *et al.*, 1989). Activation of MAP kinase through the Ras pathway appears to be the common pathway for transmission of growth signals of the ligands for TK coupled receptors. For instance, after EGF has bound to its receptor, the receptor autophosphorylates its tyrosine residues first, then this autophosphorylation will evoke a sequential activation of the guanine nucleotide exchange factor Sos, the guanosine triphosphate (GTP) binding protein Ras and the protein kinase

Raf-1, and finally an activation of MAP kinase. Sos and Raf-1 are also *c-onc* products. The activated MAP kinase can further phosphorylate transcription factors, thus protein synthesis is regulated (Wu *et al.*, 1993).

I.3.4. *Nuclear Receptors*

Thyroid hormones (Weinberger *et al.*, 1986), steroids (Evans, 1988), and also Ang II (Dzau *et al.*, 1988; Morgan and Bader, 1991) bind to the hormone-binding domain of a soluble DNA-binding protein in the cytosol of target cells. Each hormone has its specific DNA-binding protein. The hormone-receptor complex, after some structural changes, moves into the nucleus (a process called internalization) and binds to a specific regulatory site on DNA through the DNA-binding domain of the receptor protein. In this way, specific DNA transcription is regulated, and consequently the protein synthesis is modified.

I.3.5. *Summary*

Except for the hypertrophic initiating factors acting via binding directly to the nuclear receptors, most of the various extracellular stimuli and their intracellular pathways mentioned above will finally converge to a common route --- constitutive or inductive transcription of *c-onc* (Marban and Koretsune, 1990). These *c-onc* encode DNA binding proteins, which further modulate RNA transcription by binding to the controlling elements of the transcribed genes and regulate protein synthesis (Simpson *et*

al., 1989) and the assembly of these synthesized proteins (Iwaki *et al.*, 1990). The inductive transcription of *c-onc* is mediated by phosphorylation of certain nuclear proteins, called transcription factors. These phosphorylations are directly or indirectly influenced by $[Ca^{2+}]_i$, PKA, PKC or MAP kinase dependent pathways.

II. Dietary Sodium-Induced Hypertrophy

II.1. *General Review*

The correlation between sodium intake and hypertension in salt sensitive populations has been studied for quite a few years and has been well documented (Freis, 1976; Stamler *et al.*, 1989). However, the role of dietary sodium in cardiac hypertrophy or in hypertensive LVH has been emphasized just recently (Fernandez *et al.*, 1988). Initial studies (Schmieder *et al.*, 1988; Du Cailar *et al.*, 1989) indicated that in mild hypertensives, high sodium intake may be a determinant of LVH independent of other variables, such as systolic or diastolic blood pressures (SBP or DBP), body mass index, age, gender, haematocrit, and plasma catecholamine levels. Afterwards, the role of sodium intake has been confirmed in normotensive as well as in hypertensive subjects by demonstration of a positive correlation between the amount of sodium intake, designated as the urinary Na^+ excretion, and the changes in LV geometry, measured by echocardiography (Hammond *et al.*, 1988; Du Cailar *et al.*, 1992). In Schmieder's

study, urinary sodium excretion was found to be the strongest predictor for LV mass in the essential hypertensive patients (Schmieder *et al.*, 1988). Dietary sodium was also shown by Liebson *et al.* (1993) as strong a predictor for LV mass as SBP or body mass index in mild hypertensives. Kupari *et al.* (1994) suggested dietary sodium as an independent predictor but also a synergistic factor with BP for LV mass of a population sample aged 36 to 37 years old.

The positive correlation between the high sodium intake and the increase in heart mass has also been demonstrated in animal experiments. An increase in the LV mass by salt exposure was observed in normotensive young WKY rats on a high sodium diet (2% to 8% NaCl, with respectively 3 to 10 times higher NaCl content than that in the regular food) for 4 to 10 *wk* (Yuan and Leenen, 1991; Frohlich *et al.*, 1993). A similar effect was also seen in normotensive Wistar rats on a 1% NaCl solution in drinking water for 3 to 6 *wk* (Fields *et al.*, 1991). Besides studies with normotensive rats, the effect of high salt exposure on cardiac mass was also studied in some hypertensive animal models. In two-kidney, one-clip (2K, 1C) renal hypertensive rats, high dietary sodium increased and low dietary sodium decreased cardiac hypertrophy by 13 and 9%, respectively, while the level of the elevated BP was not affected (Lindpainter and Sen, 1985; Gallo *et al.*, 1990). In contrast, SHR on a 10 *wk* high sodium diet developed LVH accompanied by an increase in BP and in total peripheral resistance (TPR) (Frohlich *et al.*, 1993).

Hypertrophy, rather than hyperplasia of myocardium, is the underlying cellular process of the increased LV mass by high sodium intake. In rats, the increase in LV mass by high sodium intake is associated with an increase in the ratio of non-collagenous protein to DNA and in the total collagen content as well as a decrease in DNA

concentration (Kihara *et al.*, 1985). Based on the concept that each cell contains a constant amount of DNA, both an increased ratio of protein to DNA and a decreased DNA concentration indicate that the individual cellular mass has been increased without an increase in the cell numbers.

The LVH induced by a high sodium intake in animals shows a concentric form, similar to that induced by a pressure overload. This is clearly observed in WKY rats on 1% saline in drinking water (Fields *et al.*, 1991). High sodium diet-induced LVH in WKY rats also has a tendency for a concentric form (Yuan and Leenen, 1991). A diet with 4% NaCl for 9 *wk* increased the LV wall thickness without changing the end-diastolic dimension of the LV in Wistar rats, suggesting a similar effect of high salt exposure on the ventricular structures (de Simone *et al.*, 1993)

The hypertrophic effects of the high sodium diet in rats appears to be age and strain dependent. The hypertrophic effect is significant in young WKY rats on diet from 4 to 8 *wk* of age, to about 25% increase in LV weight, and in young Wistar rats to about 14%. It is less potent in adult WKY or Wistar rats commencing diet from 13 *wk* of age or older, and non-effective in Dahl salt-resistant rats, which showed no increase in LV weight (Yuan and Leenen, 1991). These results indicate a preferential influence of the high sodium intake on the early developmental stage of the animals.

II.2. *Possible Mechanisms*

The first question which needs to be answered is whether a pressure or volume

overload is playing a role in the salt exposure-induced cardiac hypertrophy, even though high sodium intake has already been confirmed to be a risk factor for cardiac hypertrophy independent of other hemodynamic factors (Hammond *et al.*, 1988; Schmieder *et al.*, 1988; Du Cailar *et al.*, 1992). Different strains of normotensive rats have been used in various chronic exposure protocols and the possible involvement of a load factor has been consistently ruled out. For instance, in normotensive young rats, high sodium intake causes LVH, and this LVH is not accompanied by an increase in the resting systemic BP, right atrial pressure (RAP) or LV filling pressures measured during the daytime (Kihara *et al.*, 1985; Fields *et al.*, 1991; Yuan and Leenen, 1991; Frohlich *et al.*, 1993). This LVH is not accompanied by changes in the regional hemodynamics in the heart or in peripheral organs (Frohlich *et al.*, 1993), or by an increase in RV weight (Fields *et al.*, 1991; Yuan and Leenen, 1991). These results indicate that pressure or volume overload mechanisms may not be involved in the development of sodium-induced LVH. Furthermore, the 24 *hr* average BP of WKY rats was not changed, although nighttime BP was increased significantly by a high sodium diet by 4 - 6 *mmHg* (Calhoun *et al.*, 1994).

In addition, most data do not support a primary role of an elevated sympathetic activity in the development of the high sodium intake-induced LVH. For example, the daytime LV norepinephrine turnover rate and tissue tyrosine hydroxylase activity were not increased by a high sodium intake (Fields *et al.*, 1991; Yuan and Leenen, 1991). In studies on normotensive humans, high sodium diet actually decreased the plasma and urinary catecholamine concentrations (Wood *et al.*, 1982; Egan, *et al.*, 1991).

In contrast, normotensive rats on a one or six months high sodium intake showed

an increased urinary catecholamine excretion rate (Battarbee *et al.*, 1979). But, since urinary catecholamine excretion rate is a parameter for the last step of the dynamic profile of catecholamine metabolism, this rate is influenced by a variety of factors within and outside the sympathetic nervous system, such as the rate of synthesis, release and re-uptake of catecholamines, and the plasma and interstitial levels of catechol O-methyl transferase (Ostman-Smith, 1981). Because of this, it is difficult to make a conclusion on the levels of cardiac sympathetic activity based on the change of urinary catecholamine excretion rate.

The effect of a high dietary sodium on sympathetic nervous system has also been studied in some hypertensive rat models. An elevated BP and more marked sympathetic activity are involved in rats on a DOCA-salt treatment (de Champlain and van Ameringen, 1972). In DOCA-salt treated rats, the plasma catecholamine concentrations were increased in some studies (de Champlain *et al.*, 1976). Conversely, the cardiac catecholamine content of rats with the same treatment was reduced (Krakoff *et al.*, 1967; Doyle, 1968). In the same model, the norepinephrine turnover rates appeared to be increased in the heart (Motoyama *et al.*, 1988). In SHR, a salt exposure by drinking 1% NaCl increased the turnover of norepinephrine in the heart and its excretion in the urine (Gradin *et al.*, 1988). Even though a low sodium intake does not necessarily affect sympathetic activities in a manner opposite to a high sodium intake, the depressed cardiac catecholamine in 2K 1C hypertensive rats was restored to normal levels by a restriction of sodium intake (Lindpaintner and Sen, 1985).

The predominance of α-isoform of MHC is not changed by a 4% NaCl diet for 6 *wk* (Buttrick *et al.*, 1993), suggesting no similarities to the cardiac hypertrophy induced

by an increased load or by an α-receptor activation. In the latter two conditions, MHC was switched to a ß-isoform dominant V_3 phenotype (Marban and Koretsune, 1990). In contrast, in normotensive Wistar rats, very low sodium diet (7 *mEq/kg*) for 6 *wk* increased MHC V_1 isoform by 24% and reduced V_3 by 60%, while normal sodium diet (204 *mEq/kg*) did not change the isoform distribution (Sen and Young, 1986). However, a lower sodium intake does not necessarily affect MHC isoform in a manner opposite to a high sodium intake.

The high sodium diet-induced LVH is also unlikely related to plasma-derived Ang II, since the levels of Ang II are inhibited to almost undetectable levels by a high sodium intake (Buttrick *et al.*, 1993; Osborn *et al.*, 1993). Whether local Ang II could be regulated differently from that of plasma Ang II level needs to be studied.

An other potential cellular response to salt exposure could involve the activation of cytoplasmic Na^+/H^+ exchange. However, the activation of this exchange system is not likely to be an initial mechanism for dietary sodium-induced LVH. Na^+ influx through myocardial membrane Na^+/H^+ exchange activated by an overload does stimulate cardiomyocyte protein synthesis (Kent *et al.*, 1989). However, the plasma Na^+ levels of rats on a high salt exposure are transiently increased by only 1 to 2% by a diet with 8% NaCl (Mozaffari *et al.*, 1990). Moreover, if only sodium intake is increased, without altering other mechanisms for maintaining normal extracellular sodium levels, hypernatraemia is rarely observed (Vollmer, 1984). Besides, the activity of the Na^+/H^+ exchange system is mainly regulated by its affinity to the intracellular H^+, instead of the extracellular $[Na^+]$ (Soltoff and Cantley, 1988), showing its preferential sensitivity to a change in $[H^+]_i$ than to a change in extracellular $[Na^+]$. Taken altogether, it is unlikely

that the activation of Na^+/H^+ exchange could be a mechanism for the dietary sodium-induced LVH.

The other recognized pathway influencing $[Na^+]_i$ and then $[Ca^{2+}]_i$ is through modulation of myocardial Na^+, K^+-ATPase activity. Inhibition of this enzyme increases $[Na^+]_i$, and subsequently increases $[Ca^{2+}]_i$ via a secondarily activated Na^+/Ca^{2+} exchange. High sodium diet has been shown to modify the activity of this enzyme in myocardium (McPartland and Rapp, 1982; Clough *et al.*, 1985) and kidney (Di Campo *et al.*, 1991; Nishi *et al.*, 1992), or to change the amount of the subunit isoforms of the enzyme (Sweadner *et al.*, 1994). Ouabainlike activity (OLA), an endogenous inhibitor of Na^+, K^+-ATPase, has been found to be increased by a high sodium diet in LV and other tissues from WKY rats (Leenen *et al.*, 1993). However, Na^+, K^+-ATPase inhibition by exogenous ouabain does not promote cardiac protein synthesis. On the contrary this inhibition even reduces cardiac hypertrophy by decreasing protein synthesis in the heart (Kent *et al.*, 1989). The mechanism for the decreased protein synthesis when Na^+, K^+-ATPase is inhibited may be related to a continual loss of intracellular K^+ associated with an increased Na^+ influx. This loss of intracellular K^+ will markedly impair protein translation, since intracellular K^+ in particular is required for the elongation of the synthesized peptide (Kent *et al.*, 1989). Based on all these studies, the hypothesis that an initial increase in $[Na^+]_i$ through inhibition of Na^+, K^+-ATPase and a subsequent increase in $[Ca^{2+}]_i$ is unlikely applicable in the high sodium diet-induced LVH.

II.3. *Supporting Evidence for Our Hypothesis*

Although the systemic or cardiac sympathetic activity appears to be lowered by salt exposure, several lines of evidence suggest that a change in adrenoceptor activity or sensitivity might be involved in the hypertrophic process of a high sodium intake (Woodcock *et al.*, 1979; Meggs *et al.*, 1988; Gallo *et al.*, 1990).

On the cellular level, some experiments suggest that a high sodium intake changes the cardiac adrenoceptor densities or affinities. Rats subjected to a 1% saline as the only drinking water for 6 *wk* showed an up-regulation of the myocardial α_1-receptors density (with an increase of about 40%), though with a reduced affinity (K_D, the dissociation constant = 395 *vs.* 100 *pM*) (Meggs *et al.*, 1988). High levels of dietary sodium significantly increased the ß-receptor numbers in normotensive, sham-operated rats (Gallo *et al.*, 1990).

Changes in adrenoceptor density by high sodium intake were also observed in various hypertensive models. In rats with DOCA-salt-induced hypertension, a 50% decrease in ß-receptor number in the heart with an unchanged affinity (K_D 1.43 *vs.* control of 1.59 *nM*) was found (Woodcock *et al.*, 1979). In rabbits, the maximal binding sites (B_{max}) of myocardial membrane for ß-receptor agonist was decreased significantly from 109 to 55 *fmol/mg* protein by chronic treatment with DOCA-salt (Tsuji *et al.*, 1987). Since an elevated BP and higher sympathetic activity are present in the DOCA-salt model (de Champlain and van Ameringen, 1972), these changes in ß-receptor density or affinity may not be attributable to the salt exposure only. A high sodium diet did not change the ß-receptor numbers in the heart of 2K, 1C hypertensive rats (Gallo *et al.*,

1990).

In addition to an influence on the receptor levels, salt exposure may modulate post-receptor signal pathways for the adrenoceptors as well. The activity of the intracellular mediating pathway for α_1-adrenoceptor activation, the inositol phosphate-DAG pathway can be determined by measuring the accumulation of the major product from this pathway, inositol monophosphate. The amount of inositol monophosphate was twofold higher in the atria and ventricles of DOCA-salt hypertensive rats than in the normotensive rats (Eid and de Champlain, 1988). This result suggests that in the heart of DOCA-salt-treated rats there might be a hyperactivity of the α_1-adrenoceptor or of the intracellular signalling systems responsible for the activation of the inositol phosphate-DAG pathway.

A more complicated modification of adrenoceptors by a high sodium intake was noted by measuring the ratio of α_2- to $ß_2$-adrenoceptor in blood cells from normotensive young men. The ratio was increased by a 2 *wk* high sodium diet, showing an up-regulation of α_2- and/or a concomitant down-regulation of $ß_2$-receptors in the blood cells (Skrabal *et al.*, 1988). The change of myocardial adrenoceptors in response to the salt exposure might follow a similar pattern.

In association with changes in adrenoceptor density or affinity by a high sodium intake, the pressor or chronotropic responses in humans and in animals also showed some alterations. In normotensive young men, the magnitude of BP increase in response to norepinephrine infusion was significantly increased by a 5-day high sodium diet. At the same time the threshold of the pressor response to norepinephrine was lowered, indicating an increase in both responsiveness and sensitivity to α-receptor stimulation

(Rankin *et al.*, 1981). A similar augmentation in pressor response to norepinephrine was seen in other studies on a group of young male students (Skrabal *et al.*, 1984; Sharma *et al.*, 1992). This increased pressor response is in agreement with the finding in normotensive humans that the ratio of α_2- to $ß_2$-adrenoceptors in blood cells was increased by a high sodium diet (Skrabal *et al.*, 1988). A similar pattern of the change in the ratio of these two receptor types may also occur in vascular smooth muscles of the subjects on a high sodium diet, and then result in increased vasoconstriction. Assessment of the dose for isoproterenol *in vivo* to increase HR by 25% as an indicator of ß-receptor responses, showed that the response level was not changed by a high dietary sodium for 7 days in normotensive volunteers (Volpe *et al.*, 1982). However, in another study with the similar method in a group of normotensive humans, a high sodium diet was demonstrated to increase the sensitivity to isoproterenol (Fraser *et al.*, 1981).

Although most hemodynamic parameters are not changed by a salt exposure, a short (for several *min*) but significant increase in HR and BP (by about 30% for both parameters) is recorded in normotensive WKY rats during each drinking period in the night (unpublished data from our laboratory). The increase in BP and HR was instantaneous and both parameters returned to normal levels immediately after cessation of the drinking. These features indicate that the hemodynamic changes are neurogenic rather than from a volume expansion. When the rats are given a high sodium diet, their total drinking time is significantly increased by 70% and the 24 *hr* drinking volume increased by 3-fold. Twenty-four *hr* hemodynamic monitoring showed a diurnal BP rhythm in WKY rats on a regular diet, with a 4 - 6 *mmHg* higher BP in the nighttime than during the daytime ($p < 0.05$), while this diurnal BP difference was increased to

9 - 10 *mmHg* in those on a high sodium diet (8% NaCl) ($p < 0.05$) (Calhoun *et al.*, 1994). With this increase in the day-night BP difference, the nighttime average BP of rats on the high sodium diet was 4 to 5 *mmHg* higher than those on a regular diet ($p < 0.05$). The increase in systemic BP and HR associated with each drinking suggests a transient increase in sympathetic activity. Although a decreased parasympathetic activity can result in an increase in HR, the systemic BP level is mainly determined by a sympathetic tone and the elevation of BP is therefore most probably related to an increased sympathetic activity.

II.4. *Hypothesis*

Based on the previous findings that a high sodium diet modulates adrenoceptor activities through a change in receptor density or affinity, or through an alteration in the activities of post-receptor pathways, it is hypothesized that a possible increase in cardiac adrenoceptor responses, possibly combined with intermittent increases in sympathetic activity caused by a high dietary sodium is responsible for the development of LVH.

II.5. *Objective*

We intend to assess the possible alterations in responses of α_1- and/or ß-adrenoceptor(s) after a chronic high sodium diet, and the possible role of the

adrenoceptors in the development of high dietary sodium-induced LVH.

II.6. *Approaches to Test the Hypothesis*

(1) We plan to test whether the responses of the α_1- or ß-adrenoceptors *in vivo* are changed in WKY rats during and after the establishment of the high sodium diet-induced cardiac hypertrophy, by monitoring BP, HR, cardiac output (CO), intracardiac pressures and total peripheral resistance index (TPRI), and the responses of these parameters to α_1- or ß-adrenergic stimulations after a high sodium diet for various lengths of time;

(2) We plan to test whether blockade of the α_1- or ß-receptors can prevent the development of LVH induced by a high sodium diet, by intragastrically (*ig*) administration of α_1- and/or ß-receptor antagonists throughout the whole diet regimen. Combined blockade of both α_1- and ß-receptors will be used to prevent possible excitatory influences on the other type of the adrenoceptors when one type has been blocked (Steinkraus *et al.*, 1989).

METHODS

I. General

Male WKY rats were obtained from Taconic Farms, Germantown, NY, at 3 *wk* of age. They were housed two per cage in a temperature-controlled room with a 12-*hr* light-dark cycle and had free access to tap water. All animals were in our animal facility one *wk* before starting the experiments. Diets with the following sodium concentrations were used: 1,370 μmol Na^+/g food (8% NaCl, high sodium diet), 342 μmol Na^+/g food (2% NaCl, medium high sodium diet) and 101 μmol Na^+/g food (0.6% NaCl, regular diet) for 1, 2, 4 or 6 *wk* (Table 1). The diet treatment was started at 4 *wk* of age. Water intake was monitored by measuring bottle weight change during 24 *hr* for day 1, 4 and 7 after starting the diet and thereafter, once a week, and by averaging this change for the two rats in each cage as previously described (Ely *et al.*, 1987; Fields *et al.* 1991; Yuan and Leenen, 1991). Body weight was measured one day before starting the regular or high sodium diets, and then once a week.

Four experimental protocols were used to test changes in the resting hemodynamics and in their responses to adrenergic stimulation, and to test the change in the heart weight and design after (1) 1, 2 or 6 *wk* high or medium high sodium diet; (2) 1, 2 or 4 *wk* high sodium diet with α_1-receptor antagonist; (3) 4 *wk* high sodium diet with ß-receptor antagonist; and (4) 4 *wk* high sodium diet with combined α_1- and ß-receptor antagonists (Table 1 and the following description), in comparison with rats on

Table 1. Animal Groups for Various Diets, Antagonist Treatments, Hemodynamic Recordings and Agonist Infusion

Protocol	Diet Period (*wk*)	Diet Type (% NaCl)	Adrenergic Antagonist (*mg/kg/day*)	Hemodynamic Parameters Measured	Adrenergic Agonist Infusion Rate (*μg/kg/min*) Phenylephrine	Isoproterenol
I	1	8%	--	BP, HR	1, 3, 6 (8 *min*)	0.05,0.1,0.2 (6 *min*)
	2	2% or 8%	--	BP, HR, CO	||	||
	6	2% or 8%	--	BP, HR, CO	||	||
IIA	1	8%	Terazosin (60)	BP, HR	6, 12, 24 (8 *min*)	0.05, 0.1, 0.2 (6 *min*)
			--		1, 3, 6	||
	2	8%	Terazosin (60)	LVP, RAP, HR	6, 12, 24	||
			--		1, 3, 6	||
	4	8%	Terazosin (60)	LVP, RAP, HR	6, 12, 24	||
			--		1, 3, 6	||
IIB	4	8%	Nadolol (25)	LVP, RAP, HR	--	0.1,0.2,0.4,0.8,1.6 (6 *min*)
			Nadolol (100)		--	||
			--		--	0.1, 0.2, 0.5
IIC	4	8%	Terazosin (60) + Nadolol (100)	LVP, RAP, HR	6,12,24,48 (8 *min*)	0.2,0.4,0.8,1.6 (6 *min*)
			--		1, 3, 6	0.05, 0.1, 0.2

Diet regimen started at 4 *wk* of age, without showing the regular diet (0.6% NaCl). BP, arterial blood pressure; HR, heart rate; CO, cardiac output; LVP, left ventricular (peak-systolic & end-diastolic) pressures; RAP, right atrial pressure. ||, same infusion rate as above.

a regular diet for same number of weeks (not shown in Table 1). The adrenoceptor antagonists were *ig* given by gavage, once a day in the afternoon between 2:00 to 6:00, starting 2 days before the diet regimen and with the last dose on the morning of surgery. After finishing the diet and drug treatment, rats were cannulated under halothane-nitrous oxide-oxygen anaesthesia for hemodynamic measurements. All the hemodynamic recordings were measured in conscious unrestrained rats after 3 *hr* recovery from surgery. In one protocol, arterial blood was collected for determining haematocrit right after recording of the baseline hemodynamic measurement. Immediately before assessing hemodynamic responses to α-adrenoceptor agonist, phenylephrine HCl (Sterling-Winthrop Inc., Markham, ON) or ß-adrenoceptor agonist, isoproterenol HCl (Sabex, Boucherville, QP), the ganglionic blocker, hexamethonium (Sigma, St Louis, MO), was *iv* injected to eliminate the influence of the baroreceptor reflex on the BP or HR responses.

After hemodynamic measurements, the rats were sacrificed, their hearts taken out and dissected to obtain the weights of the LV and RV, and the chamber dimensions and wall thickness of the LV.

II. Experimental Protocols

II.1. *Protocol I. Effect of the Medium High or High Sodium Diet for 1, 2 or 6 wk on the Resting Hemodynamics, Heart Chamber Weight and Dimensions and α- or ß-Adrenergic Responses*

Medium high or high sodium diet (as shown in Table 1) or regular diet (not shown in Table 1) was used for 1, 2 or 6 *wk*. At the end of diet treatment, rats were cannulated for arterial BP and HR measurements for the 1 *wk* diet group, and BP, HR and CO measurements for the 2 and 6 *wk* diet groups. After recovery from surgery for 3 *hr*, baseline BP, HR and CO were recorded. After 30 *mg/kg* hexamethonium was slowly *iv* injected and flushed with normal saline, phenylephrine was *iv* infused at a rate of 1, 3 or 6 *μg/kg/min* for 7 - 9 *min* of each dosage. After a 30 *min* recovery from phenylephrine infusion and a second dose of hexamethonium, isoproterenol was *iv* infused at a rate of 0.05, 0.1 or 0.2 *μg/kg/min*, each rate for 6 - 8 *min*. The peak responses of pressure and CO were recorded at the end of each infusion rate. The solutions of the drugs *iv* infused or injected were made up with normal saline.

II.2. *Protocol IIA. Effect of α_1-Receptor Blockade on the Hemodynamics and on the Heart Chamber Weight and Dimensions of Rats on the High Sodium Diet for 1, 2 or 4 wk*

Four groups, each of 36 rats were fed with either regular or high sodium diet for 1, 2 or 4 *wk* (Table 1). Each diet group was further divided into 2 subgroups for different treatments. One subgroup was *ig* injected with terazosin, starting at 15 for the first day, 30 for the second day and afterwards 60 *mg/kg/day* until the end of the treatment (Kyncl, 1986), the other with 1 *ml/kg/day* distilled water as a control injection. Terazosin solutions were made up with distilled water. After the last injection of

terazosin on the morning of the surgery day, rats were cannulated for arterial BP and HR measurements for the 1 *wk* group, and LV pressure (LVP) and RAP measurements for the 2 and 4 *wk* groups. About 6 *hr* after the final dose of terazosin and 3 *hr* after surgery, baseline pressures and HR were recorded. Thereafter, 30 *mg/kg* hexamethonium was *iv* injected, followed by phenylephrine *iv* infusion at a rate of 1, 3 or 6 for rats without terazosin and 6, 12 or 24 *μg/kg/min* for those with terazosin treatment, and each infusion rate lasted 7 - 9 *min*. Similarly, after a second dose of hexamethonium, isoproterenol was *iv* infused at a rate of 0.05, 0.1 or 0.2 *μg/kg/min* and each infusion rate lasted 6 - 8 *min*. The peak responses of pressures were recorded at the end of each infusion rate.

II.3. *Protocol IIB. Effect of ß-Receptor Blockade on the Hemodynamics and on the Heart Chamber Weight and Dimensions of Rats on the High Sodium Diet for 4 wk*

Forty-two rats were divided into 2 groups and fed with either regular or high sodium diet for 4 *wk* (Table 1). Each diet group was divided into 3 treatment subgroups. One subgroup was *ig* administered 1 *ml/kg/day* distilled water as a control for the injection process, the second subgroup with 25 (with the first 2 days 12.5), and the third subgroup with 100 (with the first day 25 and the second day 50) *mg/kg/day* nadolol (Lee *et al.*, 1992). Nadolol solutions were made up with distilled water. At the end of the diet regimen, on the morning of the surgery day, the last dose of nadolol was given and

LV and RA catheterizations were performed. After recovery from surgery for 3 *hr*, baseline LVP and RAP were recorded. Then isoproterenol was *iv* infused at a rate of 0.1, 0.2 or 0.4 for rats without nadolol treatment, and 0.1, 0.2, 0.4, 0.8 or 1.6 *μg/kg/min* for rats treated with nadolol. Pressure recordings were taken at the peak responses for each dosage infused.

II.4. *Protocol IIC. Effect of Combined α_1- and β-Receptor Blockade on the Hemodynamics and Heart Chamber Weight and Dimensions of Rats on the High Sodium Diet for 4 wk*

Thirty-six rats were divided into 2 diet groups and fed with either regular or high sodium diet for 4 *wk* (Table 1). Each diet group was divided into 2 subgroups and were *ig* administered 1 *ml/kg/day* distilled water as a control for the injection process, or 60 *mg/kg/day* terazosin plus 100 *mg/kg/day* nadolol. Terazosin and nadolol solutions were made up with distilled water and administered by gavage every afternoon, 2 *hr* apart. After recovery for 3 *hr* and determination of baseline LVP and RAP, arterial blood was collected from the carotid catheter without applying any sucking force. Then after *iv* injection of 30 *mg/kg* hexamethonium, phenylephrine was *iv* infused at 1, 3 and then 6 or 6, 12, 24 or 48 *μg/kg/min* for rats without or with terazosin treatment, isoproterenol at 0.1, 0.2 or 0.4, or at 0.1, 0.2, 0.4, 0.8 or 1.6 *μg/kg/min* for rats without or with nadolol treatment, respectively. Again, the pressures were recorded at the peak responses for each dosage infused.

III. Surgery and Hemodynamic Measurements

III.1. *General*

Rats were anaesthetized with halothane-nitrous oxide-oxygen mixture. A midline incision was made along the front side of the neck, and afterwards, polyethylene 50 catheters (PE-50, Clay Adams, Becton Dickinson Labware, Lincoln Park, NJ) filled with heparin saline solution (150 - 200 *IU/ml* with Hepalean, Organon Teknika, Toronto, ON) were placed into the artery or heart chambers for pressure recordings, and another one into the external jugular vein for *iv* infusion of the drugs. All the catheters were exteriorized at the back of the neck through subcutaneous tunnels and locked with a piece of coloured adhesive plaster to the skin. After surgery, rats were permitted to recover for 3 *hr* from the anaesthesia and the surgical procedures. This recovery time has been shown to be sufficient for rats to recover from the anaesthesia (Kanda and Flaim, 1984; Yamamoto *et al*, 1980). A longer recovery period is inappropriate for rats with LV cannulation, since 1 or 2 days after such a cannulation rats are associated with increasingly severe aortic insufficiency and a higher mortality (Tsoporis *et al*, 1989).

Then the rats were put into the partitioned cage for containing rats during hemodynamic measurements, with the bottom of the cage set at the same level as the transducer. After an acclimatization period of 30 *min*, the catheters to the carotid artery or heart chambers was connected to a pressure transducer (Statham P23 ID, Statham-

Gould, Oxnard, CA). For CO measurements, the thermistor and the catheter for saline injection were connected to respective instruments, as described in the following. The jugular vein catheter was connected to a syringe mounted on an infusion pump (Harvard Apparatus, Millis, MA). Baseline pressures and HR were recorded in conscious rats on a Grass 7D multichannel chart recorder (Grass Instruments, Quincy, MA). Each transducer and the connected recording channel on the chart recorder was calibrated with a sphygmomanometer one day before measurements. At the peak response of each infusion rate, the same recordings were taken. At least two samples of the recordings were taken for both the baseline and each of the infusion rate of the drugs. Mean values were calculated from the two sample recordings.

III.2. *Arterial BP and HR*

PE-50 catheters were placed into right carotid artery for BP and HR recordings and into left external jugular vein for infusion of the drugs. Baseline BP was recorded on a Grass 7D multichannel chart recorder at a setting of 50 *mmHg/cm* and paper speed at 10 *mm/sec*. SBP, the peak of the BP pulsation, DBP, the bottom of the BP pulsation and HR (count from the pulsation of the BP tracing in certain time period) were read from the polygraph recordings. MAP was calculated with the equation: MAP = DBP + (pulse pressure/3), while the pulse pressure is the difference between SBP and DBP.

III.3. *CO, BP and HR*

CO was measured by the thermodilution technique as previously described (Lin *et al.*, 1970; Yuan and Leenen, 1991). For CO measurements, a PE-50 catheter was placed into the RA via the right external jugular vein for injection of thermal tracer indicator, another PE-50 catheter into the left external jugular vein for *iv* injection or infusion of the drugs. A PE-10 catheter was inserted into the left femoral artery for monitoring BP and HR. A thermistor (0.64 *mm* OD) was advanced into the aortic arch via the left common carotid artery and connected to a computer designed for cardiac output measurements (Cardiotherm-500) through a Cardiomax-II-R Interface (Columbus Instrument, Columbus, OH) and a Probe Selector (Columbus Instruments, Columbus, OH). The tip of the thermistor was to be located ideally in the centre of blood stream at the aortic arch, without touching the aortic wall or being left far away from the arch. Its depth was adjusted by observing chart recorder (Columbus Instruments, Columbus, OH) until the baseline was stable and the CO test curve on the chart recorder recorded after each thermal tracer injection was smooth and at the largest deflection with rapid recovery. The test was repeated several times to make sure that all of these features were reproducible. Immediately after BP and HR recordings, 200 μl normal saline at room temperature (21 - 22 °C) was injected into the RA as a thermal tracer indicator, and then the temperature at the arterial blood stream close to the aortic arch was sensed by the thermistor tip. The difference between the temperature of the normal saline injected and that of the blood stream at the aortic arch was calculated by Cardiotherm-500 to obtain the digitized CO readings. This procedure was repeated twice, and the mean of

the two readings was multiplied by 4 to obtain the CO values (*ml/min*). The same recording was taken at the peak response of each infusion rate of the drugs given. From the BP tracings and CO readings, MAP and the following parameters were calculated: cardiac index (CI, *ml/min/kg*) = CO/body weight; stroke volume index (SVI, *ml/beat/kg*) = CI/HR; total peripheral resistance index (TPRI, *mmHg/ml/min/kg*) = MAP/CI.

III.4. *LV and RA Pressures*

For the measurements of LVP and RAP, rats were catheterized under anaesthesia with PE-50 cannulas inserted into the LV via the right common carotid artery, to the RA via the right external jugular vein. Another cannula was put into the left external jugular vein for infusion of drugs. Left ventricular peak-systolic pressure (LVPSP), LV end-diastolic pressure (LVEDP) and HR were recorded in conscious rats at a setting of 50 *mmHg/cm* with a paper speed at 100 *mm/sec*. The RAP was recorded at a setting of 5 *mmHg/cm*, and paper speed at 100 *mm/sec*. RAP was determined during both inspiration and expiration. The values of RAP shown were those taken at the end of the expiration phase.

IV. **Drug Infusion**

After baseline hemodynamics were recorded twice for each rat, 30 *mg/kg* hexamethonium was slowly *iv* injected through the infusion catheter and flushed with saline to block the baroreceptor reflex influences on BP and HR. Pressure or CO recordings were taken when maximal decrease in the pressures appeared. These post-hexamethonium recordings were used as new baseline values. Afterwards, phenylephrine was infused through the catheter inserted into the left jugular vein at 3 - 4 dosages (see Table 1 and the experimental protocols) and each dosage for 7 - 9 *min*. After a recovery period of 30 *min*, another dose of 30 *mg/kg* hexamethonium was injected, and then isoproterenol was infused through the same catheter at 3 - 5 dosages (see protocols), each lasting for 6 - 8 *min*. The recovery time of 30 *min* before going on to isoproterenol infusion is sufficient for eliminating the effect of phenylephrine infused, as the latter has a short half-life. If the infusion time for the first two dosages, of either phenylephrine or isoproterenol, was longer than 15 *min*, then a maintenance dose of 10 *mg/kg* hexamethonium was given before continuing infusion. After recording of the post-infusion values for each drug, the infusion catheter was slowly flushed with normal saline.

V. Heart Chamber Weight and Dimensions

After recording of the resting and responsive hemodynamics, the rats were sacrificed with *iv* injection of an overdose of sodium pentobarbital (Somnotol, MTC Pharmaceuticals, Cambridge, ON) through the infusion catheter, followed by flushing

with 1 *M* KCl solution to arrest the heart in diastole (Ruzicka *et al.*, 1993). Heart anatomy was done as described by Tsoporis *et al.* (1989). After a midline incision through the sternum, the heart was rapidly excised and placed into ice-cold saline to maintain the heart in diastole and to remove the blood. Both the atria and big vessels were trimmed off, then RV free wall was cut off carefully from the interventricular septum. The LV (LV free wall plus interventricular septum) and RV wet weights were measured on a balance after the slices being blotted dry. A transverse midlevel slice of LV was obtained by two cross-sectional cuts at one-third and two-thirds on the longitudinal axis, and was viewed under a light microscope equipped with a calibrated ocular lens (Macrometer, Olympus, Tokyo, Japan). The LV wall thickness was measured at 8 points around the circular section, and the average was calculated. The internal diameters of the LV were measured from the farthest points of the major (anterior-posterior) and minor (septal-lateral) internal diameters. The mean value of the diameters was divided by 2 to obtain the radius, which was used for calculation of the ratio of LV wall thickness to radius. When reading under the microscope, care was taken to avoid including images from epicardium, papillary muscles, or trabeculae carneae. The readings of the wall thickness and internal diameters under the microscope was transformed to *mm* by dividing them by a factor of 16.75. The RV free wall and the three LV slices were then put into an oven (60 ^{o}C) for 24 *hr*, and their dry weights were measured with the same balance used for wet chamber weight measurements. Both the wet and dry weights were normalized for individual body weight, to get the relative chamber weight for comparison between different groups. The ratio of the dry weight to corresponding wet weight of the ventricles, and both the ratio of LV wet to RV wet

weights and of LV dry to RV dry weights were calculated.

VI. Haematocrit Measurements

Haematocrit was measured according to Wiseman and Irving (1963). After baseline hemodynamic recordings were finished, the arterial blood was collected into heparinized microhematocrit capillary tubes (Fisherbrand, Allied Corporation, Fisher Scientific, Pittsburgh, PA) from the carotid catheter without applying any suction force to the cannula. The first four drops of arterial blood was discarded, and collecting was started from the fifth drop. The capillary tubes filled up with fresh arterial blood was centrifuged (Hettich Haematocrit, D-7200 Tuttlingen, Germany) for 2 *min* and haematocrit readings were taken from the scale. After collecting the blood sample, the catheter was flushed with saline with a volume similar to the blood collected, making sure that there was no visible blood trace left inside the catheter.

VII. Statistical Analysis

Body weight, water intake, haematocrit, heart chamber weight and dimensions and resting hemodynamic values are expressed as mean ± SEM for each group. Hemodynamic changes in response to agonist infusion or hexamethonium injection are expressed as $\delta \pm$ SEM for each dosage *vs.* baseline values. The data was analyzed by

analysis of variance, and the Duncan multiple range test was used for post hoc analysis. Values of $p < 0.05$ were considered statistically significant.

RESULTS

I. Experimental Protocol I: Effect of Medium High or High Sodium Diet for 1, 2 or 6 *wk* on the Resting Hemodynamics, Heart Chamber Weight and Dimensions and α- or ß-Adrenergic Responses of Rats

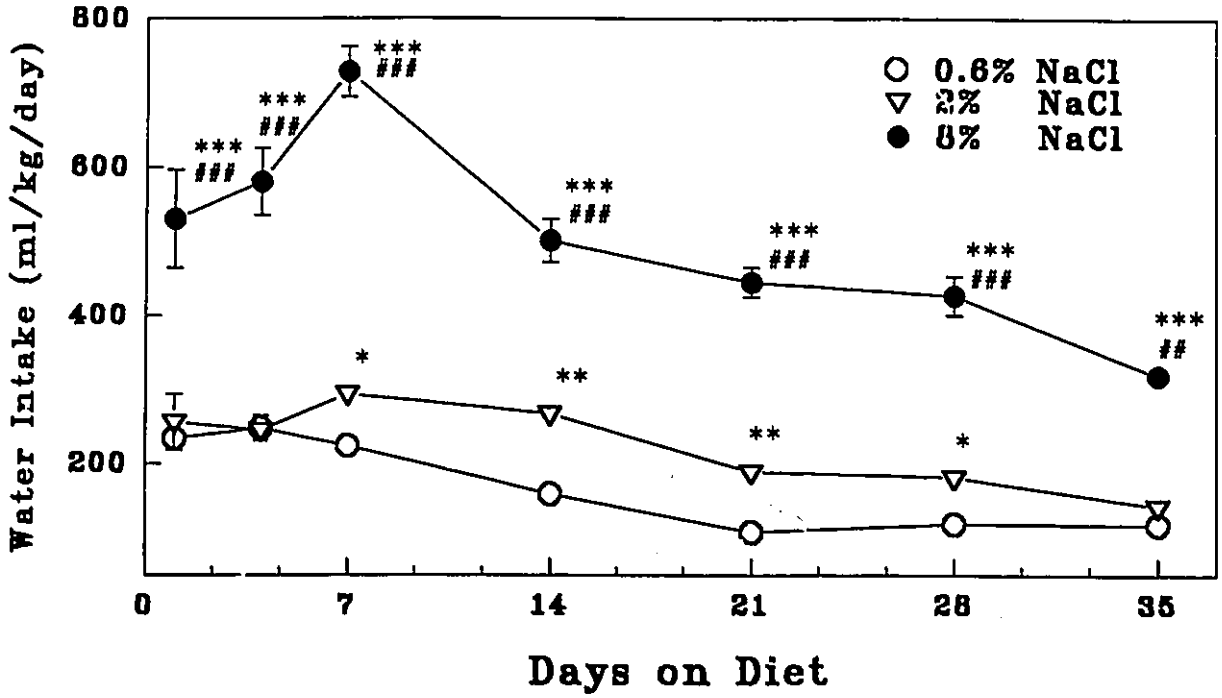
I.1. *Water Intake and Body Weight*

Four *wk* old WKY rats on a regular diet drank 234 $\pm$ 10 *ml/kg/day* during the first several days (Fig. 2). This amount gradually decreased to 118 $\pm$ 8 *ml/kg/day* after being on diet for 3 *wk*. For young WKY rats on a high sodium diet, their water intake was increased significantly by more than two-fold during the initial days. During the following 5 *wk* their water intake remained about 3 times higher than that of rats on a regular diet. The extent of increase in water intake by a medium high sodium diet was markedly smaller than that by a high sodium diet, and this increase in water intake was not significant versus the water intake of rats on a regular diet until day 7 after starting diet and thereafter remained significantly higher through the 4 *wk*. Similar changes in water intake by high or medium high sodium diets were also seen in WKY rats on a diet regimen for 1 or 2 *wk* (data not shown).

The mean body weight values measured once a week showed a significant and similar decrease by both the high and medium high sodium diets, starting from the first

Fig. 2. **Daily water intake of WKY rats on the medium high or high sodium diet for 6 *wk*.**

Twenty-four *hr* water intake (*ml/kg/day*) was monitored by measuring the bottle weight change from 14:00 to next day 14:00 on day 1, 4, or 7, and then once a week. Rats were kept 2 per cage and put on high sodium diet (8% NaCl, ●), medium high sodium diet (2% NaCl, ∇) or regular diet (0.6% NaCl, ○) from 4 to 10 *wk* of age. Data are means ± SEM (n = 9 to 10). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ medium high or high sodium diet groups *vs*. the regular diet group; ### $p < 0.001$ high sodium diet group *vs*. the medium high sodium diet group.


800
600
400
200
Water Intake (ml/kg/day)
0
7
14
21
28
35
Days on Diet
0.6% NaCl
2% NaCl
8% NaCl
*** ###
*** ###
*** ###
*** ###
*** ###
*** ###
*** ##
*
**
**
*

wk and continuing to the last *wk* of the diet (Fig. 3). The high or medium high sodium diets for 1 or 2 *wk* also showed a similar decrease in the mean body weights (data not shown). This decrease resulted from a reduction in the body weight gain by the sodium exposure, and the reduction in the gain was only seen in the first *wk* of the diet regimen.

I.2. *Heart Chamber Weight and Dimensions*

The relative LV weight (in *mg* per 100 *g* body weight) was not significantly changed by the high sodium diet for 1 *wk* (Table 2), while significantly increased by 12% in the 2 *wk* group and, by 15% in the 6 *wk* group ($p < 0.001$). In both the 2 and 6 *wk* groups, the relative RV dry weight was also increased significantly by the high sodium diet ($p < 0.05$). The relative LV dry weight was also significantly increased by the medium high sodium diet for 2 or 6 *wk*. The ratio of LV to RV weight was between 3.2 to 3.8, being similar between the groups on 1 to 6 *wk* medium high or high sodium diet and on the regular diet (Table 3). The ratio of dry weight to wet weight of both LV and RV in all the 1, 2 or 6 *wk* groups was between 24% to 26%, and it did not show any significant differences between the regular diet and the high or medium high sodium diet groups.

The high sodium diet did not change the LV internal diameters, but significantly increased the wall thickness in both the 1 and 2 *wk* groups (Table 2). The ratio of the LV wall thickness to radius was increased by the medium high and high sodium diet in the 2 *wk* group ($p < 0.05$), showing that a concentric LVH was induced by this high

Fig. 3. **Body weight of WKY rats on the medium high or high sodium diet for 6 *wk*.**

Body weight (*g*) was measured one day before starting high sodium diet (8% NaCl, ●) or medium high sodium diet (2% NaCl, ▽), and afterwards, once a week at noon. The regular diet contained 0.6% sodium chloride (0.6% NaCl, ○). Data are mean ± SEM (n = 9 to 10). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ medium high or high sodium diet groups *vs.* the regular diet group.

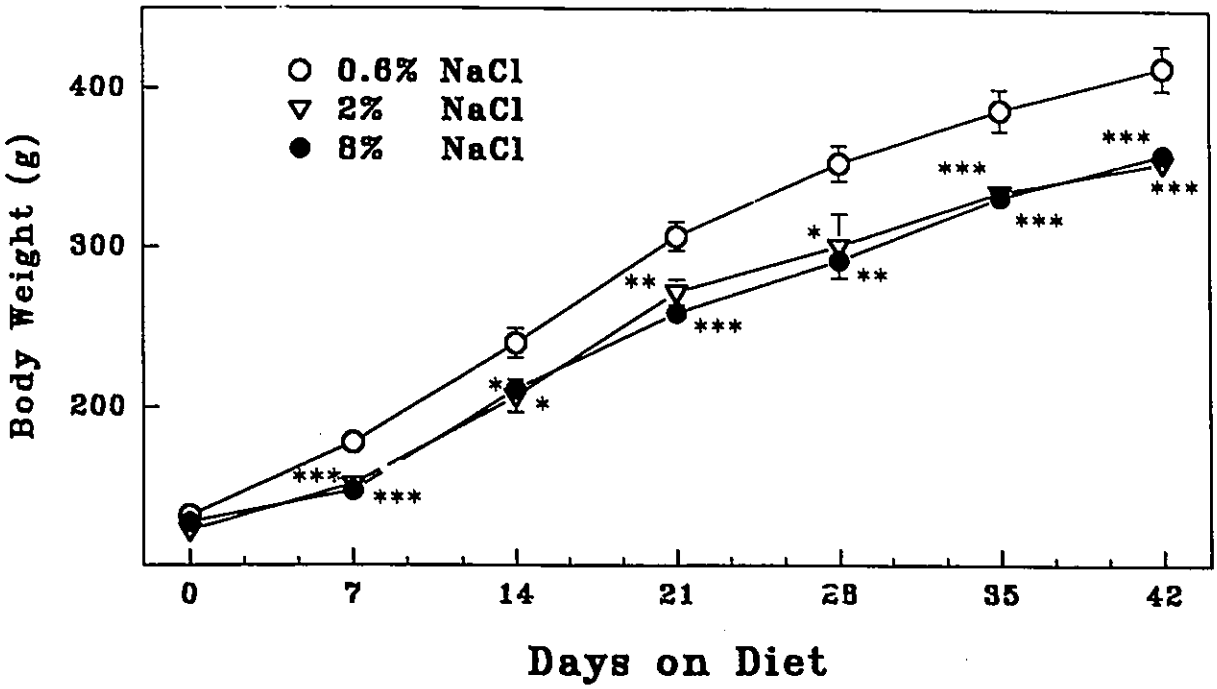

0.6% NaCl
2% NaCl
8% NaCl
Body Weight (g)
400
300
200
0
7
14
21
28
35
42
Days on Diet

Table 2. Heart Chamber Weight and Dimensions of WKY Rats on the Medium High or High Sodium Diet for 1, 2 or 6 *wk*

Group	(n)	Body Weight (*g*)	Heart Chamber Weight (*mg*/100 *g* body wt)				LV Wall Thickness (*mm*)	Internal Diameter (*mm*)	LV Wall Thickness/ Radius
			LV wet	LV dry	RV wet	RV dry			
1 week									
0.6%	(8)	202±4	216± 5	54± 2	56± 2	15.6±0.4	1.22±0.03	4.69±0.12	0.53±0.03
8%	(9)	184±4**	220± 6	57± 1	61± 2	17.2±0.6	1.35±0.04*	4.72±0.22	0.59±0.04
2 week									
0.6%	(9)	257±4	197± 5	51± 1	61± 1	16.9±0.4	1.33±0.05	5.23±0.24	0.52±0.04
2%	(9)	242±3*	217± 5**	55± 1*	63± 3	17.2±0.5	1.59±0.07**	5.38±0.18	0.59±0.04*
8%	(10)	234±4***	227± 3***	59± 1***′	65± 2	19.0±0.6**″	1.60±0.05***	5.12±0.14	0.63±0.03**
6 week									
0.6%	(10)	414±14	199± 4	48± 1	57± 2	13.5±0.4	---	---	---
2%	(10)	354±5***	213±3**	52±1***	58± 1	13.3±0.4	---	---	---
8%	(10)	359±7***	229±2***″′	55±1***″′	64± 2″	15.1±0.4*″	---	---	---

Values are mean ± SEM for n rats in each group. 0.6%, regular diet containing 0.6% sodium chloride; 2% or 8%, medium high or high sodium diet (2% or 8% NaCl).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* the regular diet group (0.6%);

′ $p < 0.05$, ″ $p < 0.01$, ″′ $p < 0.001$ *vs.* the medium high sodium diet group (2% NaCl).

--- 6 *wk* dimension data not available due to technical error.

Table 3. Heart Chamber Weight (Absolute Values) of WKY rats on the Medium High or High Sodium Diet for 1, 2 or 6 *wk*

Group	(n)	Body Weight (*g*)	Heart Chamber Weight (*mg*)				LV/RV Ratio
			LV wet	LV dry	RV wet	RV dry	
1 week							
0.6%	(8)	202±4	435± 5	110± 1	114± 3	32± 1	3.85± 0.11
8%	(9)	184±4**	406± 18	105± 3	112± 4	32± 1	3.63± 0.13
2 week							
0.6%	(9)	257±4	505± 16	131± 4	158± 4	43± 1	3.21± 0.08
2%	(9)	242±3*	523± 12	133± 2	152± 8	42± 2	3.52± 0.19
8%	(10)	234±4***	530± 10	139± 4	151± 5	45± 2	3.52± 0.19
6 week							
0.6%	(10)	414±14	820± 20	198± 6	237± 9	56± 3	3.49± 0.12
2%	(10)	354±5***	754± 15*	183± 3*	204± 7*	47± 2**	3.72± 0.08
8%	(10)	359±7***	825± 19′	200± 5′	228± 8′	54± 1′	3.63± 0.10

Values are mean ± SEM for n rats in each group. 0.6%, regular diet containing 0.6% sodium chloride 2% or 8%, medium high or high sodium diet (2 or 8% NaCl).
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* the regular diet group (0.6%);
′ $p < 0.05$ *vs.* the medium high sodium diet group (2%).

sodium intake.

In contrast to the changes in the relative heart chamber weights, the absolute values of both the LV and RV weights, either in the form of a wet or dry weight, did not show any significant effects by the high or medium high sodium diet in the 1, 2 or 6 *wk* diet groups (Table 3). The absolute LV and RV wet and dry weights were significantly lower in the 6 *wk* group on the medium high sodium diet than that on a regular diet (Table 3), likely caused by the reduced body weight gain by a medium high sodium diet.

I.3. *Resting Hemodynamics*

The medium high or high sodium diet for 1, 2 or 6 *wk* did not change the resting BP (either SBP or DBP), neither did the CI, SVI or TPRI in the 2 and 6 *wk* groups (Table 4). The HR was not changed by the high sodium diet for 1, 2 or 6 *wk*, but significantly increased by the medium high sodium diet in both the 2 and 6 *wk* groups.

I.4. *Responses to Ganglionic Blockade*

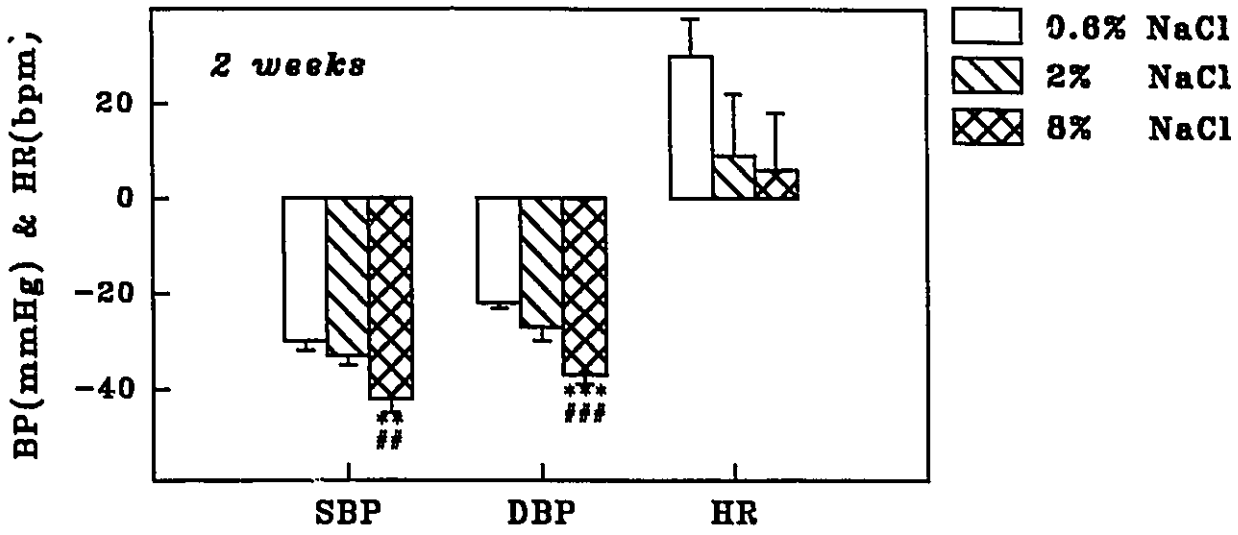
Both SBP and DBP of all the groups on various diets for 2 or 6 *wk* showed a significant decrease in responses to the *iv* injection of 30 *mg/kg* hexamethonium (Fig. 4). In comparison with the responses of the groups on regular diet, the decreases were

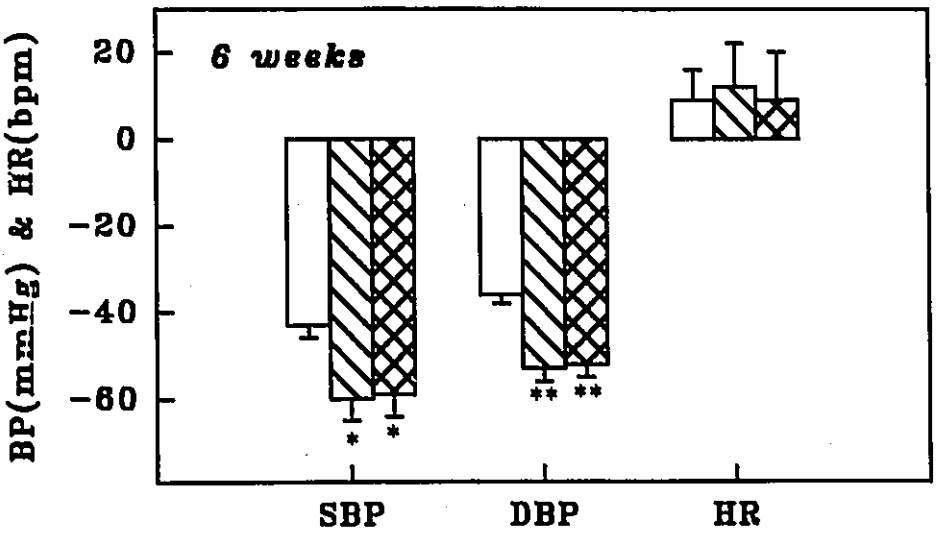
Table 4. Resting Hemodynamics of WKY Rats on the 1, 2 or 6 *wk* Medium High or High Sodium Diet

Group	(n)	SBP (*mmHg*)	DBP (*mmHg*)	HR (*bpm*)	CI (*ml/min/kg*)	TPRI (*mmHg/ml/min/kg*)	SVI (*ml/beat/kg*)
<u>1 week</u>							
0.6%	(8)	116±3	95±9	382± 5	ND	ND	ND
8%	(9)	116±2	93±2	422±19	ND	ND	ND
<u>2 week</u>							
0.6%	(9)	108±4	88±3	384±12	348±22	0.28±0.02	0.90±0.05
2%	(9)	113±5	96±3	441±15**	367±41	0.30±0.03	0.83±0.09
8%	(10)	108±4	87±4	417±12	349±24	0.29±0.03	0.83±0.05
<u>6 week</u>							
0.6%	(10)	121±4	100±2	369±8	325±24	0.35±0.03	0.89±0.07
2%	(10)	135±4	113±3**	439±17***	311±22	0.41±0.03	0.71±0.05
8%	(10)	122±6	106±5	401±10	346±15	0.33±0.03	0.87±0.05

Values are mean ± SEM for n rats in each group. 0.6%, regular diet containing 0.6% NaCl; 2% or 8%, medium high or high sodium diet (2 or 8% NaCl).
SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; CI, cardiac index; TPRI, total peripheral resistance index; SVI, stroke volume index.
** $p < 0.01$, *** $p < 0.001$ *vs.* the regular diet group (0.6%). ND, data not determined.

Fig. 4. **Arterial blood pressure and heart rate (HR) of WKY rats in response to hexamethonium after a 2 or 6 *wk* high or medium high sodium diet.** Arterial pressures (SBP and DBP) and HR were recorded via the carotid artery catheter both before and after *iv* injection of hexamethonium 30 *mg/kg*. The change of SBP, DBP and HR were calculated for rats on 2 (top panel) or 6 *wk* (bottom panel) regular diet (0.6% NaCl, blank bars), medium high sodium diet (2% NaCl, slashed bars) or high sodium diet (8% NaCl, cross-lined bars). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ medium high or high sodium diet groups *vs.* the regular diet group; # $p < 0.05$, ## $p < 0.01$ high sodium diet group *vs.* the medium high sodium diet group.


2 weeks
BP(mmHg) & HR(bpm)
20
0
-20
-40
SBP
DBP
HR
0.6% NaCl
2% NaCl
8% NaCl


6 weeks
BP(mmHg) & HR(bpm)
20
0
-20
-40
-60
SBP
DBP
HR

significantly augmented by the high sodium diet for 2 or 6 *wk*, and by the medium high sodium diet for 6 *wk* ($p < 0.05$). The decrease in BP was mainly from a decrease in TPRI (shown in Table 5 by the negative signs). After hexamethonium injection, HR was increased, but this increase was significant only in the 2 *wk* group on a regular diet (Fig. 4).

The decrease in SBP and DBP in response to hexamethonium was similar but smaller in the 1 *wk* diet groups (data not shown). Different from the results of the 2 and 6 *wk* diet groups, the high sodium diet for 1 *wk* did not augment this decrease. The HR increase in response to the hexamethonium injection was significant only in the group on a regular diet (data not shown).

I.5. *Responses to Adrenergic Stimulation*

Phenylephrine infusion significantly increased MAP in a dose-dependent manner (Fig. 5, upper panel), due to a remarkable increase in TPRI (Fig. 6, upper panel). At the doses used, phenylephrine did not show any significant effect on CI (Fig. 6, lower panel), but decreased the HR ($p < 0.05$) (Fig. 5, lower panel). Comparing the hemodynamic responses to phenylephrine infusion in rats on a high sodium diet with those on a regular diet for 1, 2 or 6 *wk*, similar patterns were observed over time. Only in rats after the 6 *wk* high sodium diet, the decrease in HR associated with high doses of phenylephrine infusion was augmented significantly, compared with the HR decrease in animals on a regular diet (Fig. 5, lower panel).

Table 5. Hemodynamic Responses to 30 *mg/kg* Hexamethonium Injection of WKY rats on the Medium High or High Sodium Diet for 2 or 6 *wk*

Group (n)	CI (*ml/min/kg*)	TPRI (*mmHg/ml/min/kg*)	SVI (*ml/beat/kg*)
<u>2 week</u>			
0.6% (9)	-29 ± 17	-0.05 ±0.02	-0.18 ±0.05
2% (9)	38 ± 37	-0.11 ±0.03	0.06 ±0.11
8% (10)	5 ± 33	-0.11 ±0.02	-0.02 ±0.10
<u>6 week</u>			
0.6% (10)	33 ± 20	-0.18 ±0.03	0.06 ±0.05
2% (10)	-65 ± 23	-0.11 ±0.02	-0.20 ±0.07 *
8% (10)	-5 ± 25	-0.15 ±0.02	-0.07 ±0.08

Values are $\delta \pm$ SEM for n rats in each group; 0.6%, regular diet containing 0.6% NaCl; 2% or 8%, medium high or high sodium diet (2 or 8% NaCl);
CI, cardiac index; TPRI, total peripheral resistance index; SVI, stroke volume index.
* $p < 0.05$ *vs.* the regular diet group (0.6%).

Fig. 5. **Mean arterial pressure (MAP) and heart rate (HR) responses to phenylephrine infusion of WKY rats after a high sodium diet for 1 to 6 *wk*.**

Phenylephrine was infused 3 *hr* after surgery at 1, 3 or 6 *μg/kg/ min*, each for 7 - 8 *min* through the left jugular vein catheter, after *iv* injection of 30 *mg/kg* hexamethonium. Responses of MAP (top panels) or HR (bottom panels) was recorded for rats on the high sodium diet (8% NaCl, ●) or regular diet (0.6% NaCl, ○) for 1 *wk* (left panels), 2 *wk* (central panels) or 6 *wk* (right panels). Data are $\delta \pm$ SEM ($n = 8$ to 10). * $p < 0.05$ high sodium diet group *vs.* the regular diet group.

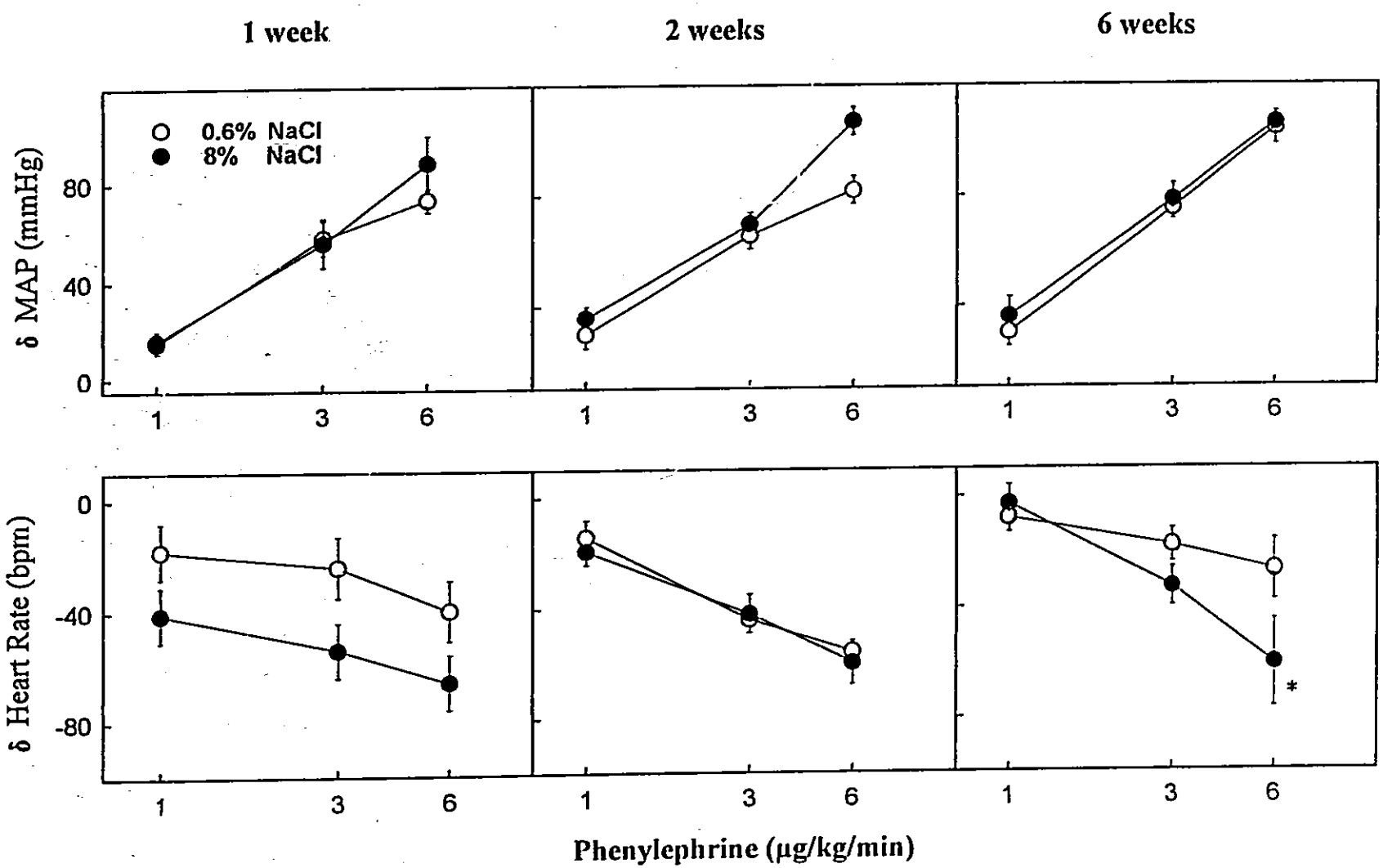
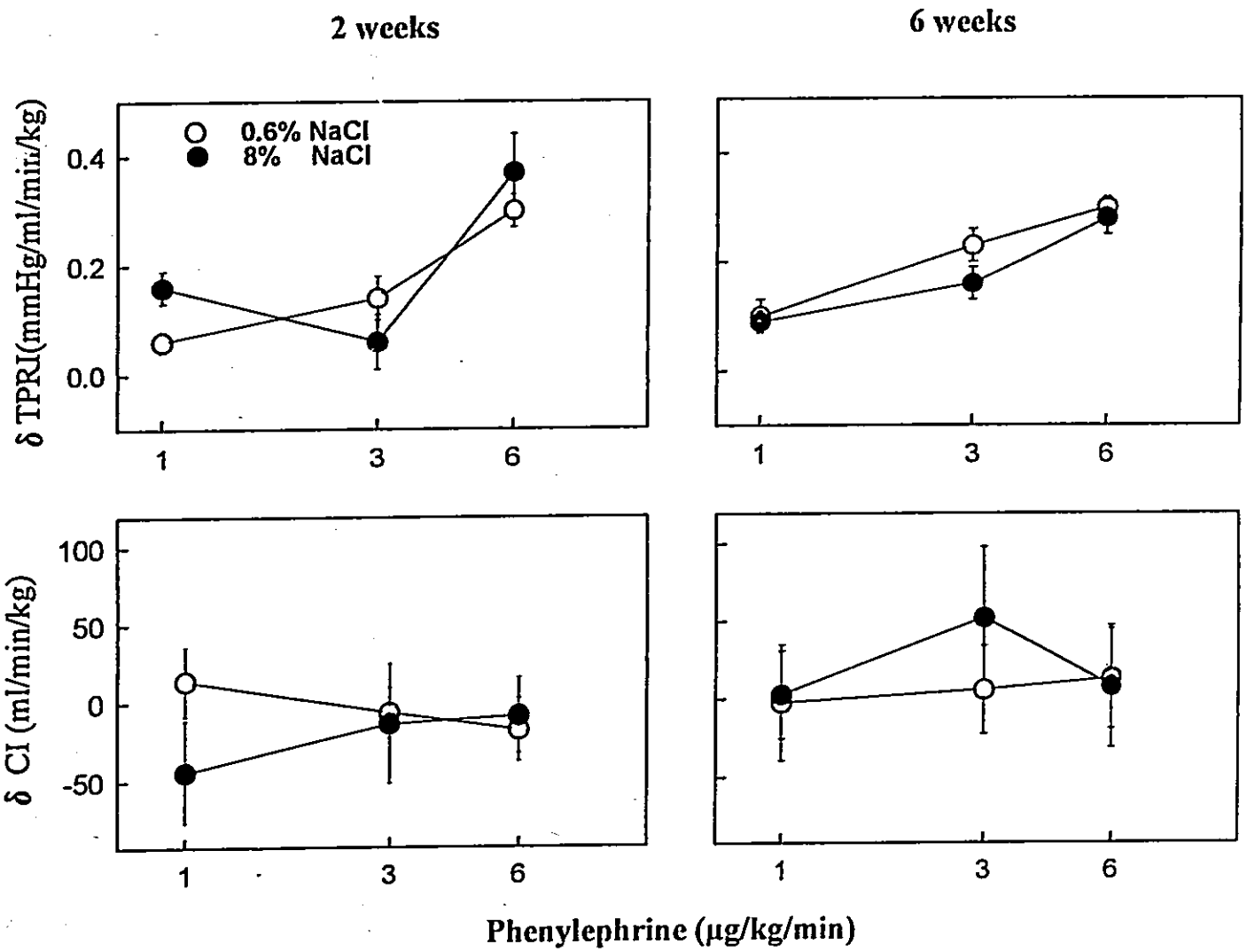

1 week
2 weeks
6 weeks
0.6% NaCl
8% NaCl
δ MAP (mmHg)
80
40
0
δ Heart Rate (bpm)
0
-40
-80
1
3
6
*
Phenylephrine (µg/kg/min)

Fig. 6. **Total peripheral resistance index (TPRI) and cardiac index (CI) responses to phenylephrine infusion of WKY rats after a high sodium diet for 2 to 6 *wk*.**

At the end of 2 (left panels) or 6 *wk* (right panels) high sodium diet (8% NaCl, ●) or regular diet (0.6% NaCl, ○), cardiac output (CO, in *ml/min*) was measured by thermodilution technique, while phenylephrine was infused (see Fig. 5 for details). Then CI (bottom panels) was calculated by dividing CO with body weight (*kg*), and TPRI (top panels) by dividing MAP with CI. Data are $\delta \pm$ SEM (n = 8 to 10).


2 weeks
6 weeks
0.6% NaCl
8% NaCl
δ TPRI(mmHg/ml/min/kg)
0.4
0.2
0.0
1
3
6
δ CI (ml/min/kg)
100
50
0
-50
Phenylephrine (μg/kg/min)

Isoproterenol infusion significantly decreased MAP and increased HR also in a dose-dependent manner (Fig. 7). The decrease in MAP was due to a decrease in TPRI ($p < 0.05$), in part offset by a significant increase in CI (Fig. 8). When comparing rats on a regular diet with those on a high sodium diet, no significant differences were seen in the BP responses to isoproterenol in the 1 or 2 *wk* groups (Fig. 7, upper panel), or in the CI responses of the 2 or 6 *wk* groups (Fig. 8, lower panel). After the 6 *wk* diet regimen, the MAP decrease in response to the high doses of isoproterenol was less pronounced in rats on a high sodium diet than that of the rats on a regular diet ($p < 0.05$, Fig. 7, upper panel). This attenuation was similar in both SBP and DBP responses to isoproterenol. Over time, no differences in HR responses were seen between the groups on a regular and on a high sodium diet.

II. Experimental Protocol IIA, B and C: Effect of α_1- and/or ß-Receptor Blockade on Hemodynamics and Heart Chamber Weight and Dimensions of Rats on the High Sodium Diet

II.1. *Water Intake, Body Weight and Haematocrit*

The water intake profile for rats on a regular diet for 4 *wk* (Fig. 9) was similar to that from protocol I (Fig. 2). This water intake profile was not changed by chronic treatment with α_1-, ß-blocker or α_1- and ß-blocker (Fig. 9).

Fig. 7. **Mean arterial pressure (MAP) and heart rate (HR) responses to isoproterenol infusion of WKY rats after a high sodium diet for 1 to 6 *wk*.**

Isoproterenol was infused 4 *hr* after surgery at 0.05, 0.1 or 0.2 *μg/kg/min* and each infusion rate for 5 - 7 *min*, through the left jugular vein catheter, after *iv* injection of 30 *mg/kg* hexamethonium. Responses of MAP (top panels) and HR (bottom panels) was recorded for rats on high sodium diet (8% NaCl, ●) or regular diet (0.6% NaCl, ○) for 1 *wk* (left panels), 2 *wk* (central panels) or 6 *wk* (right panels). Data are $\delta \pm$ SEM (n = 5 to 7). * $p < 0.05$ high sodium diet group *vs.* regular diet group.

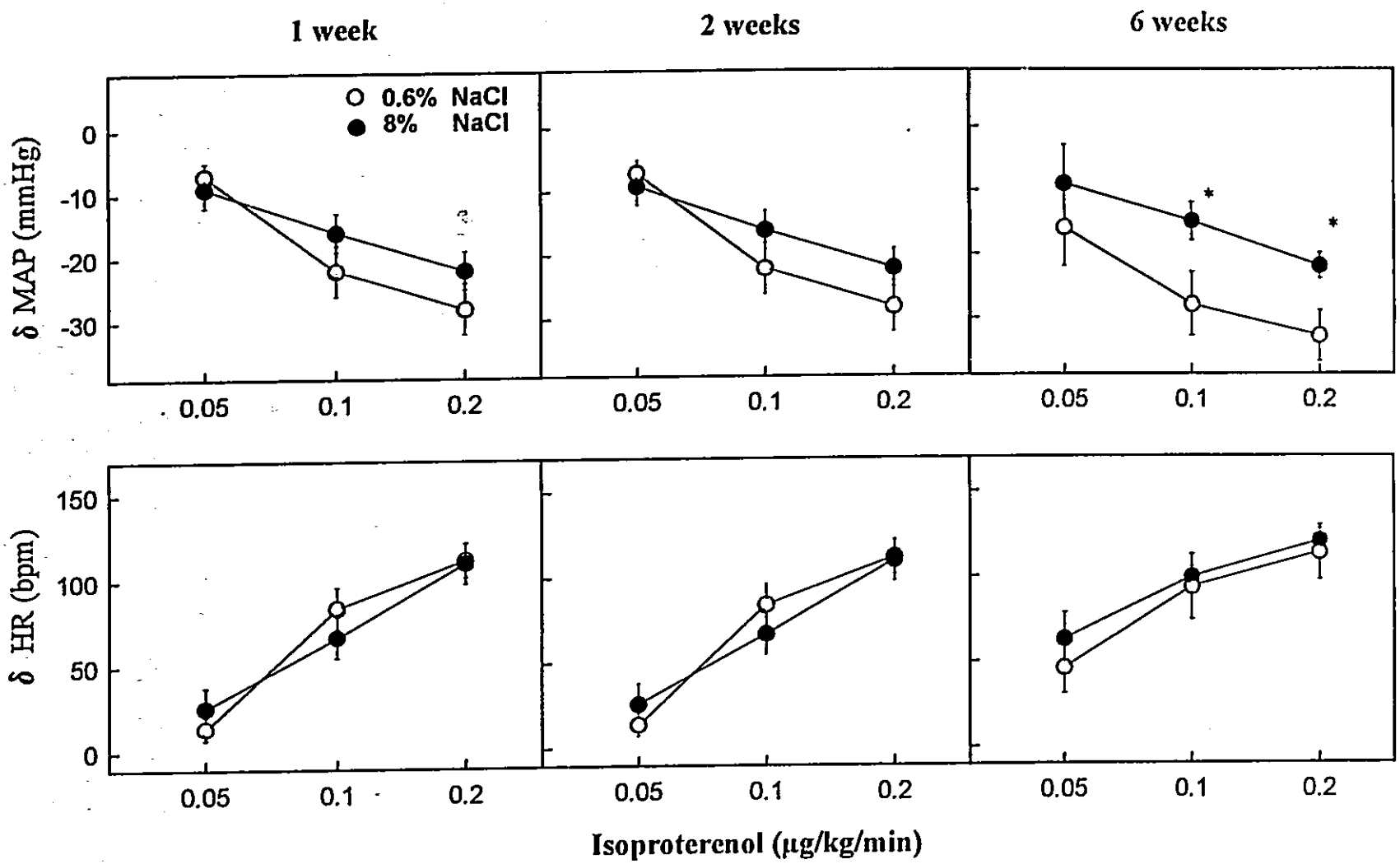

1 week
2 weeks
6 weeks
0.6% NaCl
8% NaCl
δ MAP (mmHg)
0
-10
-20
-30
*
*
0.05
0.1
0.2
δ HR (bpm)
150
100
50
0
Isoproterenol (μg/kg/min)

Fig. 8. **Total peripheral resistance index (TPRI) and cardiac index (CI) responses to isoproterenol infusion of WKY rats after a high sodium diet for 2 to 6 *wk*.**

At the end of 2 *wk* (left panels) or 6 *wk* (right panels) high sodium diet (8% (8% NaCl, ●) or regular diet (0.6% NaCl, ○), cardiac output (CO, in *ml/min*) was measured by thermodilution technique, while isoproterenol was infused (see Fig. 7 for details). CI (bottom panels) was calculated by dividing CO with body weight (*kg*), and TPRI (top panels) by dividing MAP with CI. Data are $\delta \pm$ SEM (n = 5 to 7).

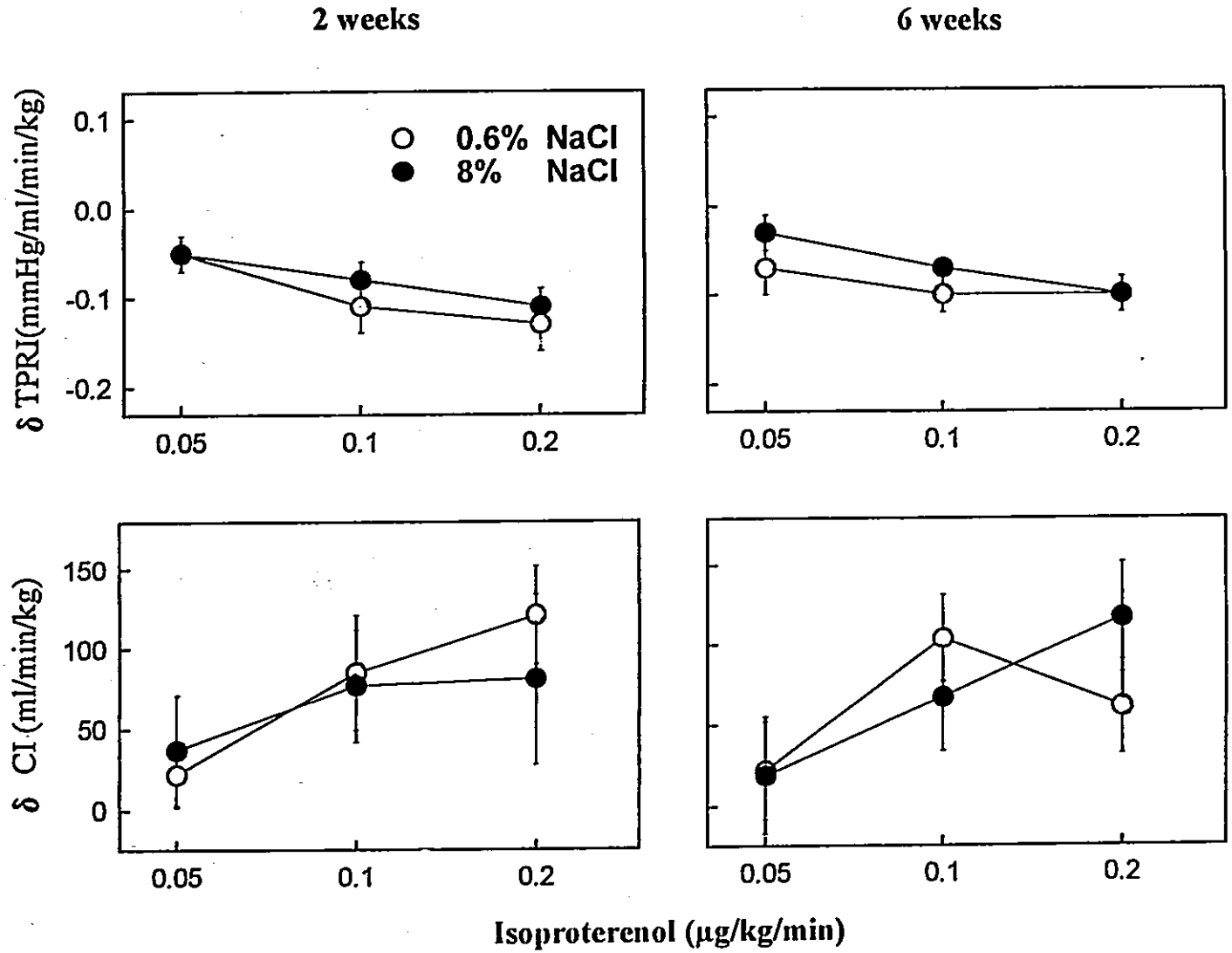
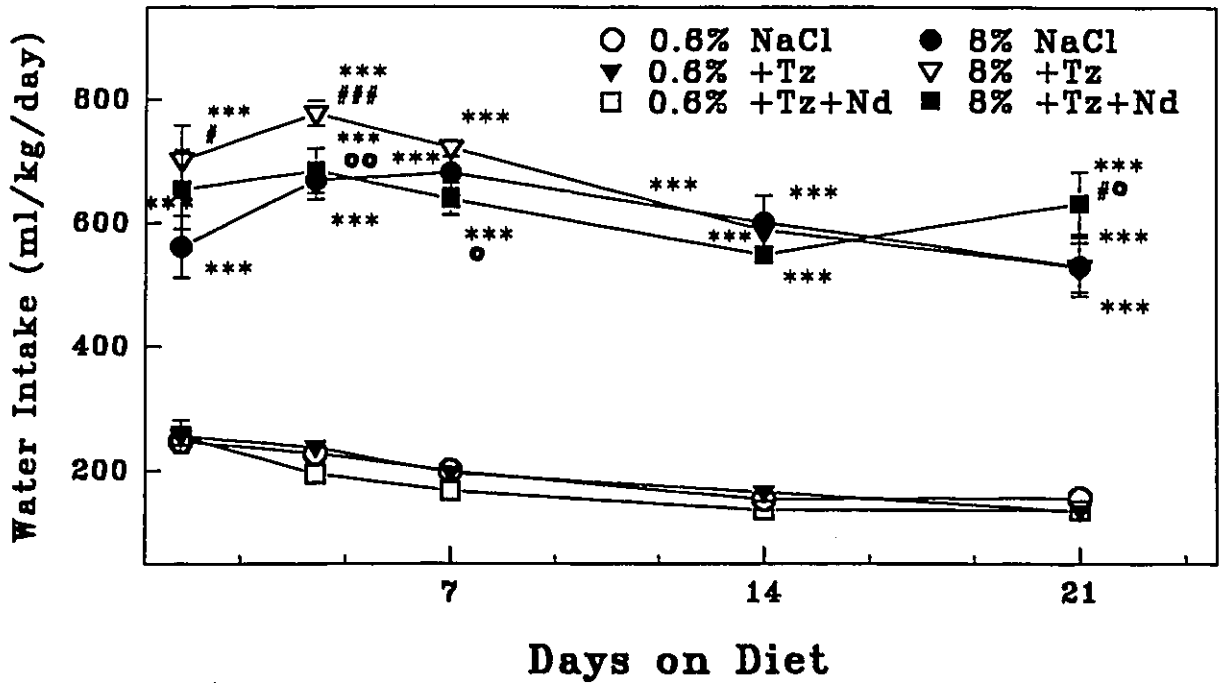

2 weeks
6 weeks
0.6% NaCl
8% NaCl
δ TPRI(mmHg/ml/min/kg)
0.1
0.0
-0.1
-0.2
0.05
0.1
0.2
δ CI (ml/min/kg)
150
100
50
0
Isoproterenol (μg/kg/min)

Fig. 9. **Daily water intake of WKY rats on the 4 *wk* high sodium diet with terazosin and/or nadolol treatment.**

Twenty-four *hr* water intake (*ml/kg/day*)was monitored by measuring the bottle weight change from 14:00 to next day 14:00 on day 1, 4, or 7, and then once a week. Rats were kept 2 per cage and put on high sodium diet (8% NaCl, ●) or regular diet (0.6% NaCl, ○) from 4 to 8 *wk* of age, with gavage injection of distilled water 1 *ml/kg/day* as a control for the injection process. In the groups on a regular or high sodium diet, terazosin 60 *mg/kg/day* (Tz) (▼ or ▽), or same dose terazosin plus nadolol 100 *mg/kg/day* (Tz + Nd) (□ or ■) were given by gavage, starting two days prior to the diet regimen and being continued for 4 *wk*. In the combined blockade groups, the injections were given 2 *hr* apart. Data are means ± SEM (n = 8 to 9). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ high sodium diet groups with terazosin and/or nadolol or distilled water injection *vs* the regular diet group with injection of the same agent; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ high sodium diet group with terazosin and/or nadolol *vs*. the high sodium diet group with distilled water injection; $^{@}p < 0.05$, $^{@@}p < 0.01$ high sodium diet group with terazosin and nadolol *vs*. the high sodium diet group with terazosin only.


0.6% NaCl
8% NaCl
0.6% +Tz
8% +Tz
0.6% +Tz+Nd
8% +Tz+Nd
Water Intake (ml/kg/day)
800
600
400
200
7
14
21
Days on Diet

For rats on a high sodium diet for 4 *wk*, the extent of increase in water intake in comparison with that of rats on a regular diet was also similar to the result from 1 to 6 *wk* diet regimen protocols. This increase in water intake was not changed by a concomitant *ig* injection of ß-blocker, nadolol (Fig. 10). Compared to the group on a high sodium diet without drug treatment, the increase was significantly augmented during the first *wk* by the α_1-blocker, terazosin ($p < 0.05$) (Fig. 9). However, the combined blockade with both nadolol and terazosin did not show such an augmentation (Fig. 9). Similar augmentation on the increase in water intake was seen in rats on a high sodium diet for both 1 and 2 *wk* (data not shown).

The mean body weight values of rats was not decreased by a 4 *wk* high sodium diet (Fig. 11). A similar result of the high sodium diet was also found in rats on a 1 or 2 *wk* diets and again another group on 4 *wk* diet when nadolol was tested (data not shown). These results showed a discrepancy with the observations on body weight from 1, 2 or 6 *wk* diet groups (Fig. 3).

The mean body weight of rats treated only with terazosin or terazosin and nadolol for 4 *wk* did not show significant changes, except for those of rats on a high sodium diet plus treatments with the combined antagonists, which was significantly lower than that of the rats on a regular diet with injection of the same blockers (Fig. 11). This lowered mean body weight was due to a decrease in the body weight gain (data not shown), which was observed only in the first *wk* starting the diet regimen and drug treatment (data not shown). However, the lowered mean body weight remained throughout the 4 *wk* of diet regimen. Conversely, rats on a high sodium diet and treated with terazosin for 1 or 2 *wk* did not show a decrease in their mean body weights (data not shown).

Fig. 10. **Daily water intake of WKY rats on the 4 *wk* high sodium diet with nadolol treatment.**

Twenty-four *hr* water intake (*ml/kg/day*) was monitored by measuring the bottle weight change from 14:00 to next day 14:00 on day 1, 4, or 7, and then once a week. Rats were kept 2 per cage and put on high sodium diet (8% NaCl, ●) or regular diet (0.6% NaCl, ○) from 4 to 8 *wk* of age, and injected by gavage with 1 *ml/kg/day* distilled water as a control for the injection process. In the regular or high sodium diet group, nadolol 25 (Nd25) (▼ or ∇) or 100 (Nd) (□ or ■) *mg/kg/day* was given by gavage, starting two days prior to diet and continuing for 4 *wk*. Data are means ± SEM (n = 5 to 7). *** $p < 0.001$ high sodium diet group with nadolol or distilled water injection *vs*. the regular diet group with the same injection.

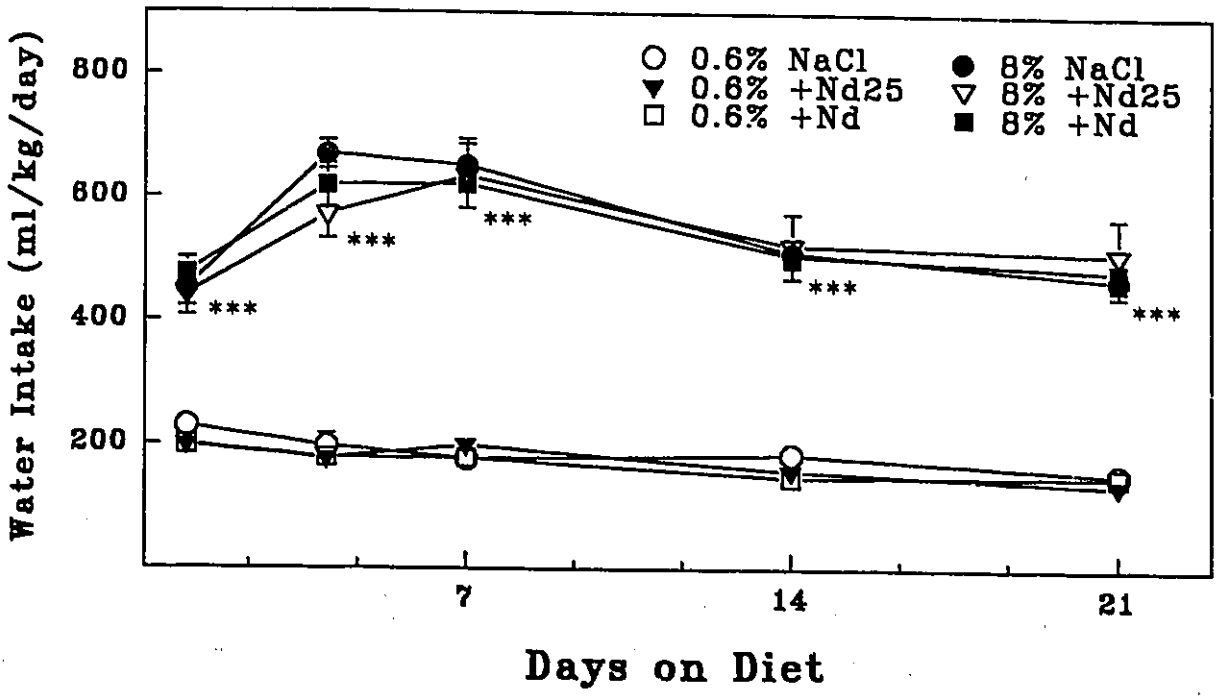
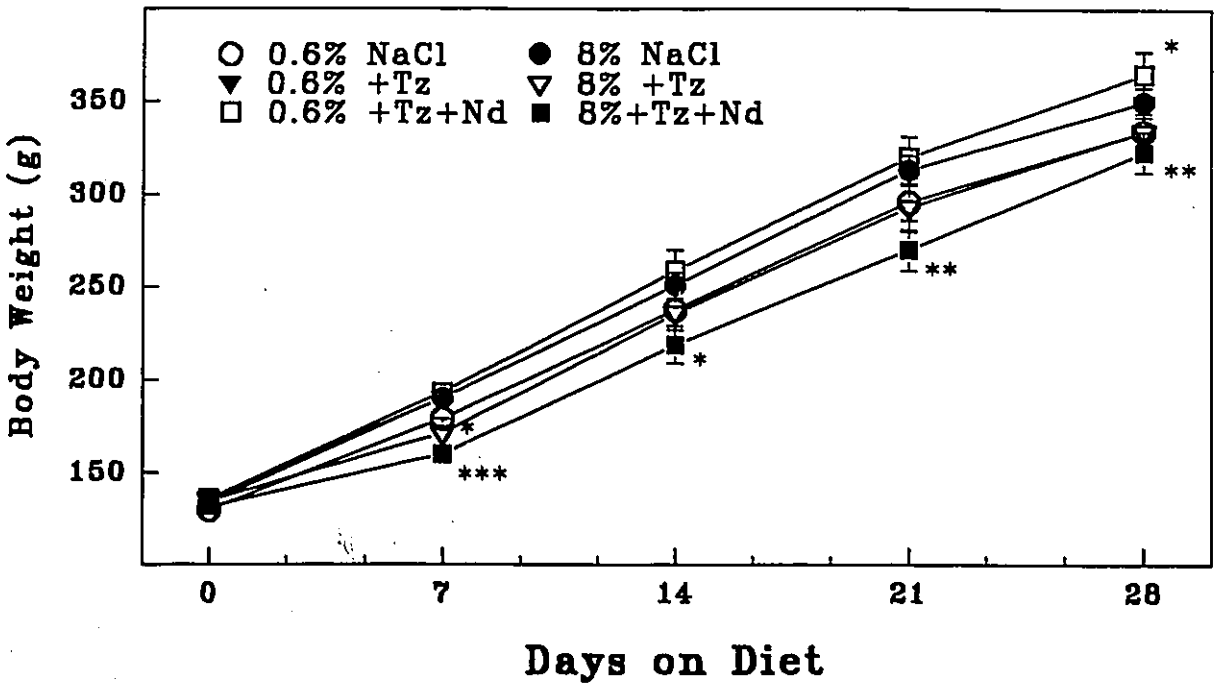

Water Intake (ml/kg/day)
800
600
400
200
0.6% NaCl
0.6% +Nd25
0.6% +Nd
8% NaCl
8% +Nd25
8% +Nd
***
***
***
***
***
7
14
21
Days on Diet

Fig. 11. **Body weight of WKY rats on the high sodium diet with terazosin and/or nadolol for 4 *wk*.**

In the groups on a regular food (0.6% NaCl) or high sodium diet (8% NaCl), body weight (*g*) was measured on the day starting terazosin (Tz) (▼ or ∇), or terazosin with nadolol (Tz + Nd) (□ or ■) or same volume distilled water injection (○ or ●), and thereafter, once a week at noon. See Fig. 9 for drug dosages. Data are mean ± SEM (n = 8 to 9). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ high sodium diet group with terazosin and/or nadolol injection *vs.* the regular diet group with injection of the same agents.


0.6% NaCl
0.6% +Tz
0.6% +Tz+Nd
8% NaCl
8% +Tz
8%+Tz+Nd
Body Weight (g)
350
300
250
200
150
0
7
14
21
28
Days on Diet
*
**
***

Similarly, rats on a high sodium diet with or without one of the two different doses of nadolol for 4 *wk* did not show a change in their weekly body weight gain, neither in their mean body weight (data not shown).

Among the 4 *wk* groups, the haematocrit values were not changed by the high sodium diet (Fig. 12). Compared with the respective control groups, the haemotocrit values were not changed by injection of terazosin or terazosin with nadolol in the regular diet groups, but reduced moderately in the group on a high sodium diet with terazosin treatment.

II.2. *Heart Chamber Weight and Dimensions*

The relative LV dry weight was significantly increased in rats on a 4 *wk* high sodium diet by 14 or 17% in the two sets of experiments (Table 6). Terazosin, nadolol or both of them together did not prevent the increase in the chamber weight. On the contrary, in comparison with the group on a high sodium diet without drug treatment, nadolol alone, or terazosin with nadolol even enhanced the increase in the LV weight by an additional 7 to 9% ($p < 0.05$). In the regular diet group, terazosin alone or terazosin with nadolol caused an 8 to 11% increase in the LV and RV dry weights respectively ($p < 0.05$), while nadolol alone did not have such an effect. After a 4 *wk* diet, compared with the group on a regular diet without drug treatment, the ratio of the LV weight to RV weight was significantly increased by a high sodium exposure only in the experiment for nadolol ($p < 0.01$), while unchanged in the experiment for terazosin (Table 7).

Fig. 12. **Haematocrit values of WKY rats on the 4 *wk* high sodium diet with terazosin and/or nadolol treatment.**

Haematocrit was measured by 2 *min* centrifuge of heparinized capillary tubes filled with fresh arterial blood at the end of 4 *wk* regular (0.6% NaCl) (left) or high sodium diet (8% NaCl) (right) with terazosin 60 *mg/kg/day* (slashed bars), terazosin 60 *mg/kg/day* and nadolol 100 *mg/kg/day* (solid bars) treatment, in comparison with distilled water injection (open bars). Data are mean ± SEM (n = 8 to 9). * $p < 0.05$, high sodium diet group with terazosin injection *vs.* the high sodium diet group with distilled water injection.

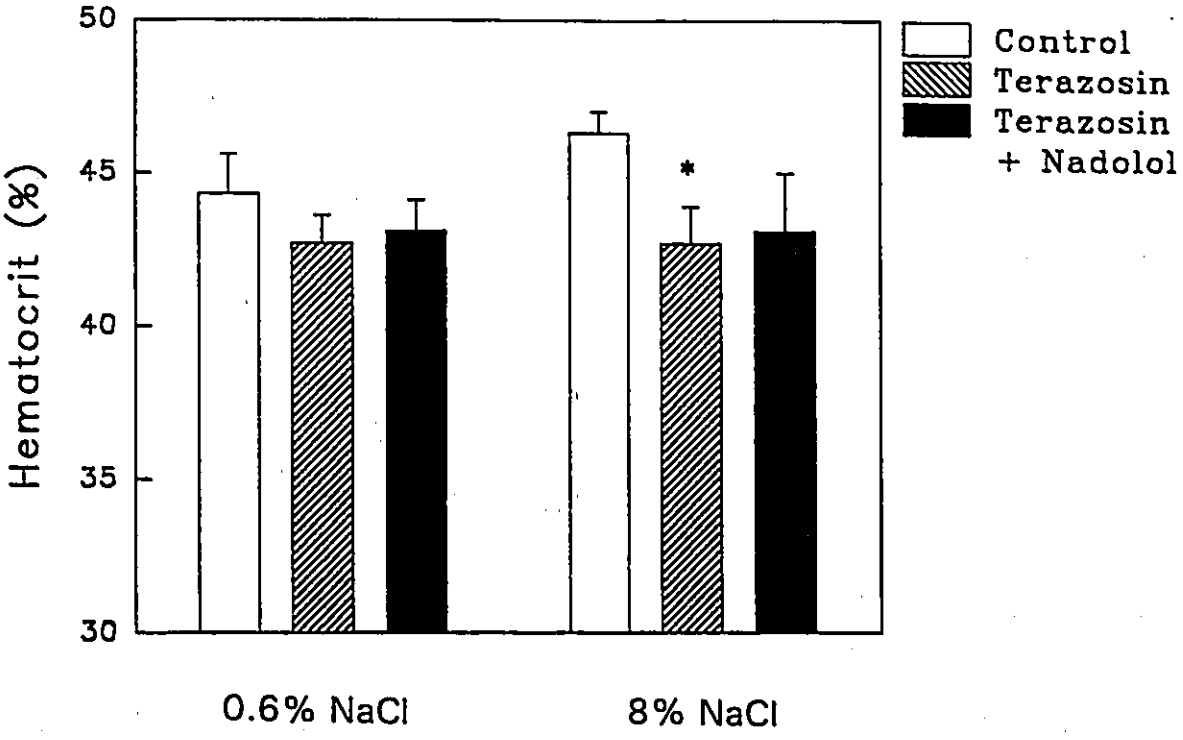

50
45
40
35
30
Hematocrit (%)
*
0.6% NaCl
8% NaCl
Control
Terazosin
Terazosin
+ Nadolol

Table 6. Heart Chamber Weight of WKY Rats on the High Sodium Diet with Terazosin and/or Nadolol for 4 *wk*

Group	(n)	Body Weight (*g*)	Heart Chamber Weight (*mg*/100 *g* body wt)			
			LV wet	LV dry	RV wet	RV dry
0.6%W	(9)	334±10	189± 4	48± 1	51± 2	14.0±0.4
0.6%Tz	(9)	347± 8	205± 4*	52± 1*	58± 2*	15.6±0.5*
0.6%TzNd	(8)	365±12*	208± 3*	53± 1*	60± 2*	15.9±0.4*
8%W	(9)	320±10	217± 7$^{\#}$	56± 3$^{\#}$	54± 2	15.4±0.6
8%Tz	(8)	334±13	236± 6*$^{\#}$	58± 1$^{\#}$	65± 3***$^{\#}$	16.9±0.7
8%TzNd	(8)	330± 9$^{\#}$	243± 5*$^{\#}$	60± 1*$^{\#}$	66± 2***$^{\#}$	17.0±0.6*
0.6%W	(7)	339±10	209± 4	51± 1	58± 2	15.6±0.5
0.6%Nd25	(7)	334± 6	210± 3	52± 1	59± 1	15.4±0.5
0.6%Nd	(7)	338±10	215± 3	53± 1	59± 2	15.7±0.8
8%W	(6)	336±16	230± 5$^{\#}$	58± 2$^{\#\#\#}$	57± 3	14.9±0.7
8%Nd25	(6)	323± 7	259±13**$^{\#\#\#}$	63± 2*$^{\#\#\#}$	68± 3**$^{\#\#}$	16.9±0.5*
8%Nd	(7)	324± 7	250± 8*$^{\#\#\#}$	63± 2*$^{\#\#\#}$	67± 2**$^{\#}$	17.6±0.9**

Values are mean ± SEM for n rats in each group; 0.6%, regular diet containing 0.6% NaCl; 8%, high sodium diet (8% NaCl); W, control group with 1 *mg/kg/day* distilled water *ig*; Tz, terazosin 60 *mg/kg/day ig*; Nd or Nd25, nadolol 100 or 25 *mg/kg/day ig*;
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* the control injection group (W) on the same diet;
$^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ *vs.* the regular diet group (0.6%) with the same injection.

Table 7. **Heart Chamber Weight (Absolute Values) of WKY Rats on the High Sodium Diet with Terazosin and/or Nadolol for 4 *wk***

Group	(n)	Body Weight (*g*)	Heart Chamber Weight (*mg*)				
			LV wet	LV dry	RV wet	RV dry	LV/RV
0.6%W	(9)	334±10	633± 20	160± 4	173±10	47± 2	3.72± 0.13
0.6%Tz	(9)	347± 8	709± 19**	180± 3*	201± 6***	54± 1*	3.53± 0.09
0.6%TzNd	(8)	365±12*	758± 26*	195± 5*	218± 8***	58± 2*	3.49± 0.06
8%W	(9)	320±10	692± 24	180± 7′	173± 6	49± 2	4.03± 0.14
8%Tz	(8)	334±13	785± 30*′	193± 8	218±12***	56± 3*	3.64± 0.14*
8%TzNd	(8)	330± 9′	799± 25*	199± 6*	217± 9***	57± 3*	3.70± 0.09*
0.6%W	(7)	339±10	706± 18	173± 4	198± 9	53± 2	3.59± 0.09
0.6%Nd25	(7)	334± 6	702± 16	178± 3	196± 5	51± 1	3.58± 0.06
0.6%Nd	(6)	338±10	726± 24	177± 4	200±10	53± 2	3.66± 0.16
8%W	(6)	336±16	770± 26	194± 6″	190± 7	50± 2	4.06± 0.13″
8%Nd25	(6)	323± 7	833± 36‴	204± 5‴	218± 9*	55± 2	3.82± 0.09
8%Nd	(7)	324± 7	809± 27′	203± 6‴	216± 7*	57± 3*	3.76± 0.06*

Values are mean ± SEM for n rats in each group; 0.6%, regular diet containing 0.6% NaCl; 8%, high sodium diet (8% NaCl); W, control injection with 1 *ml/kg/day* distilled water *ig*; Tz, terazosin 60 *mg/kg/day ig*; Nd or Nd25, nadolol 100 or 25 *mg/kg/day ig*;
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* the control injection group (W) on the same diet;
′ $p < 0.05$, ″ $p < 0.01$, ‴ $p < 0.001$ *vs.* the regular diet group (0.6%) with the same injection.

Single blockade with terazosin or high dose of nadolol, or a combination of these two agents significantly attenuated the increase in the ratio compared to the high sodium diet group without drug treatment ($p < 0.05$) (Table 7).

Similar to the results of heart anatomy from the previous protocols for the 1 to 6 *wk* medium high or high sodium diets, the ratio of the dry weight to wet weight of both the LV and RV was not changed by a 4 *wk* high sodium diet regimen. Moreover, no differences in the ratio were seen between the groups treated with terazosin or nadolol alone, or both of the antagonists together and those without drug treatment (data not shown).

In these groups, the absolute values of LV dry weight, before being normalized for the body weight, were also increased significantly by a 4 *wk* high sodium diet ($p < 0.05$) (Table 7), being parallel to the changes in the relative values of LV weight as shown in Table 6. This is different from the changes in the relative and the absolute LV weights of rats on a 2 or 6 *wk* high sodium diet, in which only the relative LV weights were increased because of a reduced mean values of their body weights (Table 2 and 3, Fig. 3). Since the gain in body weight was not reduced by a high sodium diet in the 4 *wk* groups (see Fig. 11), the changes in LV weight by a high sodium diet was seen in their absolute as well as the relative values. For the same reason, a similar consistency in the changes between the relative and absolute chamber weights was observed in the increases in the LV wet and dry weights of rats on a high sodium diet with terazosin and nadolol treatment, and in the increase of the RV wet and dry weights of rats on a high sodium diet with injection of terazosin and nadolol or of nadolol alone, compared to the group on a regular diet with distilled water injection ($p < 0.05$) (Table 6 and 7). The

increases in the absolute wet and dry weights of LV and RV by terazosin or terazosin with nadolol in the regular diet groups were also significant *vs.* the regular diet group with *ig* injection of distilled water ($p < 0.05$) (Table 7), being consistent with the changes of the relative values (Table 6).

Comparable to the results from the 1 to 6 *wk* high sodium diet protocol (Table 2), the increase in the relative LV dry weight by a 4 *wk* high sodium diet was associated with an increase in the LV wall thickness and in the ratio of wall thickness to radius ($p < 0.05$) (Table 8). However, rats on a high sodium diet and treated with terazosin, nadolol or both showed an increase in LV weight only, without a change in the ratio. This was due to a slightly smaller effect of the high sodium diet on the increase in the LV wall thickness in the blocker-treated groups than in the groups without drug treatment, as well as due to an concomitant increase in the internal diameters. This result indicates a shift of the hypertrophy from a concentric form to an eccentric one by the adrenoceptor blockers.

Similar to the results from the 4 *wk* diet groups, the relative wet and dry weights of LV were increased by a 2 *wk* high sodium diet, compared to the regular diet group ($p < 0.001$) (Table 9). In comparison with the group on a high sodium diet without drug treatment, the increase in the LV weight was enhanced by terazosin ($p < 0.05$). The relative chamber weights were also increased by terazosin injection for 2 *wk* in the regular diet group ($p < 0.05$), similar to the result from the 4 *wk* group. Neither the 1 *wk* high sodium diet nor the terazosin alone changed the relative chamber weights significantly ($p > 0.05$). However, when compared with the group on a regular diet with terazosin treatment, rats on a 1 *wk* high sodium diet together with terazosin injection

Table 8. Left Ventricular Dimensions of WKY Rats on the High Sodium Diet with Terazosin and/or Nadolol for 4 *wk*

Group	(n)	LV Wall Thickness (*mm*)	Internal Diameter (*mm*)	LV Wall Thickness/ Radius
0.6%W	(9)	1.60±0.01	6.44±0.22	0.50±0.02
0.6%Tz	(9)	1.63±0.01	6.67±0.21	0.51±0.02
0.6%TzNd	(8)	1.61±0.01	6.61±0.28	0.49±0.02
8%W	(9)	1.85±0.02′	6.23±0.17	0.59±0.02′
8%Tz	(8)	1.77±0.03′	6.95±0.25*	0.51±0.02*
8%TzNd	(8)	1.80±0.07′	6.74±0.23	0.54±0.03
0.6%W	(7)	1.65±0.06	6.43±0.25	0.52±0.04
0.6%Nd25	(7)	1.72±0.07	6.74±0.17	0.52±0.03
0.6%Nd	(6)	1.68±0.04	6.62±0.20	0.51±0.02
8%W	(6)	1.88±0.04′	5.90±0.17	0.64±0.02′
8%Nd25	(6)	1.72±0.07	6.66±0.19*	0.53±0.04*
8%Nd	(7)	1.72±0.07	6.51±0.15*	0.53±0.03*

Values are mean ± SEM for n rats in each group; 0.6%, regular diet containing 0.6% NaCl; 8%, high sodium diet (8% NaCl); W, control group with 1 *ml/kg/day* distilled water *ig*; Tz, terazosin 60 *mg/kg/day ig*; Nd or Nd25, nadolol 100 or 25 *mg/kg/day ig*;
* $p < 0.05$ *vs.* the control injection group (W) on the same diet;
′ $p < 0.05$ *vs.* respective regular diet group (0.6%) with the same injection.

Table 9. Heart Chamber Weight of WKY Rats on the High Sodium Diet with Terazosin for 1 or 2 *wk*

Group	(n)	Body Weight (*g*)	Chamber Weight (*mg*/100 *g* body wt)			
			LV wet	LV dry	RV wet	RV dry
<u>1 week</u>						
0.6%W	(8)	202± 4	216± 5	54± 2	56± 2	15.8±0.4
0.6%Tz	(9)	203± 4	218± 6	55± 1	59± 1	15.9±0.3
8%W	(9)	184± 4	220± 6	57± 1	61± 2	17.2±0.6
8%Tz	(9)	186± 2	233± 5′	59± 1′	67± 2*″	18.3±0.6″
<u>2 week</u>						
0.6%W	(8)	259±10	203± 3	51± 1	54± 2	13.5±0.6
0.6%Tz	(9)	243± 9	219± 5*	55± 1*	61± 2*	15.9±0.7**
8%W	(8)	245± 9	244± 6‴	60± 1‴	64± 2″	15.7±0.5′
8%Tz	(7)	238±10	259± 6*‴	65± 1**‴	70± 3″	18.1±0.7*′

Values are mean ± SEM for n rats in each group;
0.6%, regular diet containing 0.6% NaCl; 8%, high sodium diet (8% NaCl);
W, control injection with 1 *ml/kg/day* distilled water *ig*; Tz, terazosin 60 *mg/kg/day ig*;
* $p < 0.05$, ** $p < 0.01$ *vs.* the control injection group (W) on the same diet;
′ $p < 0.05$, ″ $p < 0.01$, ‴ $p < 0.001$ *vs.* respective regular diet group (0.6%) with the same injection.

showed an increase in both the LV and RV relative weights ($p < 0.05$).

Associated with the increase in the relative chamber weights induced by the high sodium diet, the LV wall thickness and the ratio of LV wall thickness to radius were increased by the 2 *wk* high sodium diet ($p < 0.05$) (Table 10). The groups on a 1 *wk* high sodium diet and those on a 1 *wk* high sodium diet with terazosin injection showed an increase in the LV wall thickness only ($p < 0.05$).

Parallel to the changes in the relative chamber weights, the absolute LV and RV wet and dry weights were significantly increased by the high sodium diet for 2 *wk* ($p < 0.05$) (data not shown). Only the increase of the RV wet weight in the 1 *wk* and RV dry weights in the 2 *wk* by the high sodium diet, when compared to the groups on a similar length of time high sodium diet without drug treatment, was enhanced by terazosin ($p < 0.05$, Table 9).

II.3. *Resting Hemodynamics*

The resting MAP, LVPSP and LVEDP were not changed by the high sodium diet or by terazosin or nadolol in both the regular or high sodium diet groups for 1, 2 or 4 *wk* (Table 11 and 12). In contrast, combined blockade with both terazosin and nadolol significantly decreased the LVPSP in the group on a high sodium diet ($p < 0.05$), without change to the group on a regular diet (Table 12).

In comparison with the respective regular diet groups, the resting HR was not changed by the high sodium diet for 1 *wk*, but increased by 14% in the 2 *wk* group (p

Table 10. **Left Ventricular Dimensions of WKY Rats on the High Sodium Diet with Terazosin for 1 or 2 *wk***

Group (n)	LV Wall Thickness (*mm*)	Internal Diameter (*mm*)	LV Wall Thickness/ Radius
1 week			
0.6%W (8)	1.22±0.03	4.69±0.12	0.53±0.03
0.6%Tz (9)	1.24±0.03	4.91±0.19	0.51±0.02
8%W (9)	1.35±0.04′	4.72±0.22	0.59±0.04
8%Tz (9)	1.38±0.02′	4.91±0.14	0.56±0.02
2 week			
0.6%W (8)	1.23±0.02	5.68±0.21	0.44±0.02
0.6%Tz (9)	1.22±0.01	5.44±0.17	0.45±0.02
8%W (8)	1.39±0.05″	5.35±0.15	0.52±0.03′
8%Tz (7)	1.31±0.05	5.52±0.23	0.48±0.03

Values are mean ± SEM for n rats in each group;
0.6%, regular diet containing 0.6% NaCl; 8%, high sodium diet (8% NaCl);
W, control injection with 1 *ml/kg/day* distilled water *ig*; Tz, terazosin 60 *mg/kg/day ig*;
′ $p < 0.05$, ″ $p < 0.01$ *vs.* respective regular diet group (0.6%) with the same injection.

Table 11. Resting Hemodynamics of WKY Rats on the High Sodium Diet with Terazosin for 1 or 2 *wk*

Group (n)	HR (*bpm*)	SBP (*mmHg*)	DBP (*mmHg*)	MAP (*mmHg*)
1 week				
0.6%W (8)	383 ± 5	116 ± 4	95 ± 3	102 ± 3
0.6%Tz (9)	432 ± 16*	105 ± 4	89 ± 3	94 ± 3
8%W (9)	422 ± 19	116 ± 2	93 ± 2	101 ± 2
8%Tz (9)	431 ± 20*	104 ± 4	87 ± 3	93 ± 3
	HR	**LVPSP**	**LVEDP**	**RAP**
2 week	(*bpm*)	(*mmHg*)	(*mmHg*)	(*mmHg*)
0.6%W (8)	362 ± 14	122 ± 3	4.4 ± 1.2	1.0 ± 0.2
0.6%Tz (9)	431 ± 14**	110 ± 3	5.3 ± 0.7	0.7 ± 0.3
8%W (9)	413 ± 8*	119 ± 2	5.9 ± 0.7	0.6 ± 0.3
8%Tz (7)	427 ± 23**	114 ± 5	4.1 ± 1.1	0.9 ± 0.3

Values are mean ± SEM for n rats in each group. 0.6%, regular diet containing 0.6% NaCl; 8%, high sodium diet (8% NaCl); W, control injection with distilled water *ig*; Tz, terazosin 60 *mg/kg/day ig*; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; LVPSP, left-ventricular peak-systolic pressure; LVEDP, left-ventricular end-diastolic pressure; RAP, right atrial pressure; * $p < 0.05$, ** $p < 0.01$ *vs.* regular diet & control injection group(0.6%W).

Table 12. Resting Hemodynamics of WKY Rats on the High Sodium Diet with Terazosin and/or Nadolol for 4 *wk*

Group	(n)	HR (*bpm*)	LVPSP (*mmHg*)	LVEDP (*mmHg*)	RAP (*mmHg*)
0.6%W	(9)	365 ± 10	116 ± 2	4.8 ± 0.6	0.8 ± 0.3
0.6%Tz	(9)	371 ± 20	118 ± 2	4.4 ± 1.1	1.7 ± 0.2*
0.6%TzNd	(8)	330 ± 8@	107 ± 4	2.1 ± 0.6	1.5 ± 0.3
8%W	(9)	367 ± 5	125 ± 3	3.9 ± 0.8	0.9 ± 0.2
8%Tz	(8)	380 ± 11	118 ± 5	3.7 ± 1.1	1.5 ± 0.3
8%TzNd	(8)	330 ± 11@	109 ± 6*	2.3 ± 0.7	1.5 ± 0.4
0.6%W	(7)	384 ± 13	110 ± 5	3.8 ± 1.0	0.1 ± 0.7
0.6%Nd25	(7)	331 ± 7**	112 ± 4	1.1 ± 1.0	0.3 ± 0.5
0.6%Nd	(6)	336 ± 12*	112 ± 5	-0.6 ± 0.9	0.8 ± 0.8
8%W	(6)	392 ± 15	118 ± 5	1.3 ± 1.0	0.5 ± 0.4
8%Nd25	(6)	328 ± 6**	117 ± 4	2.5 ± 0.6	0.3 ± 0.9
8%Nd	(7)	338 ± 5**	119 ± 3	1.4 ± 0.8	0.3 ± 0.6

Values are mean±SEM for n rats in each group. 0.6%, regular diet containing 0.6% NaCl; 8%, high sodium diet (8% NaCl); W, control injection with 1 *ml/kg/day* distilled water *ig*; Tz, terazosin 60 *mg/kg/day ig*; Nd or Nd25, nadolol 100 or 25 *mg/kg/day ig*; LVPSP, left-ventricular peak-systolic pressure; LVEDP, left-ventricular end-diastolic pressure; RAP, right atrial pressure; * $p < 0.05$, ** $p < 0.01$ *vs.* control injection group(W) on the same diet; @ $p < 0.05$ *vs.* single blockade group (Tz) on the same diet.

< 0.05). However, this increase was not shown in the 4 *wk* group (Table 12). When rats on either a regular or high sodium diet for 1 or 2 *wk* were treated with terazosin, their HR was significantly higher than those on a regular diet without drug treatment ($p < 0.05$, Table 11). However, within the high sodium diet group, there were no differences in the HR between the control and the terazosin-treated rats. The HR of rats treated with nadolol alone or together with terazosin was reduced by 10 to 14% *vs.* the groups without treatment on either a regular diet or a high sodium diet (Table 12).

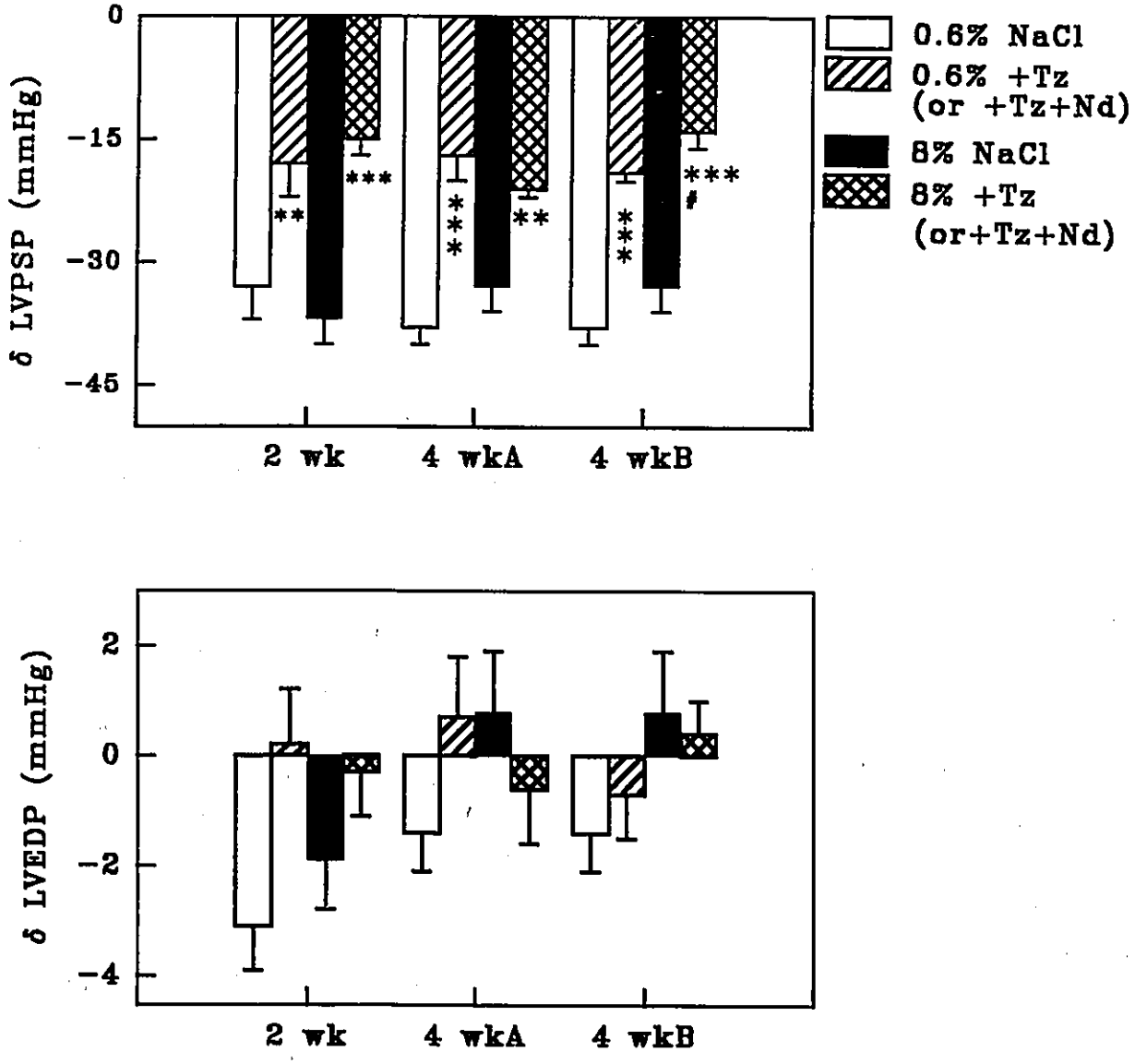
The resting RAP was not changed by nadolol, but increased by a 4 *wk* terazosin treatment in the regular diet group ($p < 0.05$) (Table 12). This increase in the resting RAP was not seen in the 2 *wk* group treated with the same dosage of terazosin (Table 11).

II.4. *Responses to Ganglionic Blockade*

Compared to the baseline values, LVPSP was decreased in response to hexamethonium in both the 2 and 4 *wk* regular diet groups ($p < 0.05$) (Fig. 13). In contrast to the augmented decreases in MAP in response to hexamethonium by a high sodium diet in the 2 and 6 *wk* groups (Fig. 4), the 4 *wk* high sodium diet here did not change the extent of this response. When compared to the groups on either a regular or high sodium diet without drug treatment, the pressure decrease in rats with treatment of terazosin alone or with terazosin and nadolol for either 2 or 4 *wk* was significantly attenuated ($p < 0.01$ to 0.001) (Fig. 13). In the 4 *wk* high sodium diet groups, this attenuation was significantly greater in those with a combined blockade (4 *wk*B) than in

Fig. 13. **Responses to hexamethonium of left ventricular (LV) pressures of WKY rats after a 2 or 4 *wk* high sodium diet.**

LV peak-systolic pressure (LVPSP) and LV end-diastolic pressure (LVEDP) were recorded both before and after injection of hexamethonium 30 *mg/kg*. The change of LVPSP (top panel) and LVEDP (bottom) were calculated for rats on 2 or 4 *wk* regular diet (0.6% NaCl, open bars), or high sodium diet (8% NaCl, solid bars) without drug treatment, or with injection by gavage of terazosin (2 *wk* and 4 *wk*A) or terazosin and nadolol (4 *wk*B, see Fig. 9 for details) (slashed bars for groups on a regular diet, cross-lined bars for groups on a high sodium diet). Data are $\delta \pm$ SEM ($n = 8$ to 9). ** $p < 0.01$, *** $p < 0.001$ terazosin or terazosin and nadolol treated group *vs.* the group without drug treatment but on the same diet; $^{\#} p < 0.05$ terazosin and nadolol treated group *vs.* the single blockade (terazosin) group on the same diet.


0
−15
−30
−45
δ LVPSP (mmHg)
**
***
***
**
***
***
#
2 wk
4 wkA
4 wkB
0.6% NaCl
0.6% +Tz
(or +Tz+Nd)
8% NaCl
8% +Tz
(or+Tz+Nd)
2
0
−2
−4
δ LVEDP (mmHg)
2 wk
4 wkA
4 wkB

the group with terazosin treatment alone (4 *wk*A) ($p < 0.05$).

The changes in LVEDP and RAP in responses to hexamethonium were variable, showing no differences from the baseline values (Fig. 13 and 14). HR was increased with hexamethonium injection only in the 4 *wk* high sodium diet group ($p < 0.05$) (Fig. 14).

II.5. *Hemodynamic Responses and the Efficacy of α_1- and ß-Blockade*

Similar to the results of hemodynamic responses in the groups on a 1 to 6 *wk* high sodium diet, a 4 *wk* high sodium diet did not change the responses of LVPSP, LVEDP (Fig. 15, top and middle panels) or RAP to phenylephrine infusion (data not shown). The HR response of rats on either a regular or high sodium diets to phenylephrine infusion was not significant and was not augmented by a high sodium diet (Fig. 15, bottom panel), in contrast to an enhanced HR reduction seen in the 6 *wk* group (see Fig. 5). Similar to the effect of a high sodium diet for 1 to 6 *wk* (see Fig. 7), a 4 *wk* high sodium diet did not alter the HR increase in response to isoproterenol (Fig. 16, bottom panel). However, the LVPSP increase in response to isoproterenol infusion was significantly augmented by the high sodium diet ($p < 0.001$) (Fig. 16 top panel), without any influence on the LVEDP responses.

The increase in LVPSP and LVEDP in response to phenylephrine infusion was antagonized by terazosin ($p < 0.001$) (Fig. 15, top and middle panels). Thus, the dose-response curves of phenylephrine were shifted to the right by terazosin. The decrease

Fig. 14. **Responses to hexamethonium of heart rate (HR) and right atrial pressure (RAP) of WKY rats after a 2 or 4 *wk* high sodium diet.**

HR and RAP were recorded both before and after injection of hexamethonium 30 *mg/kg*. The change of HR (top panel) and RAP (bottom panel) were calculated for rats on 2 or 4 *wk* regular diet (0.6% NaCl, open bars), or high sodium diet (8% NaCl, solid bars) without drug treatment, or with injection by gavage of terazosin (2 and 4 *wk*A) or terazosin with nadolol (4 *wk*B, see Fig. 9 for details) (slashed bars for the groups on a regular diet, cross-lined bars for the groups on a high sodium diet). Data are $\delta \pm$ SEM ($n = 8$ to 9). * $p < 0.05$ terazosin treated group *vs.* the groups without drug treatment but on the same diet.

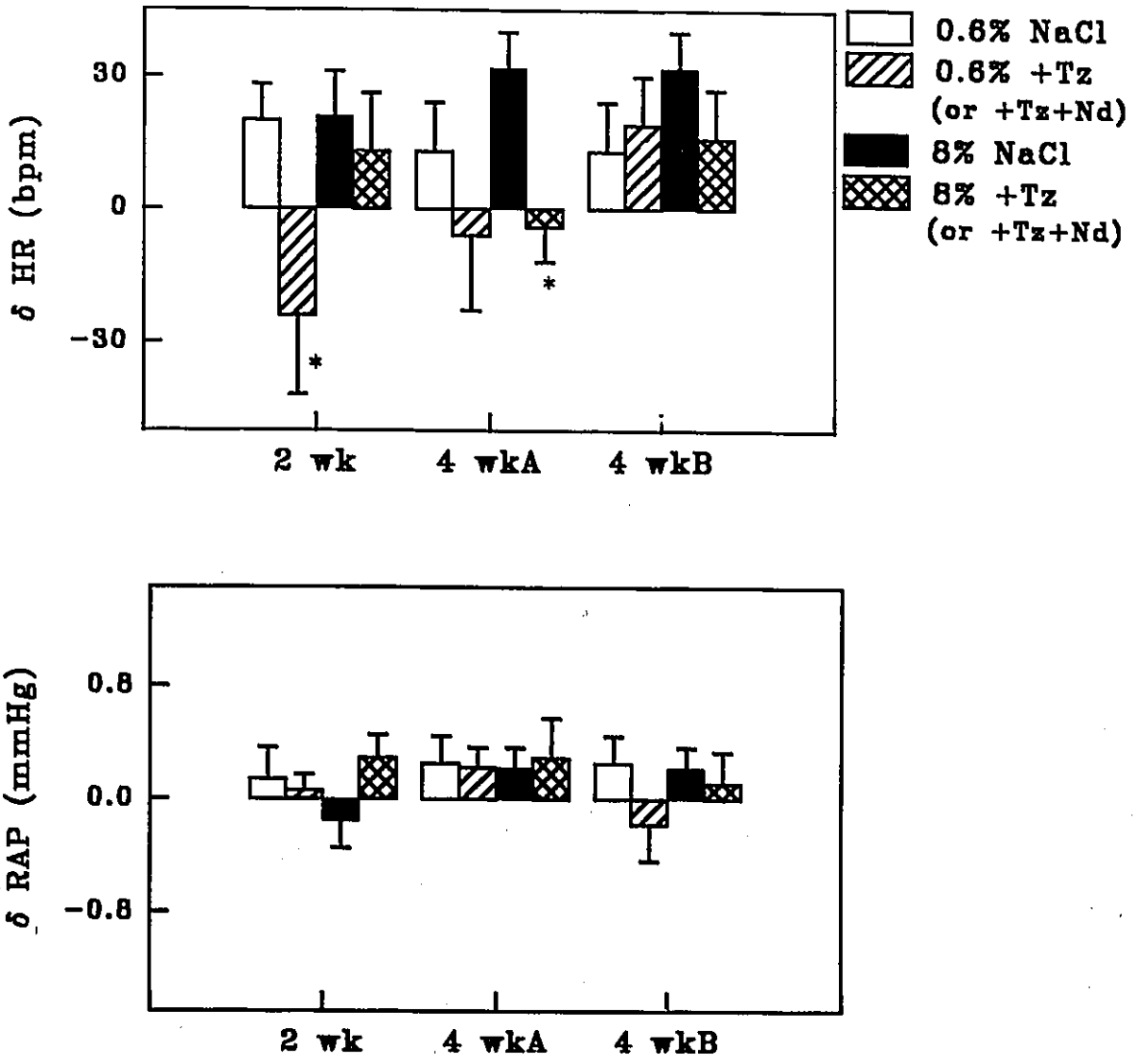

30
0
−30
δ HR (bpm)
*
*
2 wk
4 wkA
4 wkB
0.6% NaCl
0.6% +Tz
(or +Tz+Nd)
8% NaCl
8% +Tz
(or +Tz+Nd)
0.8
0.0
−0.8
δ RAP (mmHg)
2 wk
4 wkA
4 wkB

Fig. 15. **Effect of blockade with terazosin on pressure and heart rate (HR) responses to phenylephrine infusion in WKY rats.**

Terazosin 60 *mg/kg/day* (Tz) (∇, on regular diet containing 0.6% NaCl and ▼, on high sodium diet with 8% NaCl) was given by gavage, starting 2 days prior to diet initiation and being continued for 4 *wk*. Control groups were injected 1 *ml/kg/day* distilled water (○ , on regular diet and ●, on high sodium diet). Pressures (see Fig. 13 for abbreviations) and HR responses were recorded 3 *hr* after surgery when phenylephrine was infused, at 1, 3 or 6 *μg/kg/min* for rats without and 6, 12, 24 or 48 *μg/kg/min* for those with terazosin treatment, after injection of hexamethonium 30 *mg/kg*. Data are $\delta \pm$ SEM (n = 8 to 9). *** $p < 0.001$ terazosin treated groups on either the regular or high sodium diet *vs*. the respective distilled water injected groups; ## $p < 0.05$ terazosin treated group on a regular diet *vs*. the terazosin treated group on a high sodium diet.

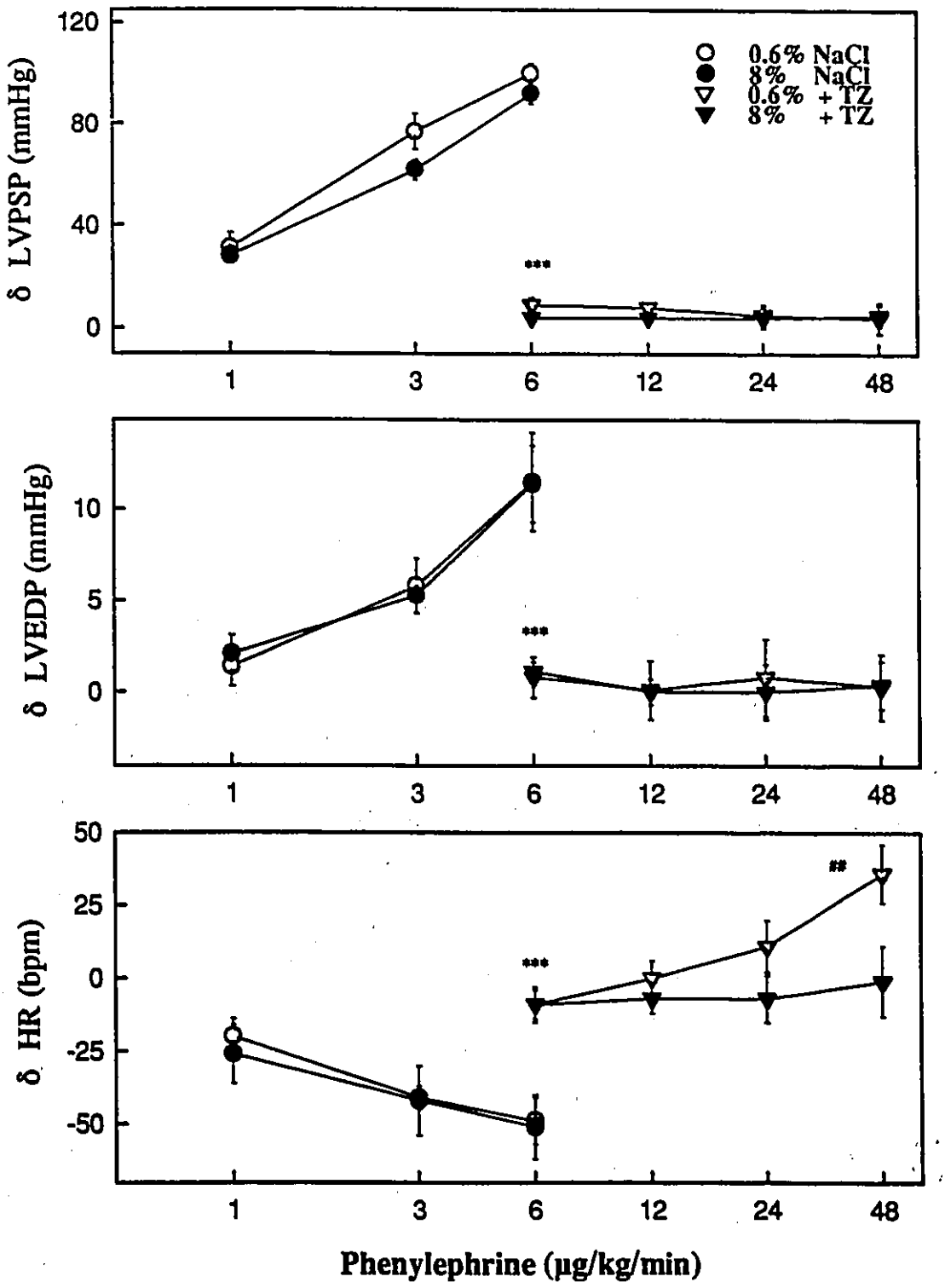
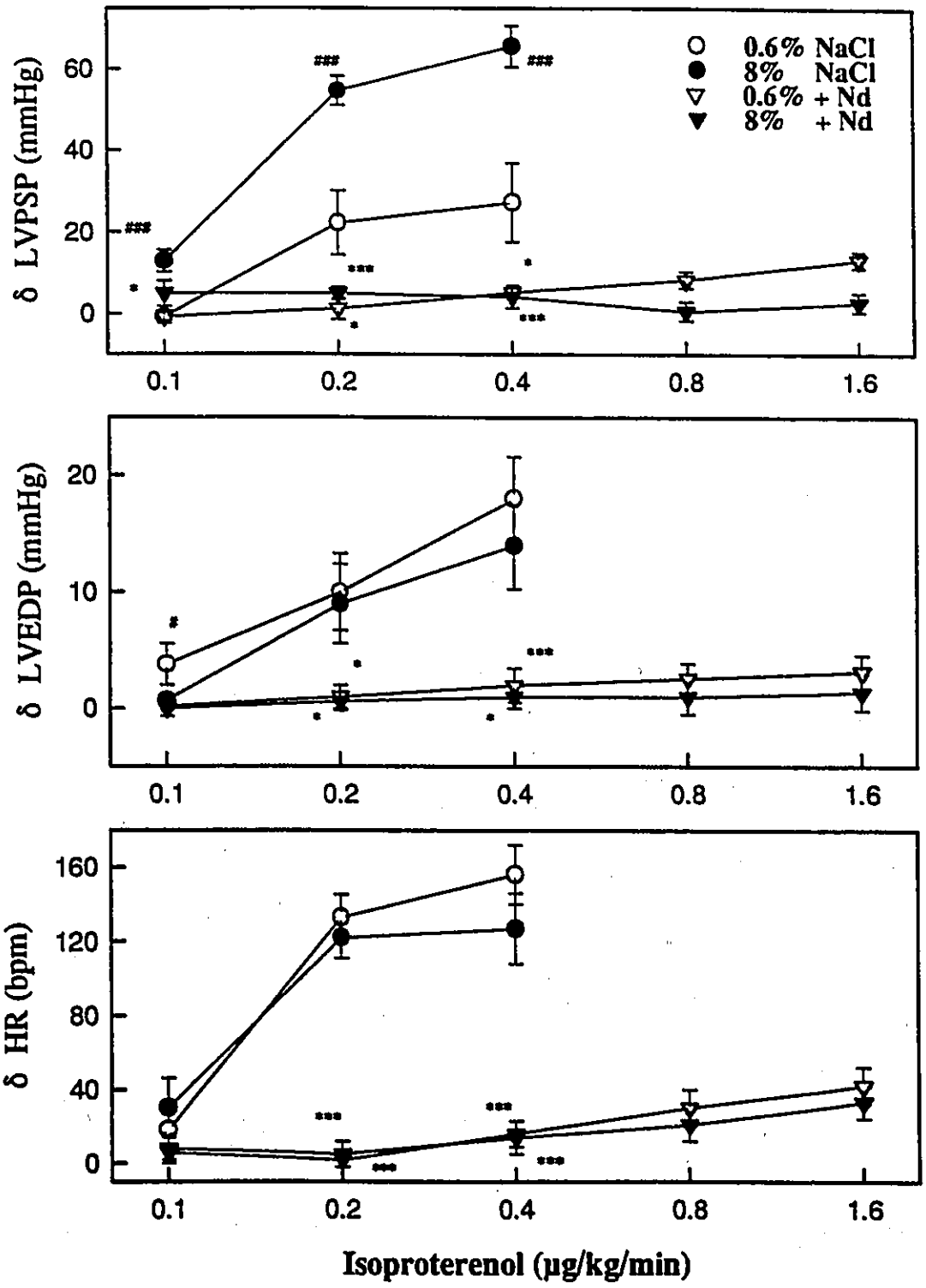

δ LVPSP (mmHg)
120
80
40
0
0.6% NaCl
8% NaCl
0.6% + TZ
8% + TZ
***
1
3
6
12
24
48
δ LVEDP (mmHg)
10
5
0
***
1
3
6
12
24
48
δ HR (bpm)
50
25
0
-25
-50
***
##
1
3
6
12
24
48
Phenylephrine (μg/kg/min)

Fig. 16. **Effect of blockade with nadolol on chronotropic and inotropic heart responses to isoproterenol infusion in WKY rats.**

Nadolol 100 *mg/kg/day* (Nd) (∇, on regular diet containing 0.6% NaCl and ▼, on high sodium diet with 8% NaCl) was given by gavage, starting 2 days prior to diet initiation and being continued for 4 *wk*. Control groups were injected 1 *ml/kg/day* distilled water (○ , on regular diet and ●, on high sodium diet). Pressure (see Fig. 13 for abbreviations) and heart rate (HR) responses were recorded 3 *hr* after surgery when isoproterenol was infused, at 0.1, 0.2 or 0.4 *μg/kg/min* for rats without and 0.1, 0.2, 0.4, 0.8 or 1.6 *μg/kg/min* for those with nadolol treatment. Data are $\delta \pm$ SEM (n = 5 to 7). * $p < 0.05$, *** $p < 0.001$ nadolol treated groups on either the regular or high sodium diet *vs*. distilled water injected groups on the same diet; # $p < 0.05$, ### $p < 0.001$ groups on a high sodium diet *vs*. those on a regular diet group with the same treatment.


δ LVPSP (mmHg)
δ LVEDP (mmHg)
δ HR (bpm)
0.6% NaCl
8% NaCl
0.6% + Nd
8% + Nd
0.1
0.2
0.4
0.8
1.6
Isoproterenol (µg/kg/min)

in HR with phenylephrine infusion was also attenuated by terazosin treatment ($p < 0.001$) (Fig. 15, bottom panel). Similarly, when compared to the group without drug treatment, the increase in HR, LVPSP and LVEDP in response to isoproterenol infusion was inhibited competitively by nadolol ($p < 0.001$), and the dose-response curves were shifted to the right by nadolol (Fig. 16). The inhibition of the responses to isoproterenol by the lower dose of nadolol (25 *mg/kg/day*) was similar, although to a smaller extent (data not shown).

DISCUSSION

I. Heart Chamber Weight and Dimensions

Cardiac mass as well as its geometrical design is dependent on the functional demand or load on the heart. Under physiological conditions, for those of the same age and sex with the same level of physical activities, the size or the weight of the heart is proportional to the whole body size or body weight, which determines the extent of the demand on the systemic circulation (Rakusan, 1984). A bigger body weight needs a higher level of systemic circulation, thus a bigger functional demand on the heart, and is associated with a bigger heart mass. Therefore, to compare the heart weight normalized for their body weights, *i.e.*, the relative heart weight, of animals at the same age will provide the accurate information of cardiac mass.

In this study, we found that the 2 to 6 *wk* high sodium diet increased the relative LV weight by 13 to 17%, in comparison to the relative heart weight of the group on a regular diet. The degree of LVH is increased with the prolongation of the high sodium diet regimen. Based on our gross anatomical data, the LVH was not significantly established at 1 *wk*; a period for a high sodium diet over 2 *wk* was needed to build up the LVH (Table 2). This increase in the relative chamber weight was reflected also in an increase in the absolute LV weight when the mean body weight values were similar in both diet groups (Table 7 and Fig. 11). The absolute LV weight did not show an increase when the gain of body weight was reduced in the high sodium diet group (Table

3 and Fig. 3). In the latter situation, the changes in the cardiac mass by a high sodium diet can only be determined when the heart weight is normalized for their body weights.

The cause for the variation in the body weight of rats on a high sodium diet is not clear, but variation has been reported in the literature (Buttrick *et al.*, 1993; Mervaala *et al.*, 1992; Huang and Leenen, 1992; Fields *et al.*, 1991). The terminal body weights of young Wistar rats on a diet with 8% NaCl were significantly reduced at the end of the 4 *wk* diet regimen (Huang and Leenen, 1992). Although the body weight gain of WKY rats was not affected by an 8 *wk* diet with 2.7% NaCl (Mervaala *et al.*, 1992), a three-month high salt exposure by giving 1% NaCl in drinking water significantly decreased the body weight (Chrysant *et al.*, 1979). In comparison, the mean body weights of rats were not changed by a 6 *wk* diet with 4% NaCl (Buttrick *et al.*, 1993), or by normal saline in drinking water for 6 *wk* (Fields, *et al.*, 1991). The different effects on the body weight of a salt exposure might be from a variability in the groups of rats, suggesting a difference in their sensitivity or tolerance to salt exposure, or from an age difference when the exposure was initiated. It might be from a difference in food preparations, such as small differences in the salt content or in taste, resulting in different influences on the appetite of the rats.

By comparing the heart weight and dimension data, the values of LV wall thickness and internal diameters obtained in this study are close to that from previous studies in this laboratory with the same microscopic method on similar age Wistar and WKY rats (Yuan and Leenen, 1991; Fields *et al.*, 1991). They are also similar to the values from studies with echocardiographic measurements validated by heart anatomy on either Wistar (de Simone *et al.*, 1993) or Sprague-Dawley rats (Pawlush *et al.*, 1993)

with renovascular hypertension.

Different initiating factors for cardiac hypertrophy can influence different heart chambers, depending on the characteristics of the stimuli imposed (Morgan and Bader, 1991). In this study, a high dietary sodium for 2 to 6 *wk* induced LVH, while the increase in RV weight was moderate and not consistent (Table 2 and 6). The ratio of LV to RV weight was slightly increased by the 2 to 6 *wk* medium high or high sodium diet (Table 3), and significantly increased in one of the two 4 *wk* high sodium diet groups (Table 7). These data suggest a preferential influence of the high sodium diet on the LV weight, being the same as observed in WKY rats on a 4% NaCl diet for 10 *wk* (Frohlich *et al*, 1993). This result is also in agreement with the effect of the high sodium diet on Wistar rats, in which only LV weight was significantly increased by a 4 *wk* diet, and also in agreement with the effect on WKY rats if the relative dry weight of heart chambers is compared (Yuan and Leenen, 1991). It is also similar to the effect of up to 7 months salt exposure by drinking normal saline (1% NaCl) on the changes in heart chamber mass of WKY rats (Kihara *et al*, 1985). The increase in the ratio of LV to RV weight suggests that pressure overload might have been involved in the development of this type of LVH, since pressure overload mainly affects the left side of the heart. Consistent with this speculation, the LVH was constantly found in this study to be in a concentric form, *i.e.*, an increase in LV weight with an increase in the wall thickness and in the ratio of the wall thickness to radius, without changes in the internal diameters. This result is similar to the LVH geometry of Wistar rats induced by drinking normal saline for 3 to 6 *wk* (Fields *et al.*, 1991). The high sodium intake-induced increase in the LV mass has been found to be associated with an increase in the ratio of non-collagenous protein to

DNA and in the total collagen content as well as a decrease in the DNA concentration (Kihara *et al.*, 1985). This finding strongly supports the notion that hypertrophy rather than hyperplasia of cardiocytes may be the underlying process for the increase in LV mass.

II. Resting Hemodynamics after High Sodium Diet

To further clarify the role of hemodynamic factors in the development of the high sodium diet-induced cardiac hypertrophy, resting BP and HR for the 1 *wk* diet group, BP, HR, CI and TPRI for the 2 or 6 *wk* diet groups and LVPSP, LVEDP and RAP for the 2 or 4 *wk* diet groups plus chronic treatment with terazosin or nadolol alone or with both of the blockers were recorded during the daytime.

In this study, neither the MAP, LVPSP, LVEDP, RAP nor the CI, TPRI measured during this resting period were changed by the high sodium intake (Table 4, 11 and 12). This is in agreement with the previous reports that BP of WKY rats was not changed by a high salt exposure for either 4 to 10 *wk* (Ely *et al.*, 1987; Fields *et al.*, 1991; Yuan and Leenen, 1991; Frohlich *et al.*, 1993) or up to 3 (Chrysant *et al.*, 1979) or 7 months (Kihara *et al.*, 1985). Although it was reported that LVEDP of normotensive rats was increased significantly by high sodium intake (Fields *et al.*, 1991), this increase happened only with prolonged salt exposure and may not have much significance in initiating the cardiac hypertrophy. Therefore, this study further confirms that the high sodium diet-induced LVH is not from a mechanism involving pressure or

volume overload, as indicated by the measurements of MAP and LVPSP, or of LVEDP and RAP, respectively. Although in one study CI was reported to be increased after a three months high sodium exposure, this happened after a long-term diet regimen and thus could be a result of the changes in the cardiac structures, or most probably a response to a reduced TPRI (Chrysant *et al.*, 1979). The resting hemodynamics in this study were measured in rats after finishing a 1 through 6 *wk* high sodium diet regimen. According to the anatomy results from this study and from others on the time-course of the changes in LV weight, this time schedule has sufficiently covered both the pre- and post-establishment of LVH by the high sodium diet.

After a 4 *wk* high sodium diet, the haematocrit values of rats was not different from that of rats on a regular diet (Fig. 12). Even a much longer salt exposure (for 3 months) did not change the haematocrit in WKY rats (Chrysant *et al.*, 1979). This unchanged haematocrit suggests that the high sodium diet for several *wk* does not increase the plasma volume. Similar results were reported from studies on Wistar rats, in which the haematocrit was not changed by a high sodium diet (4% NaCl) for 9 *wk* (de Simone *et al.*, 1993), or by drinking saline (1% NaCl) for 6 *wk* (Fields *et al.*, 1991). These negative findings on the changes in the plasma volume, together with the unchanged filling pressures, BP and TPRI from this laboratory (Fields *et al.*, 1991; Yuan and Leenen, 1991) and from others (Frohlich *et al.*, 1993), make a potential role of a pressure or volume overload in the development of this type of hypertrophy less likely.

However, the resting hemodynamics in our laboratory and in most other laboratories were measured during the daytime. Since rats are nocturnal animals, the activity of their circulation system is higher in the night. Because of this fact, the active

MAP might be more important than the resting values as a factor in determining the cardiac load. The nighttime MAP of normotensive WKY rats was increased by 4 - 5 *mmHg* by a diet with 8% NaCl for 2 *wk* ($p < 0.05$) (Calhoun *et al.*, 1994). But this increase was less than 10% of the resting BP and it appeared as very short pulses, while the daytime or 24 *hr* MAP was not changed.

In this study, the daytime resting HR was increased by a high sodium diet in the 2 *wk* group of the protocol for α_1-blocker studies; this increase disappeared in the 4 and 6 *wk* groups (Table 4, 11 and 12). HR was also significantly increased by a medium high sodium diet in both the 2 and 6 *wk* groups (Table 4). The increase in HR by a sodium exposure could suggest either an increased cardiac sympathetic activity, increased sinus ß-receptor responses or a decrease in the cardiac parasympathetic control. However, the HR increase by the sodium exposure was at a moderate level (14%) and not consistently observed in all the protocols of this study or in experiments by others with a similar high sodium diet regimen (Yuan and Leenen, 1991). The finding of the increase in HR from this study is even opposite to the result reported from another laboratory that HR of WKY rats was reduced by a high sodium exposure (Calhoun *et al.*, 1994). The discrepancy in the effects of salt exposure on the resting HR could be due to a difference in the age of rats used or, most probably, due to a difference in the time schedule for the hemodynamic measurements (see section V.1. in discussion).

III. Sodium Diet and Sympathetic Activity

III.1. *Effects of Ganglionic Blockade*

High sodium diet modulates arterial baroreceptor reflex control of HR and renal or lumbar sympathetic nerve activity (Calhoun *et al.*, 1991; Huang and Leenen, 1992) and this modulation is more pronounced in young than in adult or old rats (Huang and Leenen, 1992). This study did not address the baroreceptor reflex control. The responses of BP, HR, CI and TPRI to hexamethonium reflect the extent of sympathetic and parasympathetic control of the peripheral resistance and of cardiac function.

With the injection of hexamethonium, BP (both SBP and DBP) was significantly lowered in all the groups on various diets (Fig. 4). The lowering of BP resulted mainly from a decrease in the peripheral resistance, as indicated by the significant decrease in the TPRI (Table 5). This effect is from a blockade of the sympathetic tone on the resistance vessels by the drug (Salem, 1978). The decrease in BP became more pronounced by a prolonged high sodium diet regimen. Compared with the responses of rats in the groups on a regular diet, there was a significantly greater BP decrease in rats on a high sodium diet in the 2 or 6 *wk* groups, while the augmented decrease by a medium high sodium diet did not become significant until the diet exposure lasted for 6 *wk* (Fig. 4). The effect of the high sodium diet on the BP decrease by hexamethonium is different from the effect of a salt exposure by giving normal saline as the drinking water to WKY rats. The latter did not show an augmented hypotensive effect of hexamethonium (Fields *et al.*, 1991).

While the decrease in BP was augmented, the decrease in TPRI in response to hexamethonium was not altered by the high sodium diet (Table 5). SVI and CI was

significantly decreased in response to hexamethonium in the 6 *wk* medium high sodium diet group (Table 5). However, the changes in SVI and CI in the groups on high sodium diet for 6 *wk* or on either the medium high or high sodium diet for 2 *wk* were variable and nonsignificant (Table 5).

In response to hexamethonium, the HR was increased because the predominant vagal control on HR is blocked by the drug (Salem, 1978). In the high sodium diet groups the increase was more variable (Fig. 4 for 2 and 6 *wk*; Fig. 14 for 2 and 4 *wk*; data for 1 *wk* not shown). The high sodium diet did not affect the LVPSP decrease by hexamethonium compared to the group on a regular diet (Fig. 13). The change in LVEDP in response to hexamethonium was variable and nonsignificant, and the change was not affected by a high sodium exposure (Fig. 13). Similar to the systemic BP, LVPSP is a parameter influenced by many factors, such as cardiac function, peripheral resistance and venous return, but it is an alternative for the maximal rate of intracardiac pressure rise (dP/dt) or other related measurements in determining cardiac contractility. LVEDP is a measure for the venous return, the compliance of ventricular wall as well as the efficiency of the LV ejection, and is commonly referred to as the preload. The results from the hemodynamic measurements in general suggest a smaller effect of the high sodium diet on cardiac contractility compared to its influence on the peripheral resistance. However, since all these measurements were done in untethered conscious rats, the results from pressure measurements are more reliable and reproducible than that from CO measurements, which can be easily changed by the alteration of thermistor locations from any small postural changes (see section V.3. for discussion). Because of this limitation, it becomes difficult to make a definitive conclusion on the changes in the

regulation of the cardiac functions associated with the salt exposure. Besides SVI and CI, TPRI is also calculated from parameters involving CO measurements. As mentioned earlier, the changes in TPRI were not parallel to the changes in BP, which might be from a lower sensitivity or reproducibility of the CO measurements. Therefore, these results may indicate that the sympathetic control on the peripheral resistant arterioles may have been augmented by a high dietary sodium exposure.

III.2. *Adrenoceptor Responsiveness*

After blocking the ganglionic transmission with hexamethonium, infusion of phenylephrine increased MAP in a dose-dependent manner (Fig. 5). This MAP increase is mainly through an increase in the TPR by its activation of vascular α_1-receptors, while the CI was not significantly changed (Fig. 6).

With the increase in MAP, HR was decreased dose-dependently (Fig. 5), although the baroreceptor reflex control on the HR was supposed to have been blocked already by hexamethonium. The decrease in HR could be via an incomplete blockade in the baroreflex, or more probably from a direct activation of cardiac α_1-receptors by phenylephrine (Terzic *et al.*, 1993). Activation of cardiac α_1-receptor in adult animals caused a negative chronotropy (Steinberg *et al.*, 1985). This negative chronotropic effect is blocked by CEC, a selective α_{1B}-antagonist (del Balzo *et al.*, 1990). Since phenylephrine is a non-selective α_1-agonist, it can directly activate cardiac α_{1B}-adrenoceptor and then induce a negative chronotropy.

The increase in MAP in response to phenylephrine infusion was not changed by the high sodium diet, in either the slope or magnitude of the response curves (Fig. 5). This is not in agreement with the results from studies on healthy humans, in which an augmentation by a high sodium diet of the MAP increase in response to norepinephrine was consistently shown (Rankin *et al.*, 1981; Skrabal *et al.*, 1984; Sharma *et al.*, 1992). These latter studies suggest an increased response of the vascular α_1-receptor to adrenergic stimulations. However, all the human studies were done under conditions where the baroreceptor reflex control was not eliminated. Therefore, the pressor responses were complicated with the involvement of reflex reactions of both the sympathetic and parasympathetic nervous system to the original adrenergic stimulations. In particular, the pressor responses may have been compounded by a reflex change in the cardiac functions.

The decrease in HR associated with phenylephrine infusion was not changed by a high sodium diet (Fig. 5), except for the 6 *wk* group. Only in this group, the decrease in HR at higher doses of phenylephrine was significantly augmented by the high sodium diet when compared to the decrease in the regular diet group. The augmented HR decrease by a longer salt exposure may indicate an increase in the sinoatrial node α_{1B}-adrenoceptor responsiveness, since an activation of this subtype will evoke a negative chronotropy, as discussed above (del Balzo *et al.*, 1990). This augmented response was only seen in rats on a longer diet regimen and thus the LVH was already established. Because of this, the alteration of the HR responses is likely not related to the mechanisms causing hypertrophy.

Isoproterenol was used in this study to test the chronotropic and inotropic

responses of the heart as well as the vasodilatation in the resistance arteries. After elimination of the baroreceptor reflex control by hexamethonium, isoproterenol increased the HR markedly, together with an increase in the CI (Fig. 7 and 8). LVPSP was also increased dose-dependently by isoproterenol (Fig. 16). These hemodynamic responses are a reflection of a direct activation of the cardiac $ß_1$- and $ß_2$-receptors by the drug.

The effects of isoproterenol on HR and CI were not changed by a high sodium diet, but the increase in LVPSP was enhanced by the sodium exposure (Fig. 16). This is in agreement with the report that the responses of HR to isoproterenol infusion was not changed in normotensive volunteers after a 7-day high sodium diet (Volpe *et al.*, 1982). It is opposite to the other report that the cardiac sensitivity of normal humans to isoproterenol was increased by a 10-day high dietary sodium (Fraser *et al.*, 1981). Since our study was done in rats without the influence of baroreceptor reflex, the effect of salt exposure on the chronotropic and inotropic responses tested here more likely reflects the actual effect of this exposure on the sensitivities of the cardiac ß-receptors. The enhanced LVPSP increase in response to isoproterenol in the 4 *wk* high sodium diet group may indicate a decreased vasodilation by the sodium exposure, in agreement with the reduced BP decrease found in the 6 *wk* high sodium diet group (Fig. 7).

With the infusion of isoproterenol, MAP was significantly decreased and this decrease was not altered by the 1 or 2 *wk*, but significantly attenuated by the 6 *wk*, high sodium diet (Fig. 7). The decrease of MAP in response to isoproterenol is via an activation of the vascular $ß_2$-receptors, as shown by the significant decrease in TPRI (Fig. 8). From the attenuated decrease in MAP by a high sodium diet, it can be speculated that the salt exposure may have decreased the $ß_2$-receptor density or affinity in the

resistance vessels. This speculation is in agreement with the results from receptor binding studies, in which an up-regulation of the α_2- and a down-regulation of $ß_2$-receptors in the blood cells from normal humans by a salt exposure was found (Skrabal *et al.*, 1988). If a similar pattern of changes in the adrenoceptor subtypes has also occurred in the vascular smooth muscle cells by a chronic salt exposure, the vasodilating response to isoproterenol could be attenuated. However, there are no other *in vivo* studies supporting this speculation.

The results of the hemodynamic measurements from this study in general do not suggest a change in the cardiac α_1- or ß-receptor responsiveness of rats by a high sodium diet, although several other studies on humans showed that the pressor responses to norepinephrine or chronotropic responses to isoproterenol were increased by a high sodium diet (Fraser *et al.*, 1981; Rankin *et al.*, 1981; Skrabal *et al.*, 1984; Sharma *et al.*, 1992).

There are several possible causes for the differences in the observed effect of salt exposure on the α- or ß-receptor responses between the experiments of ours and of others. One potential cause could be the species difference, since different species can have different sensitivities to salt exposure. Thus, the extent and time-course of the possible changes in the adrenoceptor responses can be different between humans and rats. This discrepancy could be from a difference of the protocols as well. Since the hemodynamics in all the human studies were determined in the daytime, the subjects are active in this period and have higher sympathetic activity; while our measurements on rats were done also in the daytime, during which the rats are resting and have less physical activity, less feeding and lower sympathetic activity. The difference in the

sodium diet regimen between our study and others could also be a contributing factor. In most human studies (Fraser *et al.*, 1981; Wood *et al.*, 1982), there was a 40-fold difference in the sodium content between the low or normal and high sodium diets. And in some of the studies (Rankin *et al.*, 1981), there was even an 80-fold difference in the sodium content of the diets. In contrast, the high sodium diet in our study was only 13 times higher in NaCl content than that of the regular diet. The other possible factor for the difference between our results and others might be related to whether a ganglionic blocker is used to prevent the confounding effects of baroreceptor reflex control. As mentioned earlier, a high sodium diet modulates baroreceptor reflex function (Calhoun *et al.*, 1991; Huang and Leenen, 1992) and in particular, attenuates the HR and renal sympathetic nervous responses to BP increase (Huang and Leenen, 1992). It is possible that the cardiac sympathetic reactions to the BP increase might have been attenuated by a high sodium diet as well. If this assumption is correct, the reflex mechanism by which cardiac function is reduced in response to the α-receptor stimulation-increased BP (*i.e.*, by reducing HR or CI), could be attenuated in rats on a high sodium diet. Thus a higher pressor responses than in rats on a regular sodium diet could be expected in experiments not using a ganglionic blocker. Conversely, if a ganglionic blocker has been used, the pressor response becomes a more specific indicator for vascular α-receptor responses and will not be complicated by the baroreceptor reflex modulation on the cardiac functions. Therefore the experiment with ganglionic blockers should be able to more adequately assess the difference in pressor responses between different diets or between different treatment groups.

The general effects of salt exposure on the density or affinity of the cardiac

adrenoceptors are variable. As referred to earlier in introduction, an increase in the myocardial α_1-receptor density by giving 1% saline as drinking water to normotensive rats (Meggs *et al.*, 1988), or no change in the normotensive or 2K 1C hypertensive rats (Gallo *et al.*, 1990) or a decrease in DOCA-salt-treated rabbits or hypertensive rats (Woodcock *et al.*, 1979; Tsuji *et al.*, 1987) in the cardiac ß-receptor density by high sodium diet has been reported. It is difficult at this point to reach a conclusion on the actual changes in the cardiac adrenoceptors from a dietary sodium exposure.

III.3. *Effect of Adrenergic Blockade on the Hemodynamics*

In this study, a chronic treatment by *ig* injection of terazosin or nadolol was carried out throughout the entire diet regimen. Besides the effect of this chronic treatment on the high sodium diet-induced LVH, which will be discussed in the following section, the analysis of the effects of this treatment on the resting and responsive hemodynamics may be helpful in revealing a difference of the adrenergic activities among the various diet groups.

Only one dosage of the selective α_1-antagonist terazosin, 60 *mg/kg/day* was given for the 1, 2 and 4 *wk* diet groups. The dosage used was at a higher range of the doses used for rats and other laboratory animals (Kyncl, 1986). At this dosage, the pressor responses to phenylephrine were competitively antagonized (Fig. 15) because of a potent blockade of the post-synaptic α_1-receptors in the arteries (Kyncl, 1986). The extent of this blockade did not differ between the two different diet groups. The resting MAP,

LVPSP and LVEDP measured during the daytime were not changed by terazosin in either the regular or high sodium diet groups (Table 11 and 12). The lack of influence on the peripheral and LV pressures by terazosin may indicate a proper compensation in the hemodynamics despite the chronic oral administration of the drug. This is different from the effect of terazosin *ip* injected for 20 days in Sprague-Dawley rats, in which MAP was significantly decreased (O'Rourke and Reibel, 1992), or from the response to terazosin acutely *iv* infused, which decreased BP, LVPSP and TPR in normotensive dogs (Kyncl, 1986). It is also different from a 3 *wk sc* administration of 0.1 *mg/kg/day* bunazosin, which significantly decreased LVPSP of guinea pig with aortic binding (Tamai *et al.*, 1989). In addition to the difference in the routes by which the drug was given, in the latter two studies, hemodynamics were measured in anaesthetized animals, and BP may have been more dependent on the sympathetic activity.

In normotensive Sprague-Dawley rats, chronic treatment with the α_1-antagonist, prazosin, resulted in a salt-sensitive hypertension (Osborn *et al.*, 1993). This effect has been suggested to be from a mechanism related to the central nervous system, involving a possible regulation of vasopressin release or of the drinking behaviour. The possible central effect of prazosin may induce more release of vasopressin and thus fluid retention. In comparison, long-term terazosin treatment with a high sodium diet for 4 *wk* in this study did not change the MAP or LVPSP (Table 12), which may reflect a difference in the salt sensitivity between Sprague-Dawley and WKY rats (Osborn *et al.*, 1993). The different effects on arterial pressure may also be related to the difference in the dose and potency of the α_1-blockers used.

Different from the effect on the pressures, the resting HR was significantly

increased by terazosin in both the 1 and 2 *wk* groups on either a regular or high sodium diet (Table 11). But the increase in the HR by terazosin was not seen in the 4 *wk* groups (Table 12). The increase in HR by terazosin treatment may indicate a reflex tachycardia in the early stage of the drug treatment. In contrast, the HR of normotensive rats measured under anaesthesia was not changed by 2 *mg/kg/day* terazosin, even though their MAP was reduced (O'Rourke and Reibel, 1992). HR of rats with aortic constriction was not changed by 0.1 *mg/kg/day* bunazosin, despite a significant decrease in LVPSP (Tamai *et al.*, 1989). Similarly, terazosin rarely causes a reflex tachycardia in patients (Hoffman and Lefkowitz, 1993b; Moser, 1986). The discrepancy between this study and others could be from a difference in the doses used. Terazosin at very high doses, as used in this study, might have lost its seiectivity for α_1-receptors, and might have blocked both the α_1- and α_2-receptors (Kyncl, 1986). Blocking of the pre-synaptic α_2-receptors will reduce the feed-back inhibitory effects of the released transmitters on norepinephrine release, and will then induce more release of transmitters and thus a higher sympathetic activity and an increase in HR (Kyncl, 1993).

Regarding the parameters for venous return, the RAP, was not changed at 2, but significantly increased at 4 *wk* by terazosin in the regular diet group. Since the LVEDP at this time did not show an increase, the increased resting RAP in the regular diet group may not have much significance in indicating a change in the venous sympathetic activity. It is difficult to explain why RAP was not increased in rats exposed to a high sodium diet plus terazosin treatment. One possibility could be related to the diurnal fluctuations of the magnitude of the RAP increase in the high salt-exposed rats, with an expression of a higher magnitude in the night and a lower one during the day-time, since

both water intake and arterial pressure of rats on a high sodium diet showed higher diurnal variances than those of rats on a regular diet (Calhoun *et al.*, 1994). Because of this fluctuation, the daytime RAP measurements in the high sodium diet group may become less sensitive.

Although the decrease in LVPSP in response to hexamethonium was significant in both the terazosin-treated and control groups in comparison with their respective baseline values, the decrease was attenuated significantly by the chronic treatment with terazosin in rats on either the regular diet or high sodium diet. The attenuation of the LVPSP responses may be from a decrease in the sympathetic tone on the resistance arteries associated with terazosin treatment via its direct α_1-receptor blockade. Under this situation, even though the resting LVPSP was not lowered by terazosin, the decrease of LVPSP in response to hexamethonium was attenuated. However, the extent of the attenuation in LVPSP responses by terazosin was similar in both the regular and high sodium diet groups. Therefore it does not indicate a change in the adrenergic activities by the salt exposure.

Two dosages of the non-selective ß-antagonist nadolol, at either 25 or 100 *mg/kg/day*, were used throughout the entire 4 *wk* diet regimen. The dosages were applied in reference to the regular dosages used in animal studies (Lee *et al.*, 1992). The chronotropic and inotropic effects of isoproterenol were significantly antagonized by the chronic treatment at these dosages. With this treatment, the dose-response curves to isoproterenol were shifted markedly to the right (Fig. 16, data of the lower dosage nadolol treatment not shown). In contrast to the increase in HR by terazosin, the resting HR was decreased by 20 to 30% by nadolol in both the high sodium and regular diet

groups, through its direct blocking effect on the sinus $ß_1$- and $ß_2$-receptors (Frishman, 1981). With this pronounced inhibition on the resting HR, however, in both the regular and high sodium diet groups, nadolol did not change the resting LVPSP, LVEDP or RAP *vs.* control (Table 12). This result is in agreement with an unchanged BP despite a significantly reduced HR in WKY rats after over 20 *wk* nadolol administration at a similar daily dosage through drinking water (Lee *et al.*, 1992), and in either SHR or WKY rats after 4 *wk* propranolol treatment (Sen and Tarazi, 1983). Even with an *iv* injection, nadolol failed to change BP of the anaesthetized, open-chest dogs, although HR, venous return and CO were all decreased (Kawada *et al.*, 1986). It is possible that the hemodynamic functions in general may have been well compensated, probably from a slight increase in the peripheral resistance because of the blockade of arterial $ß_2$-receptors.

Chronic treatment with the ß-adrenoceptor antagonist propranolol increases the density of myocardial α_1-adrenoceptors (Steinkraus *et al.*, 1989), and *vice versa*. Since terazosin may increase cardiac sympathetic activity secondary to its reduction in TPR, the decrease in LVPSP by terazosin alone may be compensated for by an increase in the cardiac functions. When nadolol is used at the same time, the secondary cardiac effect of terazosin will be inhibited and the decrease in LVPSP can be demonstrated. Because of this reason, combined blockade of both the α_1- and ß-adrenoceptors was also applied in one experimental group. The combined blockade by nadolol with terazosin significantly decreased the resting LVPSP in the high sodium diet group, without changes in the regular diet group. The differential effects of the combined blockade on LVPSP in the regular and high sodium diet groups may indicate an augmented sympathetic

control of the cardiac function and peripheral resistance by the salt. The HR of both the regular and high sodium diets groups were decreased by the combined blockade. Meanwhile, the resting LVEDP or RAP in either the regular or high sodium diet groups were not changed by the combination of these two antagonists (Table 12).

III.4. *Summary*

Previous studies from our laboratory showed that the resting sympathetic activity was not increased by a high sodium diet, as demonstrated by the findings that the daytime LV norepinephrine turnover rate or tissue tyrosine hydroxylase activity were not increased (Fields *et al.*, 1991; Yuan and Leenen, 1991). This result does not exclude a change in the sympathetic activity at night associated with the high sodium diet. In normotensive humans high sodium diet for 4 *wk* decreased the plasma and urinary catecholamine concentrations (Wood *et al.*, 1982), indicating a decreased sympathetic activity by the salt exposure. Generally, most of the current reports suggest that resting sympathetic activity is either not changed or even decreased by a high dietary sodium in the normotensive rats or humans.

In this study the resting HR was increased by the salt exposure only in the early stages of the diet regimen. Although this increase diminished with extended time of salt exposure, it may indicate that an increase in the cardiac sympathetic activity occurred in the initial period of the diet regimen. In agreement with this assumption, the decrease in SBP and DBP in response to hexamethonium was augmented by the salt exposure,

indicating a higher sympathetic tone on the resistance vessels with the salt exposure. However, the possible increase in the cardiac and arterial sympathetic activities might not be so remarkable as to change the resting MAP or LVPSP. It is possible that in general the cardiac functions and peripheral resistance during the resting time were compensated well. Single α_1- or ß-adrenoceptor blockade did not change the resting LVPSP or LVEDP. When a combined blockade was used, the LVPSP was significantly decreased only in rats exposed to high dietary sodium, but not changed in those on a regular diet. This result also indicates that an augmented sympathetic control of cardiac contractility as well as of the peripheral resistance arteries may have been induced by a high sodium diet.

The only changes in the hemodynamic responses by a salt exposure observed in this study was the significantly attenuated BP decrease and the significantly enhanced LVPSP increase in response to isoproterenol. These two phenomenon demonstrate an attenuation in vasodilatation responses and may indicate a salt-induced decrease in the sensitivities of the vascular $ß_2$-receptors to adrenergic stimulation. Except for these two pieces of data, most results from this study do not suggest a change in rat cardiac α_1- or ß-receptor responsiveness by a high sodium diet. Studies in humans show that the pressor responses to norepinephrine or chronotropic responses to isoproterenol were increased by a high sodium diet (Fraser *et al.*, 1981; Rankin *et al.*, 1981; Skrabal *et al.*, 1984; Sharma *et al.*, 1992). The difference between this study and those on humans could be from a difference in the species used as the experimental subjects, because it is evident that the salt sensitivity can be greatly different even within one species (Skrabal *et al.*, 1984; Osborn *et al.*, 1993). It could be from a difference in the protocols for

hemodynamic measurements and most probably from a difference in whether the arterial baroreceptor reflex is blocked when the hemodynamic responses are measured. Since hexamethonium was used in this study, the pressor responses to phenylephrine, and the chronotropic and inotropic responses to isoproterenol should reflect more precisely the actual levels of the activities of the vascular α_1- or cardiac β_1- and β_2-adrenoceptors, respectively.

IV. Mechanisms of High Sodium Diet-Induced LVH

IV.1. *Possible Role of Adrenergic Responses*

Stimulation of α_1-adrenoceptor with norepinephrine induces cardiac hypertrophy *in vivo* (Laks *et al.*, 1973) and myocyte protein synthesis *in vitro* (Simpson *et al.*, 1982). The α_{1A}-receptors appear to be mainly responsible for the increased protein synthesis of cardiomyocytes stimulated by noradrenaline or phenylephrine (Simpson *et al.*, 1990; Knowlton *et al.*, 1993), while the α_{1B}-receptor is not. However, the response mediated mainly by one subtype of the α_1-receptor can also be blocked by a non-selective antagonist. For instance, the stimulated protein synthesis in neonatal rat cardiocytes via α_1-receptor activation blocked by a selective α_{1A}-receptor antagonist, was equally blocked by the non-selective α_1-receptor antagonists terazosin or prazosin (Simpson *et al.*, 1990). Based on this, it can be deduced that if an activation or an increased response of one

subtype of the α_1-receptors plays a major role in the high sodium diet-induced LVH, this LVH should be blocked by terazosin. The result from this study is negative, so it is less likely that α_1-receptor is primarily involved in the high sodium diet-induced LVH.

The potent blockade of the α_1-adrenoceptors by terazosin did not prevent LVH induced by the high sodium diet, instead, it was augmented by about 9% (Table 6). However, with terazosin treatment the increase in LV wall thickness by the high sodium diet alone was eliminated, resulting in a reduction of the ratio of the LV wall thickness to radius. Terazosin therefore switched the high sodium diet-induced LVH from a concentric form to an eccentric form. The increase in the ratio of LV to RV weight by the high sodium diet was significantly attenuated by terazosin or terazosin with nadolol. These changes in LVH features suggest that additional factors might have been involved in the increase in LV weight in the regular diet group and also in the exacerbation of the high dietary sodium-induced cardiac hypertrophy when terazosin was given.

In the high sodium diet group, terazosin augmented the increase in water intake in the initial period of the diet regimen (Fig. 9). This result is similar to the augmentation of high sodium diet-increased water intake in Sprague-Dawley rats by prazosin (Osborn *et al.*, 1993). The exaggeration of the dietary sodium-increased water intake by terazosin may also have increased the plasma volume, as the haematocrit was reduced moderately in the group with terazosin and high sodium diet *vs.* the group with a high sodium diet only (Fig. 12). These effects of chronic terazosin treatment may reflect a fluid retention similar to the long-term treatment with prazosin (Hoffman and Lefkowitz, 1993b). Taken together, the augmented increase in the water intake in the high sodium diet group by terazosin may suggest an involvement of volume overload in

the LV weight increase induced by a high sodium diet plus terazosin . The shift of LVH from a concentric to an eccentric form and the attenuation of the high sodium diet-induced increase in the ratio of LV to RV weight by terazosin (Table 6, 7 and 8) also support the speculated involvement of a volume overload. However, the LVEDP as well as the RAP was not increased by the high sodium diet throughout the 2 to 4 *wk* diet regimen (Table 11 and 12), indicating no measurable effect of the high sodium diet alone or combined with terazosin on the preload. The reasons for an unchanged RAP and LVEDP by sodium exposure plus terazosin is unknown. As discussed in the section on the effect of adrenergic blockade on hemodynamics, the possible bigger fluctuation of the magnitude of the filling pressures associated with high sodium intake may occur in the night. In general, from both the hemodynamics and gross heart anatomy, the data suggest that volume overload might be involved during high dietary sodium exposure plus terazosin treatment.

Blockade of the ß-adrenoceptors by nadolol did not prevent the high sodium diet-induced LVH. Instead, the LVH was aggravated by an additional 9% increase in the relative LV dry weight (Table 6). The LV weight of rats in the regular diet group was not changed by nadolol in this study. Similarly, nadolol at a lower dose (25 *mg/kg/day*) for 30 days did not change the weight or total protein content of the heart in Wistar rats (Paulin *et al.*, 1991). Chronic treatment with propranolol for 4 *wk* did not change the ratio of ventricular to body weight of WKY rats (Sen and Tarazi, 1983), while in another study, it even reduced the relative ventricular weight of rabbits (Vaughan-Williams *et al.*, 1975). There are no other known reports looking into the effects of the combination of salt exposure and ß-blocker treatment, therefore, it is difficult to compare results in this

respect. Similar to the change after using terazosin, the dietary sodium-induced LVH was switched from a concentric form to an eccentric form after the chronic nadolol treatment. As shown in Table 7, the high sodium diet-increased LV wall thickness was attenuated while the LV internal diameters were significantly increased by nadolol, resulting in a significant decrease in the ratio of the wall thickness to radius. Different from the effect of terazosin, nadolol did not increase the LV weight in the regular diet group, being similar to the result after over 20 *wk* chronic nadolol administration to WKY rats (Lee *et al.*, 1992). In this study nadolol did not augment the increased water intake induced by high sodium diet. This lack of LV weight change in the regular diet group is in agreement with the findings that nadolol did not increase the resting RAP or LVEDP, while it produced a significant bradycardia. However, the additional increase in LV weight by nadolol in rats on the high sodium diet is difficult to explain. Chronic nadolol treatment significantly increases norepinephrine levels in the heart of WKY rats (Lee *et al.*, 1992). This increased catecholamine levels may indicate a secondary increase in sympathetic activity after ß-receptor blockade, or a reduced release of the transmitters. For the first reason, combined blockade was performed (see the following discussion). Although the resting LVPSP in this study was not decreased, the general cardiac effect of nadolol is inhibitory and a direct effect of the drug to increase the heart weight is unlikely.

It has been demonstrated that increased cAMP levels will inhibit the intracellular pathway (Ras pathway) for growth factors, such as EGF, in fibroblasts, fat cells or smooth muscle cells, and because of this inhibition the cellular growth stimulation of the growth factors is blocked (Marx, 1993; Cook and McCormick, 1993). By analogy, the

aggravated LVH by ß-antagonist might be mediated through its reduction in cAMP, and hence less inhibition on the Ras growth pathway. However, the inhibitory communication between Ras pathway and cAMP has been demonstrated only in cell types other than cardiocytes. An increased intracellular cAMP in the cardiac cells was shown to promote cellular growth and protein synthesis (Anversa *et al.*, 1986; Xenophontos *et al.*, 1989).

To prevent a possible stimulation of one type of adrenoceptors while the other is blocked, a combined blockade was applied, but this combined blockade also failed to prevent the high dietary sodium-induced LVH. As shown in Table 6, the LV weight increase by a high sodium diet was not prevented by a combined blockade of both the α_1- and ß-adrenoceptors with terazosin and nadolol, instead, an extra 7% LV weight increase *vs.* the group on a high sodium diet alone was added. However, the combined blockade did not significantly decrease the ratio of LV wall thickness to radius (Table 7), a phenomenon seen in single blockade with either terazosin or nadolol. The results from experiments with α_1- or ß-antagonists, or with both do not support a primary involvement of the adrenergic receptors in the development of the high sodium diet-induced LVH. Besides, since combined blockade failed to prevent the LVH although it significantly decreased the resting LVPSP in the group on a high sodium diet (Table 12), this will once again exclude the possible role of an overload mechanism in this type of hypertrophy.

IV.2 *Other Potential Mechanisms*

IV.2.1. **Ang II.** Ang II has been demonstrated *in vivo* and *in vitro* to be a potent hypertrophic factor for myocardium (Sadoshima and Izumo, 1993c). Since the systemic renin activity is inhibited by a sodium exposure (Buttrick *et al.*, 1993), the circulating Ang II is hardly involved in the high sodium diet-induced LVH. Whether the cardiac renin activity is regulated differently or the AT receptor responses will be changed by high dietary sodium exposure needs further study.

IV.2.2. **Growth Factors.** There are no data at present focusing on the interaction of a salt exposure with growth factors on cardiomyocyte growth. The release of growth factors has been proposed as a major mechanism for the dietary sodium intake-induced growth of vascular smooth muscle cells (Aviv, 1990).

IV.2.3. **Change in $[Ca^{2+}]_i$.** $[Ca^{2+}]_i$ plays a central role in gene activation and protein synthesis in cardiac hypertrophy induced by pressure overload or other factors (Morgan and Bader, 1991). $[Ca^{2+}]_i$ as well as $[Na^+]_i$ might be involved in the high sodium diet-induced LVH since this type of LVH in WKY rats was prevented by potassium-magnesium-enriched food (Mervaala *et al.*, 1992). The increase in $[Ca^{2+}]_i$ can follow either a Na^+ influx through the Na^+/H^+ exchange (Soltoff and Cantley, 1988; Kent *et al.*, 1989), or through an inhibition of the Na^+, K^+-ATPase. However, the plasma Na^+ level is only transiently increased by 1 to 2% by a sodium exposure (Mozaffari *et al.*, 1990), hypernatraemia is rarely induced by increasing sodium intake alone (Vollmer, 1984), plus the activity of Na^+/H^+ exchanger is mainly regulated by its affinity for the intracellular H^+ but not extracellular $[Na^+]$ (Soltoff and Cantley, 1988). The second

pathway is also unlikely since inhibition of this enzyme by exogenous ouabain even reduces cardiac hypertrophy (Kent *et al.*, 1989).

V. Limitations of the Study

V.1. *Time Schedule for Hemodynamic Measurements*

All hemodynamics were measured 3 *hr* after finishing the cannulations. According to previous observations, this time period is sufficient for rats to recover from anaesthesia (Yamamoto *et al*, 1980; Kanda and Flaim, 1984) and has been routinely used in many studies (Yuan and Leenen, 1991; Frohlich et al., 1993). The other reason for adopting the 3 *hr* recovery schedule in this study was to reduce the post-operative mortality of rats with intraventricular cannulations. However, the hemodynamic recordings obtained through such a schedule might have been influenced or offset by a change in the physical and humoral conditions due to the traumatic effects of surgery and anaesthesia.

Stress responses to such a severe trauma as major surgery and general anaesthesia will certainly involve elevated sympathetic activities, an activation of adrenomedullary system and probably an increased function of the hypothalamus-pituitary-adrenal cortex system. Glucocorticosteroids released from the adrenal cortex can facilitate the action of catecholamines. This might be the physiological basis causing the difference in the

recordings of resting HR between our study and others. There was an increase, though not consistent in all the groups, in the resting HR by a high sodium diet in this study. In contrast, a decreased HR in rats on a similar level of high dietary sodium was found in another study (Calhoun *et al.*, 1994). In Calhoun's study, the hemodynamic measurements were done on day 7 after surgery. This longer recovery time will certainly be beneficial for the animals in getting over the traumatic period and may also help in eliminating the influence of the elevated catecholamines and corticosteroids on the resting HR.

Since reports describing the effects of a high sodium diet on hemodynamic responses to adrenergic stimulation are rare, it is not possible to determine whether there is, and how much the influence there will be, from a shorter post-operative recovery time on the hemodynamic recordings. However in each protocol of this study, there was a control group on regular diet or a group without drug treatment, which may reduce the possible influences of this shorter recovery time between surgery and hemodynamic measurements.

V.2. *Sensitivity and Significance of Hemodynamic Measurements*

The objective of this study was to look into the possible changes of cardiac adrenoceptor responses and the role of these possible changes in the high sodium diet-induced cardiac hypertrophy. Since only *in vivo* pharmacological approaches were used to examine the adrenoceptor responses, by measuring pressor responses to phenylephrine

and chronotropic and inotropic responses to isoproterenol, the measurements might not be sensitive enough to reveal a small change of these responses. As the pressor responses to phenylephrine are an estimate of the responses of vascular α_1-adrenoceptors, it is difficult to extrapolate these findings to cardiac α_1-adrenoceptor function. More sensitive parameters of LV function include ejection fraction and dP/dt, which can be determined in rats with echocardiography (de Simone *et al.* 1990) or Millar catheter (Liu *et al.* 1991), respectively. These two parameters can provide information of the muscular contraction properties of the ventricle.

Although this methodological approach in this study is closer to physiological conditions than *in vitro* methods, the measurements are influenced by many other factors. It is also difficult with this method to pinpoint any changes in the receptor characteristics, such as density, affinity or general reactivity. To more accurately determine the changes in the receptor activities, receptor binding assays, immunochemical assays or molecular biological assays could be used to look into the changes in the levels of receptor protein, intracellular signal pathways and immediate early-genes or mRNA.

V.3. *Accuracy of Hemodynamic Parameters Based on CO Measurements*

Since all the hemodynamic measurements were done in untethered conscious rats, the results from the pressure measurements are more stable and reliable than those from CO measurements. In this study, CI, SVI and TPRI were all derived from the original measurements of CO. However, CO measurements were easily influenced by the

movement of thermistor tip location from any small postural changes. Because of this, the variability of CO measurements was higher than pressure measurements.

For improvement in the accuracy of this measurement, one solution is to cut down the numbers of animals tested each day. This will leave the rats more time for recovery from surgery and from previous manipulations, such as infusion or injection of drugs or connection and disconnection of the cannulas. The other possible solution is to apply other techniques for CO measurements, in which the readings are less influenced by animal movement and have smaller variance between individual measurements.

V.4. *Specificity of the Adrenergic Blockade*

The adrenergic blockers used do not have tissue selectivity or high adrenoceptor subtype specificity. Since nadolol is a non-selective ß-antagonist, it can block both $ß_1$- and $ß_2$-receptor (Frishman, 1981). It has not been clarified yet which subtype of the ß-receptor is more important in cardiac hypertrophy. This non-selective effect can also influence vascular function by its blockade of the $ß_2$-receptor on the vessels. Because of this, the systemic effects of nadolol might have complicated its effects on the heart. Although terazosin is a selective α_1-antagonist, it blocks α_1-receptor in both the myocardium and the vasculatures (Kyncl, 1986). The hemodynamic effects from blocking the vascular receptors may have similarly complicated the direct effect of terazosin on the heart. Besides, terazosin blocks both α_{1A}- and α_{1B}-receptors (Simpson *et al.*, 1990). If the high dietary sodium-induced hypertrophy shares some similar

mechanisms with that induced by exogenous norepinephrine, in which α_{1A}-receptors have been demonstrated to be mainly responsible for the hypertrophic effect, terazosin would not selectively block this subtype. Since α_{1B}-receptors are more important than α_{1A}-receptors in the α_1-adrenoceptor-mediated negative inotropic effects (Terzic *et al.*, 1993), this subtype may have different significance in high salt exposure-induced hypertrophy. Therefore, the actual roles of the cardiac adrenoceptors in the development of high sodium diet-induced LVH need to be further clarified, by using specific antagonists for different subtypes of the α_1- and ß-adrenoceptors.

CONCLUSION

The high sodium diet-induced LVH was in a concentric form and well established after a 2 *wk* diet regimen. From resting hemodynamic measurements, this study further confirmed that pressure or volume overload do not appear to be involved in the development of this LVH.

In either the early or late stages of the high sodium diet regimen, the *in vivo* α_1-, and ß-receptor responses of WKY rats to phenylephrine or to isoproterenol were not changed. This result suggests that high sodium diet does not significantly alter the adrenoceptor responses in the rats. Although an attenuated vasodilatation in response to isoproterenol and an augmented negative chronotropy in response to phenylephrine were found to be associated with salt exposure, these changes occurred only in the 6 *wk* group when LVH was well established. Therefore, the potential changes in the vascular $ß_2$- or cardiac α_1-adrenoceptors suggested from these changes in the responses may not be essential in initiating the LVH. Experiments with hexamethonium and with adrenergic blockers suggest an augmented sympathetic control on the peripheral resistance arteries by salt exposure, as suggested by an augmented decrease in blood pressure by hexamethonium or a significant reduction in LVPSP by a combination of nadolol and terazosin only in rats after a prolonged high sodium diet. The variable increase in the resting HR by salt exposure indicates an increased cardiac sympathetic activity, or a decreased vagal activity. In contrast, most other resting hemodynamic parameters, MAP, TPRI, LVEDP and LVPSP were not changed by the high sodium diet.

The high sodium diet-induced LVH was not prevented by either single or combined blockade with chronic α_1-, ß-receptor antagonists. These results may indicate that activation of α_1- or ß-receptors might not be involved in the high sodium diet-induced LVH. However, the ratio of the LV wall thickness to radius was reduced by single blockade with terazosin or nadolol, indicating that LVH is shifted from a concentric to an eccentric form. Since terazosin aggravated the salt-increased water intake, shifted the LVH from a concentric form to an eccentric one, attenuated the high sodium diet-increased ratio of LV to RV weight, and probably expanded the plasma volume, it is possible that volume overload may have occurred with terazosin treatment. The possible mechanism for the volume overload may be related to terazosin-induced fluid retention via an action on kidneys or central nervous system. The volume overload may have induced a hypertrophy, which may have masked a reduction in the high sodium diet-induced hypertrophy.

REFERENCES

Aardal, S. and K. B. Helle. 1991. Comparative aspects of the endocrine myocardium. *Acta Physiol. Scand.* **599**(Suppl.): 31-46.

Aceto, J. F., and K. M. Baker. 1990. [Sar^1] Angiotensin II receptor-mediated stimulation of protein synthesis in chick heart cells. *Am. J. Physiol.* **258**:H806-H813.

Anversa, P., R. Ricci, and G. Olivetti. 1986. Quantitative structural analysis of the myocardium during physiologic growth and induced cardiac hypertrophy: a review. *J. Am. Coll. Cardiol.* **7**:1140-1149.

Atkins, D. L., J. K. Rosenthal, P. A. Krumm, and W. J. Marvin, Jr. 1992. Differential growth of neonatal WKY and SHR ventricular myocytes within sympathetic co-cultures. *J. Mol. Cell. Cardiol.* **24**:1479-1489.

Aviv, A. 1990. Salt sensitivity: trophic effect of growth and vasoactive factors (Letter). *Hypertension* **16**:103.

Baker, K. M., G. W. Booz, and D. E. Dostal. 1992. Cardiac actions of angiotensin II: role of an intracardiac renin-angiotensin system. *Annu. Rev. Physiol.* **54**:227-241.

Battarbee, H. D., D. P. Funch, and J. W. Dailey. 1979. The effect of dietary sodium and potassium upon blood pressure and catecholamine excretion in the rat. *Proc. Soc. Exp. Biol. Med.* **161**:32-37.

Berridge, M. J., and R. F. Irvine. 1989. Inositol phosphates and cell signalling. *Nature* (London) **341**:197-205.

Brown, Jr, A. L. 1971. Morphologic factors in cardiac hypertrophy. *In* Cardiac Hypertrophy. N. Alpert, editor. Academic Press, New York. 11-18.

Buttrick, P., M. Kaplan, M. J. F. Camargo, G. de Simone, J. H. Laragh, D. Wallerson, and R. B. Devereux. 1993. The influence of dietary salt and plasma renin activity on myosin heavy chain gene expression in rat hearts. *Am. J. Hypertens.* **6**:579-585.

Calhoun, D. A., S. Zhu, J. M. Wyss, and S. Oparil. 1994. Diurnal blood pressure variation and dietary salt in spontaneously hypertensive rats. *Hypertension* **24**:1-7.

Calhoun, D. A., J. M. Wyss, and S. Oparil. 1991. High NaCl diet enhances arterial baroreceptor reflex in NaCl-sensitive spontaneously hypertensive rats. *Hypertension* **17**:363-368.

Chrysant, S. G., G. M. Walsh, D. C. Kem, and E. D. Frohlich. 1979. Hemodynamic and metabolic evidence of salt sensitivity in spontaneously hypertensive rats. *Kidney Int.* **15**:33-37.

Clough, D. L., M. B. Pamnani, S. J. Huot, and F. J. Haddy. 1985. Myocardial Na^+, K^+-ATPase activity in Dahl salt-sensitive and resistant rats. *Clin. Exp. Hyper.-Theory and Practice* **A7**:573-584.

Cohen, J. 1974. Role of endocrine factors in the pathogenesis of cardiac hypertrophy. *Circ. Res.* **34/35**(Suppl. II):49-57.

Cook, S. J., and F. McCormick. 1993. Inhibition by cAMP of Ras-dependent activation of Raf. *Science* **262**:1069-1072.

Cooper, G., R. L. Kent, C. E. Uboh, E. W. Thompson, and T. A. Marino. 1985. Hemodynamic versus adrenergic control of cat right ventricular hypertrophy. *J. Clin. Invest.* **75**:1403-1414.

de Champlain, J., L. Farley, D. Cousineau, M. van Ameringen. 1976. Circulating catecholamine levels in human and experimental hypertension. *Circ. Res.* **38**:109-114.

de Champlain, J., and M. van Ameringen. 1972. Regulation of blood pressure by sympathetic nerve fibres and adrenal medulla in normotensive and hypertensive rats. *Circ. Res.* **31**:617-628.

del Balzo, U., M. Rosen, F. Malfatto, L. M. Kaplan, and S. F. Steinberg. 1990. Specific α_1-adrenergic receptor subtypes modulate catecholamine-induced increases and decreases in ventricular automaticity. *Circ. Res.* **67**:1535-1551.

de Simone, G., R. B. Devereux, M. J. F. Camargo, D. C. Wallerson, and J. H. Laragh. 1993. Influence of sodium intake on in vivo left ventricular anatomy in experimental renovascular hypertension. *Am. J. Physiol.* **264**:H2103-H2110.

de Simone, G., D. C. Wallerson, M. Volpe and R. B. Devereux. 1990. Echocardiographic measurement of left ventricular mass and volume in normotensive and hypertensive rats. Necropsy validation. *Am. J. Hypertens* **3**:688-696.

Di Campo, V., L. M. Henriquez, T. Proverbio, R. Marin, and F. Proverbio. 1991. Na^+-ATPase activity, cell ion and water contents of kidney cortex slices from rats on a high Na^+ diet. *Biomed. Biochim. Acta* **50**:1213-1216.

Doyle, A. E. 1968. Endogenous-catecholamine content of cardiac muscle in sodium-loaded and sodium-depleted rats. *Lancet* **29**:1399-1400.

Dubus, I., J. L. Samuel, F. Marotte, C. Delcayre, and L. Rappaport. 1990. ß-Adrenergic agonists stimulate the synthesis of noncontractile but not contractile proteins in cultured myocytes isolated from adult rat heart. *Circ. Res.* **66**:867-874.

Du Cailar, G., J. Ribstein, J. Daures, and A. Mimran. 1992. Sodium and left ventricular mass in untreated hypertensive and normotensive subjects. *Am. J. Physiol.* **263**:H177-H181.

Du Cailar, G., J. Ribstein, R. Grolleau, and A. Mimran. 1989. Influence of sodium intake on left ventricular structure in untreated essential hypertensives. *J. Hypertens.* 7(Suppl. 6): S258-S259.

Dzau, V. J., D. W. Burt, and R. E. Pratt. 1988. Molecular biology of the renin-angiotensin system. *Am. J. Physiol.* **255**:F563-F573.

Egan, B. M., A. B. Weder, J. Petrin, and R. G. Hoffman. 1991. Neurohumoral and metabolic effects of short-term dietary sodium and NaCl restriction in men. Relationship to salt-sensitive status. *Am. J. Hypertens.* **4**:416-421.

Eid, H., and J. de Champlain. 1988. Increased inositol monophosphate production in cardiovascular tissues of DOCA-salt hypertensive rats. *Hypertension* **12**:122-128.

Ely, D. E., P. Thoren, J. Weigand, and B. Folkow. 1987. Sodium appetite as well as 24-h variations of fluid balance, mean arterial pressure and heart rate in spontaneously hypertensive (SHR) and normotensive (WKY) rats, when on various sodium diets. *Acta Physiol. Scand.* **129**:81-91.

Evans, R. M. 1988. The steroid and thyroid hormone receptor superfamily. *Science* **240**:889-895.

Fernandes, M., G. Onesti, R. Fiorentini, K. E. Kim, and C. Swartz. 1976. Effect of chronic administration of propranolol on the blood pressure and heart weight in experimental renal hypertension. *Life Sci.* **18**:967-970.

Fernandez, D, P. Bolli, W. Snedden, S. Vasdex, and P. G. Fernandez. 1988. Modulation of left ventricular hypertrophy by dietary salt and inhibition of angiotensin converting enzyme. *J. Hypertens.* **6**(Suppl. 4):S145-S147.

Ferrans, V. J. 1984. Cardiac hypertrophy: morphological aspects. *In* Growth of the Heart in Health and Disease. R. Zak, editor. Raven Press, New York. 187-240.

Fields, N. G., B. Yuan, and F.H.H. Leenen. 1991. Sodium-induced cardiac hypertrophy: cardiac sympathetic activity versus volume load. *Circ. Res.* **68**:745-755.

Fraser, J., J. Nadeau, D. Robertson, and A. J. J. Wood. 1981. Regulation of human leukocyte ß-receptors by endogenous catecholamines. *J. Clin. Invest.* **67**:1777-1784.

Freis, E. D. 1976. Salt, volume and the prevention of hypertension. *Circulation* **53**:589-595.

Frishman, W. H. 1981. Nadolol: a new ß-adrenoceptor antagonist. *N. Eng. J. Med.* **305**:678-682.

Frodin, M., P. Peraldi, and E. Van Obbergher. 1994. Cyclic AMP activates the mitogen-activated protein kinase cascade in PC12 cells. *J. Biol. Chem.* **269**:6207-6214.

Frohlich, E. D., Y. Chien, S. Sesoko, and B. L. Pegram. 1993. Relationship between dietary sodium intake, hemodynamics, and cardiac mass in SHR and WKY rats. *Am. J. Physiol.* **264**:R30-R34.

Frohlich, E. D. 1989. Left ventricular hypertrophy, cardiac disease and hypertension: recent experience. *J. Am. Coll. Cardiol.* **14**:1587-1594.

Fuller, S. J., C. J. Gaitanaki, and P. H. Sugden. 1990. Effects of catecholamines on protein synthesis in cardiac myocytes and perfused heart isolated from adult rats. Stimulation of translation is mediated through the alpha$_1$-adrenoceptor. *Biochem. J.* **266**:727-736.

Gallo, A., C. M. Taquini, M. Fontan, R. Campissi, I. Kuraja, H. G. Llambi, and A. C. Taquini. 1990. Sodium intake modulates the development of cardiac hypertrophy in two-kidney, one-clip rats. *Hypertension* **15**(Suppl. I):I-157-I-160.

Gradin, K., H. Nissbrand, F. Ehrenstom, M. Henning, and B. Persson. 1988. Adrenergic mechanisms during hypertension induced by sucrose and/or salt in the spontaneously hypertensive rat. *Naunyn-Schmiedebergs Arch. Pharmacol.* **337**:47-52.

Grossman, W. 1980. Cardiac hypertrophy: useful adaption or pathologic process? *Am. J. Med.* **69**:576-584.

Hammond, I. W., R. B. Devereux, M. H. Alderman, and J. H. Laragh. 1988. Relation of blood pressure and body build to left ventricular mass in normotensive and hypertensive employed adults. *J. Am. Coll. Cardiol.* **12**:996-1004.

Haneda, T., P. A. Watson, and H. E. Morgan. 1989. Elevated aortic pressure, calcium uptake, and protein synthesis in rat heart. *J. Mol. Cell. Cardiol.* **21**(Suppl.I):131-138.

Hoffman, B. B., and R. J. Lefkowitz. 1993a. Catecholamines and sympathomimetic drugs. Section II, Drugs Acting at Synaptic and Neuroeffector Junctional Sites, *In* The Pharmacological Basis of Therapeutics. Gilman, A. G., T. W. Rall, A. S. Nies and P. Taylor, editors. McGraw-Hill, New York. 187-220.

Hoffman, B. B., and R. J. Lefkowitz. 1993b. Adrenergic receptor antagonists. Section II, Drugs Acting at Synaptic and Neuroeffector Junctional Sites, *In* The Pharmacological Basis of Therapeutics. Gilman, A. G., T. W. Rall, A. S. Nies and P. Taylor, editors. McGraw-Hill, New York. 221-243.

Hoh, J. F. Y., P. A. McGrath, and P. T. Hale. 1977. Electrophoretic analysis of multiple forms of rat cardiac myosin: effects of hypophysectomy and thyroxine replacement. *J. Mol. Cell. Cardiol.* **10**:1053-1076.

Huang, B. S., and F. H. H. Leenen. 1992. Dietary Na, age, and baroreflex control of heart rate and renal sympathetic nerve activity in rats. *Am. J. Physiol.* **262**:H1441-H1448.

Ikeda, U., Y. Tsuruya, and T. Yaginuma. 1991. α_1-Adrenergic stimulation is coupled to cardiac myocyte hypertrophy. *Am. J. Physiol.* **260**:H953-H956.

Ito, H., M. Hiroe, Y. Hirata, H. Fujisaki, S. Adachi, H. Akimoto, Y. Ohta, and F. Marumo. 1994. Endothelin ET_A receptor antagonist blocks cardiac hypertrophy provoked by hemodynamic overload. *Circulation* **89**:2198-2203.

Iwaki, K., V. P. Sukhatme, H. E. Shubeita, and K. R. Chien. 1990. α- And ß-adrenergic stimulation induce distinct patterns of immediate early gene expression in neonatal rat myocardial cells: *fos/jun* expression is associated with sarcomere assembly; *Egr*-1 induction is primarily an α_1-mediated response. *J. Biol. Chem.* **265**:13809-13817.

Kanda, K. and S. F. Flaim. 1984. Effects of nifedipine on total cardiac output distribution in the conscious rat. *J. Pharmacol. Exp. Ther.* **228**:711-718.

Katz, A. M. 1992. Altered gene expression in the chronically overloaded, failing heart. Chapter 25 Heart Failure, *in* Physiology of the Heart. Katz, A. M. editor. Raven Press, New York. 654-666.

Kawada, M., K. Satoh, and N. Taira. 1986. Cardiohemodynamic effects of nipradilol (K-351) in the dog: comparison with propranolol, nadolol and prazosin. *Japan. J. Pharmacol.* **42**:9-18.

Kent, R. L., K. J. Hoober, and G. Cooper IV. 1989. Load responsiveness of protein synthesis in adult mammalian myocardium: Role of cardiac deformation linked to sodium influx. *Circ. Res.* **64**:74-85.

Kihara, M., N. Utagawa, M. Mano, Y. Nara, R. Horie, and Y. Yamori. 1985. Biochemical aspects of salt-induced, pressure independent left ventricular hypertrophy in rats. *Heart Vessels* **1**:212-215.

King, B. D., D. Sack, M. R. Kichuk, and T. H. Hintze. 1987. Absence of hypertension despite chronic marked elevations in plasma norepinephrine in conscious dogs. *Hypertension* **9**:582-590.

Knowlton, K. U., M. C. Michel, M. Itani, H. E. Shubeita, K. Ishihara, J. H. Brown and K. R. Chien. 1993. The α_{1A}-adrenergic receptor subtype mediates biochemical, molecular, and morphologic features of cultured myocardial cell hypertrophy. *J. Biol. Chem.* **268**:15374-15380.

Kojima, M., I. Shiojima, T. Yamazaki, I. Komuro, Y. Zou, Y. Wang, T. Mizuno, K. Ueki, K. Tobe, T. Kadowaki, R. Nagai, and Y. Yazaki. 1994. Angiotensin II receptor antagonist TCV-116 induces regression of hypertensive left ventricular hypertrophy in vivo and inhibits the intracellular signalling pathway of stretch-mediated cardiomyocyte hypertrophy in vitro. *Circulation* **89**:2204-2211.

Kolbeck-Ruhmkorff, C., A. Horban, and H-G. Zimmer. 1993. Effect of pressure and volume overload on proto-oncogene expression in the isolated working rat heart. *Cardiovasc. Res.* **27**:1998-2004.

Krakoff, L. R., J. de Champlain, J. Axelrod. 1967. Abnormal storage of norepinephrine in experimental hypertension in the rat. *Circ. Res.* **21**:583-591.

Krebs, E. G. 1989. Role of the cyclic AMP-dependent protein kinase in signal transduction. *JAMA* **262**:1815-1818.

Kupari, M., P. Koskinen, and J. Virolainen. 1994. Correlates of left ventricular mass in a population sample aged 36 to 37 years, focus on lifestyle and salt intake. *Circulation* **89**:1041-1050.

Kyncl, J. J. 1993. Pharmacology of terazosin: An alpha$_1$-selective blocker. *J. Clin. Pharmacol.* **33**:866-899.

Kyncl, J. J. 1986. Pharmacology of terazosin. *Am. J. Med.* **80**(Suppl. 5B):12-19.

Laks, M. M., F. Morady, and H. J. C. Swan. 1973. Myocardial hypertrophy produced by chronic infusion of subhypertensive doses of norepinephrine in the dog. *Chest* **64**:75-78.

Lee, R. M. K. W., J. Tsoporis, R. R. J. Wang. 1992. Influence of chronic nadolol treatment on blood pressure and vascular changes in spontaneously hypertensive rats. *Can. J. Physiol. Pharmacol.* **70**:1261-1270.

Leenen, F. H. H., E. Harmsen, H. Yu, and C. Ou. 1993. Effects of dietary sodium on central and peripheral ouabainlike activity in spontaneously hypertensive rats. *Am. J. Physiol.* **264**:H2051-H2055.

Leenen, F. H. H., and E. Harmsen. 1991. Antihypertensive drugs and cardiac trophic mechanisms. *J. Cardiovasc. Pharmacol.* **17**(Suppl. 2):S50-S57.

Levy, D., R. J. Garrison, D. D. Savage, W. B. Kannel, and W. P. Castelli. 1990. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham heart study. *N. Eng. J. Med.* **322**:1561-1566.

Liebson, P. R., G. Grandits, R. Prineas, S. Dianzumba, J. M. Flack, J. A. Cutler, R. Grimm, and J. Stamler. 1993. Echocardiographic correlates of left ventricular structure among 844 mildly hypertensive men and women in the treatment of mild hypertension study (TOMHS). *Circulation* **87**:476-486.

Lin, Y. C., C. A. Dawson, E. R. Nadel, and S. M. Horvath. 1970. Reliability of cardiac output measured by thermodilution method in small animals. *Comp. Biochem. Physiol.* **34**:245-250.

Lindpaintner, K., and S. Sen. 1985. Role of sodium in hypertensive cardiac hypertrophy. *Circ. Res.* **57**:610-617.

Liu, Z., D. R. Hilbelink, W. B. Crockett, and A. M. Gerdes. 1991. Regional changes in hemodynamics and cardiac myocyte size in rats with aortocaval fistulas. 1. Developing and established hypertrophy. *Circ. Res.* **69**:52-58.

Long, C. S., C. J. Henrich, and P. C. Simpson. 1991. A growth factor for cardiac myocytes is produced by cardiac nonmyocytes. *Cell Regul.* 2:1081-1095.

Marban, E., and Y. Koretsune. 1990. Cell calcium, oncogenes, and hypertrophy. *Hypertension* 15:652-658.

Marx, J. 1993. Two major signal pathways linked. *Science* 262:988-990.

McPartland, R. P., and J. P. Rapp. 1982. (Na^+, K^+)-activated adenosinetriphosphatase and hypertension in Dahl salt-sensitive and -resistant rats. *Clin. Exp. Hyper.-Theory and Practice* A4:379-391.

Meggs, L. G., J. Ben-Ari, D. Gammon, and A. I. Goodman. 1988. Myocardial hypertrophy: the effect of sodium and the role of sympathetic nervous system activity. *Am. J. Hypertens.* 1:11-15.

Mervaala, E. M. A., J. Himberg, J. Laakso, P. Tuomainen, and H. Karppanen. 1992. Beneficial effects of a potassium- and magnesium-enriched salt alternative. *Hypertension* 19:535-540.

Moalic, J. M., J. Bercovici, and B. Swynghedauw. 1981. Protein synthesis during systolic and diastolic cardiac overloading in rat hearts: a comparative study. *Cardiovasc. Res.* 15:515-521.

Morgan, H. E., and K. Bader. 1991. Cardiac hypertrophy, mechanical, neural and endocrine dependence. *Circulation* **83**:13-25.

Morgan, H. E., E. E. Gorden, Y. Kira, B. H. L. Chua, L. A. Russo, C. J. Peterson, P. J. McDermott, and P. A. Watson. 1987. Biochemical mechanisms of cardiac hypertrophy. *Annu. Rev. Physiol.* **49**:533-543.

Moser, M. 1986. Advances in the management of hypertension: focus on terazosin, a new alpha-1-adrenergic antagonist. *Am. J. Med.* **80**(suppl. 5B):1-11.

Motoyama, T., H. Sano, T. Miki, H. Suzuki, K. Kawaguchi, Y. Furuta, and H. Fukuzaki. 1988. The role of chloride in the sympathetic nervous system in DOCA-salt hypertension. *Am. J. Hypertens.* **1**:287-290.

Mozaffari, M. S., S. Jirakulsomchok, S. Oparil, and J. M. Wyss. 1990. Changes in cerebrospinal fluid Na^+ concentration do not underlie hypertensive responses to dietary NaCl in spontaneously hypertensive rats. *Brain Res.* **506**:149-152.

Mukherjee, C., M. G. Caron, and R. J. Lefkowitz. 1975. Catecholamine-induced subsensitivity of adenylate cyclase associated with loss of ß-adrenergic receptor binding sites. *Proc. Natl. Acad. Sci. USA* **72**:1945-1949.

Nadal-Ginard, B., and V. Mahdavi. 1989. Molecular basis of cardiac performance. Plasticity of the myocardium generated through protein isoform switches. *J. Clin. Invest.* **84**:1693-1700.

Nagai, R., R. B. Low, W. S. Stirewalt, N. R. Alpert, and R. Z. Litten. 1988. Efficiency and capacity of protein synthesis are increased in pressure overload cardiac hypertrophy. *Am. J. Physiol.* **255**:H325-H328.

Nishi, A., A. M. Bertorello, and A. Aperia. 1992. High salt diet down-regulates proximal tubule Na^+, K^+-ATPase activity in Dahl salt-resistant but not in Dahl salt-sensitive rats: evidence of defective dopamine regulation. *Acta Physiol. Scand.* **144**:263-267.

O'Rourke, B., and D. K. Reibel. 1992. Effects of adrenoceptor blockade on cardiac hypertrophy and myocardial phospholipids. *Proc. Soc. Exp. Biol. Med.* **200**:95-100.

Osborn, J. W., B. J. Provo, J. S. Montana III, and K. A. Trostel. 1993. Salt-sensitive hypertension caused by long-term α-adrenergic blockade in the rat. *Hypertension* **21**:995-999.

Ostman-Smith, I. 1981. Cardiac sympathetic nerves as the final common pathway in the induction of adaptive cardiac hypertrophy. *Clin. Sci.* **61**:265-272.

Ostman-Smith, I. 1976. Prevention of exercise-induced cardiac hypertrophy in rats by chemical sympathectomy (guanethidine treatment). *Neuroscience* **1**:497-507.

Otani, H., H. Otani, T. Uriu, M. Hara, M. Inoue, K. Omori, E. J. Cragoe, Jr., and C. Inagaki. 1990. Effects of inhibitors of protein kinase C and Na^+/H^+ exchange on α_1-adrenoceptor-mediated inotropic responses in the rat left ventricular papillary muscle. *Br. J. Pharmacol.* **100**:207-210.

Paulin, A., J. Lalonde, and Y. Deshaies. 1991. ß-Adrenergic blockade and lipoprotein lipase activity in rat tissues after acute exercise. *Am. J. Physiol.* **261**:R891-R897.

Pawlush, D. G., R. L. Moore, T. I. Musch, and W. R. Davidson, Jr. 1993. Echocardiographic evaluation of size, function, and mass of normal and hypertrophied rat ventricles. *J. Appl. Physiol.* **74**:2598-2605.

Rakusan, K. 1984. Assessment of cardiac growth. *In* Growth of the Heart in Health and Disease. R. Zak, editor. Raven Press, New York. 25-40.

Rankin, L. I., F. C. Luft, D. P. Henry, P. S. Gibbs, and M. H. Weinberger. 1981. Sodium intake alters the effects of norepinephrine on blood pressure. *Hypertension* **3**:650-656.

Ruzicka, M., B. Yuan, E. Harmsen, and F. H. H. Leenen. 1993. The renin-angiotensin system and volume overload-induced cardiac hypertrophy in rats. Effects of angiotensin converting enzyme inhibitor versus angiotensin II receptor blocker. *Circulation* **87**:921-930.

Sadoshima, J., and S. Izumo. 1993a. Molecular characterization of angiotensin II-induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. Critical role of AT_1 receptor subtype. *Circ. Res.* **73**:413-423.

Sadoshima, J., Y. Xu, H. S. Slayter, and S. Izumo. 1993. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes *in vitro*. *Cell* **75**:977-984.

Sadoshima, J., and S. Izumo. 1993b. Mechanical stretch rapidly activates multiple signal transduction pathways in cardiac myocytes, potential involvement of an autocrine/ paracrine mechanism. *EMBO J.* **12**:1681-1692.

Sadoshima, J., and S. Izumo. 1993c. Signal transduction pathways of angiotensin II induced *c-fos* gene expression in cardiac myocytes *in vitro*: roles of phospholipid-derived second messengers. *Circ. Res.* **73**:424-438.

Sadoshima, J., L. Jahn, T. Takahashi, T. J. Kulik, and S. Izumo. 1992. Molecular characterization of the stretch-induced adaptation of cultured cardiac cells: an *in vitro* model of load-induced cardiac hypertrophy. *J. Biol. Chem.* **267**:10551-10560.

Salem, M.R. 1978. Therapeutic uses of ganglionic blocking drugs. *Int. Anesthesiol. Clin.* **16**:171-200.

Schmieder, R. E., F. H. Messerli, G. E. Garavaglia, and B. D. Nunez. 1988. Dietary salt intake. A determinant of cardiac involvement in essential hypertension. *Circulation* **78**:951-956.

Schreiber, S. S., M. Oratz, and M. A. Rothschild. 1971. Initiation of protein synthesis in the acutely overloaded perfused heart. *In* Cardiac Hypertrophy. N. Alpert, editor. Academic Press, New York. 215-246.

Sen, S., and D. R. Young. 1986. Role of sodium in modulation of myocardial hypertrophy in renal hypertensive rats. *Hypertension* **8**:918-924.

Sen, S., and R. C. Tarazi. 1983. Regression of myocardial hypertrophy and influence of adrenergic system. *Am. J. Physiol.* **244**:H97-H101.

Sen, S., R. C. Tarazi, and F. M. Bumpus. 1981. Reversal of cardiac hypertrophy in renal hypertensive rats: medical *vs.* surgical therapy. *Am. J. Physiol.* **240**:H408-H412.

Sen, S., R. C. Tarazi, P. A. Khairallah, and F. M. Bumpus. 1974. Cardiac hypertrophy in spontaneously hypertensive rats. *Circ. Res.* **35**:775-781.

Sharma, A. M., S. Schattenfroh, H. M. Thiede, W. Oelkers, and A. Distler. 1992. Effects of sodium salts on pressor reactivity in salt-sensitive men. *Hypertension* **19**:541-548.

Simpson, P. C., R. G. Cuenco, M. O. Paningbatan, and M. D. Murphy. 1990. An α_1-adrenergic receptor subtype sensitive to WB-4101 transduces cardiac myocyte growth. (Abstract) *Circulation* **82**(Suppl. III):561.

Simpson, P. C., C. S. Long, L. E. Waspe, C. J. Henrich, and C. P. Ordahl. 1989. Transcription of early developmental isogenes in cardiac myocyte hypertrophy. *J. Mol. Cell. Cardiol.* **21**(Suppl. V):79-89.

Simpson, P. C. 1988. Proton-oncogenes and cardiac hypertrophy. *Annu. Rev. Physiol.* **51**:189-202.

Simpson, P. C. 1985. Stimulation of hypertrophy of cultured neonatal rat heart cells through an α_1-adrenergic receptor and induction of beating through an α_1- and β_1-adrenergic receptor interaction. Evidence for independent regulation of growth and beating. *Circ. Res.* **56**:884-894.

Simpson, P. C. 1983. Norepinephrine-stimulated hypertrophy of cultured rat myocardial cells is an α_1-adrenergic response. *J. Clin. Invest.* **72**:732-738.

Simpson, P. C., A. McGrath, and S. Savion. 1982. Myocyte hypertrophy in neonatal rat heart cultures and its regulation by serum and by catecholamines. *Circ. Res.* **51**:787-801.

Skrabal, F., P. Kotanko, B. Meister, P. Doll, and G. Gruber. 1988. Augmented rise of $\alpha_2/ß_2$ adrenoceptor ratio induced by salt and essential hypertension. *Kidney Int.* **34**(Suppl. 25):S23-S27.

Skrabal, F., H. Herholz, M. Neumayr, L. Hamberger, M. Ledochowski, H. Sporer, H. Hortnagl, S. Schwarz, and D. Schonitzer. 1984. Salt sensitivity in humans is linked to enhanced sympathetic responsiveness and to enhanced proximal tubular reabsorption. *Hypertension* **6**:152-158.

Soltoff, S. P., and L. C. Cantley. 1988. Mitogenes and ion fluxes. *Annu. Rev. Physiol.* **50**:207-223.

Spaulding, S. W. 1993. The ways in which hormones change cyclic adenosine 3', 5'-monophosphate-dependent protein kinase subunits, and how such changes affect cell behaviour. *Endocrine Rev.* **14**:632-650.

Stamler, J., G. Rose, R. Stamler, P. Elliott, A. Dyer, and M. Marmot. 1989. INTERSALT study findings: public health and medical care implications. *Hypertension* **14**:570-577.

Stanton H. C., G. Brenner, and E. D. Mayfield, Jr. 1969. Studies on isoproterenol-induced cardiomegaly in rats. *Am. Heart J.* **77**:72-80.

Starksen, N. F., P. C. Simpson, N. Bishopric, S. R. Coughlin, W. M. F. Lee, J. A. Escobedo, and L. T. Williams. 1986. Cardiac myocyte hypertrophy is associated with *c-myc* proto-oncogene expression. *Proc. Natl. Acad. Sci. USA* **83**:8348-8350.

Steinberg, S. F., E. D. Drugge, J. P. Bilezikian, and R. B. Robinson. 1985. Acquisition by innervated cardiac myocytes of a pertussis toxin-specific regulatory protein linked to the α_1-receptor. *Science* **230**:186-188.

Steinkraus, V., M. Nose, H. Scholz, and K. Thormahlen. 1989. Time course and extent of the α_1-adrenoceptor density changes in rat heart after ß-adrenoceptor blockade. *Br. J. Pharmacol.* **96**:441-449.

Suzuki, J., H. Matsubara, M. Urakami, and M. Inada. 1993. Rat angiotensin II (type 1A) receptor mRNA regulation and subtype expression in myocardial growth and hypertrophy. *Circ. Res.* **73**:439-447.

Sweadner, K. J., V. L. M. Herrera, S. Amato, A. Moellmann, D. K. Gibbons, and K. R. H. Repke. 1994. Immunologic identification of Na^+, K^+-ATPase isoforms in myocardium: isoform change in deoxycorticosterone acetate-salt hypertension. *Circ. Res.* **74**:669-678.

Tamai, J., M. Hori, T. Kagiya, K. Iwakura, K. Iwai, A. Kitabatake, Y. Watanabe, H. Yoshida, M. Inoue, and T. Kamada. 1989. Role of α_1-adrenoceptor activity in progression of cardiac hypertrophy in guinea pig hearts with pressure overload. *Cardiovasc. Res.* **23**:315-322.

Terzic, A., M. Puceat, G. Vassort, and S. M. Vogel. 1993. Cardiac α_1-adrenoceptors: an overview. *Pharmacol. Rev.* **45**:147-175.

Tomanek, R. J., R. K. Bhatnagar, P. Schmid, and M. J. Brody. 1982. Role of catecholamine in myocardial cell hypertrophy in hypertensive rats. *Am. J. Physiol.* **242**:H1015-H1021.

Tomanek, R. J., J. W. Davis, and S. C. Anderson. 1979. The effect of alpha-methyldopa on cardiac hypertrophy in spontaneously hypertensive rats: ultrastructural stereological, and morphometric analysis. *Cardiovasc. Res.* **13**:173-182.

Tsoporis, J., B. Yuan, and F. H. H. Leenen. 1989. Arterial vasodilators, cardiac volume load, and cardiac hypertrophy in normotensive rats. *Am. J. Physiol.* **256**:H876-H880.

Tsuji, K., S. Tsutsumi, K. Ogawa, Y. Miyazaki, and T. Satake. 1987. Cardiac alpha$_1$ and beta adrenoceptors in rabbits: effects of dietary sodium and cholesterol. *Cardiovasc. Res.* **21**:39-44.

Vaughan-Williams, E. M., A. E. G. Raine, A. A. Cabrera, and J. M. Whyte. 1975. The effects of prolonged ß-adrenoceptor blockade on heart weight and cardiac intracellular potentials in rabbits. *Cardiovasc. Res.* **9**:579-592.

Vollmer, R. R. 1984. Effects of dietary sodium on sympathetic nervous system control of cardiovascular function. *J. Auton. Pharmacol.* **4**:133-144.

Volpe, M., B. Trimarco, B. Ricciardelli, L. Sacca, B. Ungaro, F. Rengo, and M. Condorelli. 1982. Effects of oral salt loading on beta-adrenergic receptor responsiveness in normal and hypertensive subjects. *Cardiovasc. Res.* **16**:732-737.

Waspe, L. E., C. P. Ordahl, and P. C. Simpson. 1990. The cardiac ß-myosin heavy chain isogene is induced selectively in α_1-adrenergic receptor-stimulated hypertrophy of cultured rat heart myocytes. *J. Clin. Invest.* **85**:1206-1214.

Weinberger, C., C. C. Thompson, E. S. Ong, R. Lebo, D. J. Gruol, and R. M. Evans. 1986. The *c-erb-A* gene encodes a thyroid hormone receptor. *Nature* **324**:641-646.

Wiseman, R., and C. C. Irving. 1963. A rapid micro blood volume method in laboratory animals. *Proc. Soc. Exp. Biol. Med.* **114**:728-731.

Wood, A. J. J., R. Feldman, and J. Nadeau. 1982. Physiological regulation of beta-receptors in man. *Clin. Exp. Hyper.-Theory and Practice* **A4**:807-817.

Woodcock, E. A., J. W. Funder, and C. I. Johnston. 1979. Decreased cardiac ß-adrenergic receptors in deoxycorticosterone-salt and renal hypertensive rats. *Circ. Res.* **45**:560-565.

Wu, J., P. Dent, T. Jelinek, A. Wolfman, M. J. Weber, and T. W. Sturgill. 1993. Inhibition of the EGF-activated MAP kinase signalling pathway by adenosine 3', 5'-monophosphate. *Science* **262**:1065-1069.

Xenophontos, X. P., P. A. Watson, B. H. L. Chua, T. Haneda, and H. E. Morgan. 1989. Increased cyclic AMP content accelerates protein synthesis in rat heart. *Circ. Res.* **65**:647-656.

Yamamoto, J., N. C. Trippodo, S. Ishise, and E. D. Frohlich. 1980. Total vascular pressure-volume relationship in the conscious rat. *Am. J. Physiol.* **238**:H823-H828.

Yonezawa, K., G. Endemann, K. S. Kovacina, J. E. Chin, C. Stover, and R. A. Roth. 1990. Substrates of the insulin receptor kinase. *In* The Biology and Medicine of Signal Transduction. Y. Nishizuka, editor. Raven Press, New York. 266-272.

Yuan, B., and F. H. H. Leenen. 1991. Dietary Na^+ intake and left ventricular hypertrophy in normotensive rats. *Am. J. Physiol.* **261**:H1397-H1401.

Zak, R. 1984. Overview of the growth process. *In* Growth of the Heart in Health and Disease. R. Zak, editor. Raven Press, New York. 1-24.

Zierhut, W., and H. G. Zimmer. 1989. Significance of myocardial α- and ß-adrenoceptors in catecholamine-induced cardiac hypertrophy. *Circ. Res.* **65**:1417-1425.

Zimmer, H. G., and H. Peffer. 1986. Metabolic aspects of the development of experimental cardiac hypertrophy. *Basic Res. Cardiol.* **81**(Suppl. 1):127-137.