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Effects of Dietary Sodium on Cardiac Responses to Adrenergic Stimulation in Vitro

by

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to my dear husband, my brother

Abdul Hussain and my kids

ABSTRACT

Background: In hypertensive patients, high sodium intake not only increases blood pressure, and thus causes left ventricular hypertrophy, but also appears to increase left ventricular hypertrophy independent of this increase in blood pressure. In both normo- and hyper-tensive rats the hypertrophic effect of increased dietary sodium intake on the heart has been established. High sodium intake increases left ventricular wall thickness relative to internal diameter, resembling pressure overload- rather than volume overload-induced cardiac hypertrophy. How high dietary sodium induces cardiac hypertrophy is still unknown. Several mechanisms have been postulated: Among others, the sympathetic nervous system may play a role in dietary sodium-induced cardiac hypertrophy through enhanced sensitivity to α_1- or ß-adrenoceptor stimulation.

Objective: To evaluate the role of α_1- or ß-adrenoceptors in dietary sodium-induced cardiac hypertrophy, we assessed 1) the effects of high sodium diet (8% NaCl) on the responses of hearts isolated from young WKY rats to α_1- or ß-adrenergic stimulation after 1, 2, or 6 weeks of high sodium intake and 2) the effects of high sodium diet on responses of hearts isolated from young WKY rats after 2 weeks of high sodium intake to α_{1a}- or α_{1b}-adrenoceptor subtype stimulation.

Methods: The Langendorff technique was used to perfuse the isolated rat hearts with Krebs-Henseleit buffer. This approach was used to minimize the factors and variations which can occur in in vivo studies and which could influence cardiac responses.

Methoxamine in incremental concentrations ranging from 10^{-7} to 10^{-5} M was used as a selective α_1-agonist, isoproterenol in a concentration range from 10^{-9} to 10^{-7} M was

used as a non-selective ß-agonist. Methoxamine (10^{-7} to 10^{-5} M) with 10^{-5} M urapidil (α_{1a}-adrenoceptor blocker) was used to evaluate the responses to α_{1b}-adrenoceptor stimulation, and methoxamine (10^{-7} to 10^{-5} M) with $0.25*10^{-5}$ M chloroethylclonidine (α_{1b}-adrenoceptor blocker) was used to evaluate the responses of the isolated hearts to α_{1a}-adrenoceptor stimulation. Developed pressure, heart rate, and coronary flow of hearts isolated from rats on a control or high sodium diet in respect to α_1-, α_{1a}-, α_{1b}, or ß-adrenergic stimulation have been used as indices for comparing the responses of the two diet groups.

Results: 1) Cardiac hypertrophy: A significant increase in heart dry weight and heart dry weight/body weight ratio occurred in the young WKY rats on high sodium intake: 9%, 12%, and 15% hypertrophy occurred after 1, 2, and 6 weeks of high sodium intake respectively.

2) α_1 and ß-Adrenergic stimulation: Both methoxamine and isoproterenol evoked significant increases in systolic pressure of isolated hearts, while only isoproterenol significantly decreased diastolic pressure. α_1-adrenergic stimulation mostly caused small non-significant decreases in heart rate and small non-significant increases in coronary flow, while-ß-adrenoceptor stimulation significantly increased heart rate and coronary flow. No significant differences between the two dietary groups were observed in respect to the response of these parameters to α_1- or ß-adrenergic stimulation.

3) α_{1a} and α_{1b}-adrenoceptor subtype stimulation: The α_{1a}-adrenoceptor blocker urapidil inhibited 85% of the methoxamine-induced-increase in developed pressure. It had no significant effect on the methoxamine-induced decreases in heart rate and prevented

the small methoxamine-induced increases in coronary flow of hearts isolated from control rats. Hearts isolated from high sodium treated rats showed an identical inhibition in methoxamine-induced-increase in developed pressure and the small methoxamine-induced-increase in coronary flow following α_{1a}-blockade. Unlike control hearts, methoxamine-induced-decrease in heart rate of the isolated hearts was significantly augmented following urapidil treatment in hearts from salt treated rats.

Pretreatment of the isolated hearts from control rats with the irreversible α_{1b}-adrenoceptors blocker chloroethylclonidine (CEC) inhibited 60% of methoxamine-induced-increase in developed pressure. CEC treatment also significantly inhibited the small methoxamine-induced-increases in coronary flow of hearts isolated from rats on a control diet. This treatment had no significant effect on the methoxamine-induced, small decrease in heart rate of control hearts. Hearts isolated from rats on high sodium diet showed similar changes to control hearts in developed pressure, coronary flow and heart rate in responses to methoxamine infusion following CEC, but tended to show smaller decreases in heart rate following α_{1b}-blockade compared with control hearts. No significant differences between the two diet groups subjected to CEC pretreatment were found.

Conclusions: Studies using methoxamine as an α_1-agonist, as an α_{1a}-adrenoceptor agonist in the presence of CEC, or as an α_{1b}-adrenoceptor agonist in the presence of urapidil or the use of isoproterenol as a non-selective ß-agonist revealed no significant differences in the sensitivity of ventricular α_1- adrenoceptor subtypes or ß-adrenoceptors between hearts from rats on a control or high sodium diet at any duration of feeding. A possible effect of high sodium diet on atrial α_{1a}- or α_{1b}-adrenoceptors is suggestive. The

unchanged ventricular contractile responses to α_1 or ß-adrenoceptor stimulation in the hypertrophied hearts suggest that changes in ventricular adrenergic receptors responsiveness do not play a role in dietary sodium-induced left ventricular hypertrophy. One can not exclude a dissociation of intracellular signals linked to α_1- or ß-adrenoceptors associated with dietary sodium induced cardiac hypertrophy from those associated with contractility.

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INTRODUCTION

Part 1. The myocardium and Hypertrophy.

The myocardium comprises of many different cells. Cardiac myocytes (cardiocytes), the larger of these cells occupy 75% of its structural space; although they constitute only one third of the cell population. Other cells (the nonmyocytes) include endothelial cells, vascular smooth muscle cells, cardiac fibroblasts, macrophages and mast cells {Zak, 1973}. Soon after birth, ventricular myocytes lose the ability to undergo cell division and as the heart grows and approaches maturity, myocardial cell enlargement becomes the principle process by which the heart as a whole enlarges. Under normal conditions the half-life of most cardiac proteins is 4-10 days {Morano et al. 1988}, although collagen turnover rates are substantially longer {Karim et al. 1991}. When adult ventricular myocardium is exposed to a growth stimulating factor, the ventricular myocardium enlarges by hypertrophy caused by enhanced protein synthesis {Zak, 1973}. In addition to this alteration in the rate of protein synthesis, cardiac hypertrophy may also be associated with a different expression patterns of the ventricular isomyosins such as myosin ATPase {Waspe et al. 1990; Gustafson et al. 1986}. Two sarcomeric myosin heavy chain (MHC) isogenes, designated α and ß are known to be expressed in cardiac muscle. The proteins encoded by these genes associate in pairs with identical light chains to form three myosin isoforms designated V_1 ($\alpha\alpha$), V_2 (αß), and V_3 (ßß) in the order of decreasing electrophoretic mobility and myosin ATPase activity (V_1 is associated with the highest ATPase activity). In certain species, the relative proportions of myosin isoforms vary during development. In rats for example, during the latter part of gestation, V_3-

myosin accounts for 80-90% of fetal ventricular myosin. After birth, a transition begins between V_3 and V_1, and by 3 weeks of age there is 100% V_1 {Morgan and Baker, 1991}. Myosin isoform shift may occur during hypertension, low sodium diet, treatment with ß-blockers {Sen and Young, 1986}, or in α_1-adrenoceptor stimulation {Waspe et al. 1990}.

Left ventricular hypertrophy (LVH) can be either of concentric type which is characterized by increased left ventricular wall thickness relative to internal diameter, a pattern normally associated with pressure overload {De Simone et al. 1993} or it could be of eccentric type which is associated with volume overload and characterized by an increase in ventricular internal diameter relative to wall thickness {Tsoporis et al. 1989; Ruzicka et al. 1993; Lavandero et al. 1993}. Cardiac hypertrophy involves a sequence of events, including initiating signals (**Extracellular Signals**), coupling mechanisms (**Intracellular Signals**), and **Regulation of Gene Expression** that can regulate and direct protein synthesis which finally creates an increment in mass of the myocardium.

(A) Extracellular Signals of Hypertrophy.

Cardiac hypertrophy usually follows an increase in work load that is imposed on the heart as a result of *Mechanical Factors*, but, it can also be the result of the direct effect of some *Endocrine and Growth Factors* or *Neural Factors* on cardiac myocytes.

A-I Mechanical factors.

Increased mechanical load on the heart by volume or pressure overload is usually associated with accelerated growth and cardiac hypertrophy. Volume overload imposed

by aortic insufficiency {Gerova et al. 1993} or aortocaval shunt {Ruzicka et al. 1993; Lavandero et al. 1993} produces a significant increase in heart weight and heart weight to body weight ratio in rabbits and rats within a month after surgery. In normotensive rats volume overload-induced cardiac hypertrophy has also been reported during long-term treatment with arterial vasodilators {Tsoporis et al. 1989; Ruzicka et al. 1993}. In all these cases eccentric left ventricular hypertrophy develops which indicates an increase in ventricular diameter relative to wall thickness.

Pressure overload-induced cardiac hypertrophy develops in all models of human hypertension such as the two-kidney, one clip (2K,1C) hypertension model {Lindpainter and Sen, 1985; Gallo et al. 1990} in which hypertension is induced by renal arterial narrowing without removal of the opposite kidney {Leenen and Myers, 1984}, in spontaneously hypertensive rats (SHR), a genetic model of hypertension {Mertens et al. 1992}, coarctation of the abdominal aorta in normotensive rats {de la Bastie et al. 1990}. The type of cardiac hypertrophy associated with pressure overload is mainly characterized by an increase in left ventricular wall thickness rather than its diameter.

A-II Endocrine and Growth factors.

A variety of growth factors such as insulin, endothelin, and others stimulate cell growth {Schneider and Parker, 1990; Engelmann et al. 1989} and may play a role in the development of cardiac hypertrophy. Serum from dogs with high-pressure induced cardiac hypertrophy increased protein synthesis in an acceptor dog heart, indicating a possible role for circulating growth factors in the development of cardiac hypertrophy

{Hommond et al. 1979}. The receptors for insulin and other peptide hormones that regulate growth, differentiation, and development (e.g. epidermal growth factor, platelet-derived growth factor and certain lymphokines) are frequently protein kinases that act by phosphorylating target protein on tyrosine residues. Thyroid hormone may directly mediate increased protein synthesis and development of cardiac hypertrophy. Administration of triiodothyronine (T_3) to rats induced metabolic and hemodynamic changes and resulted in development of cardiac hypertrophy. ß- or α-adrenoceptor blockade reduced hemodynamic changes but had no effect on T_3-induced cardiac hypertrophy which supports a possible direct effect for this hormone on cardiac growth {Zierhut and Zimmer, 1989}. T_3-induced cardiac hypertrophy is also characterized by increased myocardial content of V_1-myosin while the synthesis of V_3-myosin is depressed. Thyroid hormone can directly attach to a receptor on the nucleus, these receptors are soluble DNA-binding proteins, thus mediating or modulating transcriptional processes, which can result in the regulation of specific genes. This hormone causes an accumulation of α-myosin heavy chain mRNA and inhibits expression of ß-myosin heavy chain mRNA {Gustafson et al. 1986}. Angiotensin II (AII) can stimulate cardiac growth independent on hemodynamic changes. A possible direct growth effect of angiotensin II on the left ventricle has been demonstrated in the development of pressure overload- or volume overload-induced cardiac hypertrophy {Baker et al. 1990; Ruzicka et al. 1993}. Furthermore, treating animals having abdominal coarctation with enalapril (an ACE inhibitor) completely prevented LVH without changing carotid artery pressures {Baker et al. 1990}. In isolated cardiac myocytes, saralasin or losartan (AII receptor blockers)

completely inhibited or suppressed stretch-induced c-fos expression {Sadoshima et al. 1993}. In cardiovascular tissues, all components of the renin angiotensin system (angiotensinogen, renin, angiotensin I, angiotensin converting enzyme [ACE], angiotensin II, and angiotensin receptors) were found {Paul and Ganten, 1992} and a significant increase in cardiac renin activity was associated with the development of volume or pressure overload-induced cardiac hypertrophy {Schunkert et al. 1990; Ruzicka et al. 1993; Bore et al. 1994}. These trophic effects of AII are mediated through the AT_1 receptors { Dostal and Baker, 1992; Sadoshima and Izumo, 1993; Ruzicka et al. 1993}.

A-III Neural factors.

Catecholamines have long been implicated in the pathogenesis of LVH {Gans and Cater, 1970}. Chronic elevation of norepinephrine (NE) in conscious dogs for 28 days {Stewart et al. 1992}, infusion of subhypertensive dose of NE in dogs {Chiba et al. 1989}, or constant intravenous infusion of NE in rats for 3 days {Zierhut and Zimmer 1989} all resulted in a various degree of cardiac hypertrophy. In vitro studies on isolated myocytes from neonatal or adult rat hearts supported the in vivo studies and demonstrated a clear trophic effect of catecholamines on these cells {Simpson et al. 1985; Schluter and Piper 1992; Knowlton et al. 1993; Clark et al. 1993}. This trophic effect of NE could be mediated through stimulation of ß- or α-adrenergic receptors. Rats received a constant intravenous infusion of norepinephrine for 3 days showed increased left ventricular weight which was antagonized partially by the α_1-blocker prazosin or the ß-blocker metoprolol but it was prevented by simultaneous administration of both blockers {Zierhut and

Zimmer, 1989}. In vivo daily injection of isoproterenol to the rats produced a cardiac hypertrophy of about 15-20% {Leenen and Harmsen, 1991}. However, in contrast to these in vivo studies, treatment of isolated ventricular cardiomyocytes from adult rats with 1 or 10 μM isoproterenol did not stimulate protein synthesis {Schluter and Piper, 1992}. In addition, in these isolated cells NE-induced acceleration in protein synthesis was not inhibited by ß-adrenergic blockade {Meidell et al. 1986}. Similar results were obtained using neonatal rat heart muscle cells {Simpson, 1985; Meidell et al. 1986}. Due to the varied hemodynamic effects induced by catecholamines *in vivo*, it is difficult to implicate them with certainty in the pathogenesis of left ventricular hypertrophy. This inconsistency in the results between the in vivo and in vitro studies makes the role of ß-adrenoceptor stimulation in cardiac hypertrophy unclear.

α_1-adrenergic stimulation has been reported to play an important role in the development of cardiac hypertrophy. Cardiac α_{1a}-adrenoceptors may be involved in the induction of embryonic gene expression in ventricular cell hypertrophy, whereas α_{1b}-adrenoceptors mediate positive inotropic effects in rat heart {Michel et al. 1990}. In cultured neonatal rat cardiomyocytes different α_1-agonists such as norepinephrine, phenylephrine, or methoxamine increased myocyte surface area and cell protein content {Simpson, 1985; Meidell et al. 1986}. Similar results has been reported in adult (8-10 weeks old) rat ventricular cells {Ikeda et al. 1991; Schluter and Piper 1992}. This hypertrophy in culture of neonatal rat heart muscle cells was associated with a selective increase in the cellular levels of ß-myosin heavy chain and the V_3 (ßß) isoform became predominant {Waspe et al. 1990}. It was also associated with an increase in myofibrillar

protein synthesis without an effect on protein degradation {Meidell et al. 1986}, and an increase in ANF expression {Sei et al. 1991}. Pharmacological and receptor cloning studies have demonstrated the existence of at least three α_1-adrenoceptor subtypes {Lomasney et al 1991; Michel et al. 1992}, and in rats co-existence of α_{1a}- and α_{1b}-adrenoceptors in the myocardium has been demonstrated {Kinami et al. 1992} in a ratio of an approximately 20:80 in adult rat {Hanft et al. 1989; Michel et al. 1994} and 35:65 in neonate rats {Knowlton et al. 1993}. These two subtypes of α_1-adrenoceptors can be distinguished on the basis of their sensitivity toward selective antagonists {Kinami et al. 1992}. The α_{1a}-subtype has a higher affinity than the α_{1b}-subtype for the agonist urapidil and some of its derivatives which are substituted at the 5-position of the uracil moiety {Hanft and Gross 1989}, for the agonist, 2-(2,6-dimethoxyphenoxyethyl)-amino-methyl-1,4-benzodioxane (WB-4101), or for the novel prazosin derivative SZL-49 {Minneman, 1988 }. The α_{1b}-subtype is irreversibly alkylated by chloroethylclonidin (CEC) {Han et al. 1987; Minneman et al. 1988}.

Recent studies have reported that α_1-adrenoceptor mediated hypertrophic effects are linked to the stimulation of α_{1a}-adrenoceptor subtype {Simpson et al. 1990}. In cultured neonatal rat cardiomyocytes, the α_1-adrenergic agonist phenylephrine increased cell size, ANF expression and protein synthesis. These effects were inhibited by WB-4101, an α_{1a}-adrenoceptor antagonist but not by the α_{1b}-adrenoceptor blocker CEC {Knowlton et al. 1993}. Niguldipine, another α_{1a}-antagonist in low concentration inhibited the transcriptional activation of ANF-Luciferase gene expression (a marker for embryonic gene expression associated with the hypertrophic response) induced by NE in

the presence of propranolol {Michel et al. 1990}.

All the above mentioned factors (Mechanical, Endocrine, Growth, and Neural) exert their effects mostly on the cellular membrane, but some on nuclear receptors resulting in generation of a wide range of **intracellular mechanisms or signals of cardiac hypertrophy.**

(B) Intracellular Mechanisms of Hypertrophy.

Protein synthesis and hence hypertrophy can be mediated by several intracellular signals that may be activated as a result of *cell-deformation* induced by diastolic wall stress (i.e. volume overload) or systolic wall stress (i.e. pressure overload), or it could be the result of a *hormone-, growth factor- or neurotransmitter- receptor interaction.*

B-1 Cell Deformation-related Intracellular Signals.

Cellular stretch or stress may modify protein synthesis by modifying the intracellular contents of different signalling compounds, including Na^+, Ca^{2+}, H^+, cyclic adenosine 3, 5-monophosphate (cAMP), and inositol phosphates which are normally involved in the process of protein synthesis. Stretching of the myocytes induces increase in Na^+ influx {Bustamante et al. 1991}, increase in Ca^{2+} influx {Watson et al. 1989}, stimulation of Na^+ / H^+ exchange {Schwartz et al. 1990}, raising cAMP content {Xenophontos et al. 1989; Watson et al. 1989}, and stimulation of phosphatidylinositol turnover {Harsdorf et al. 1988; Harsdorf et al. 1989}. Increased Na^+ influx may stimulate protein synthesis as a result of increased intracellular levels of Na^+ {Kent et al. 1989},

increased intracellular pH {Fuller et al 1990}, or probably through a secondary increase in myoplasmic Ca^{2+} caused by enhanced Na^{+}-Ca^{2+} exchanger expression {Kent et al. 1993}. Increased intracellular Ca^{2+} levels could augment protein synthesis via Ca^{2+} / calmodulin-dependent kinases or PKC that activate a variety of proteins some of which might act as transcription factors {Marban and Koretsune 1990}. Stimulation of Na^{+} / H^{+} exchange increases intracellular pH with an increase in protein synthesis {Gaitanaki et al. 1990}.

Cell deformation-induced elevated levels of cAMP may increase intracellular Ca^{2+} levels and activate protein kinase A (PKA) {Krebs, 1989} both of which may play a role in phosphorylation of transcription factors. The phosphatidylinositol turnover pathway involves receptor stimulation of phospholipase C, which selectively cleaves a plasma membrane lipid, phosphatidylinositol-4,5(bis)phosphate (IP_2). This cleavage generates two biologically active intracellular messengers, 1,2-diacylglycerol (DAG) and inositol-1,4,5(tris)phosphate (IP_3). DAG stimulates the membrane-bound, phospholipid-dependent, calcium-dependent protein kinase C (PKC), while IP_3 can increase intracellular free calcium from intra- and extra-cellular sources {Berridge and Irvine 1989}. Activation of PKC could lead to phosphorylation of one or more of transcription factor(s) {Terzic et al. 1993}. PKC can also activate the Na^{+} / H^{+} exchanger { Otani et al. 1990} resulting in an increased H^{+} efflux with a concomitant increase in the intracellular pH. This latter condition might increase protein synthesis {Cooper IV et al. 1989}.

B-II Endocrine, Growth factors, and Neural control of cardiac Hypertrophy.

Interaction of a hormone, growth factor or neurotransmitter with appropriate receptors generates several intracellular signals. Thyroid hormones can directly attach to a receptor on the nucleus. The resultant receptor internalization could promote interaction with regulatory sites on DNA, thus modifying and stimulating protein synthesis. {Dzau et al. 1988; Morgan and Baker 1991}. Stimulation of tyrosine kinase coupled receptors by growth factors (e.g., the insulin or heparin receptor) can activate several target proteins. This target protein may be an enzyme, a regulatory protein, or structural protein, but phosphorylation is assumed to alter their individual activities {Yarden and Ullrich 1988} which can stimulate or modify protein synthesis directly. Furthermore, these activated kinases influence phosphoinositol lipase, and so enhance IP_3 and DAG formation {Simpson, 1989}. The potential mediators of angiotensin II or endothelin signal transduction include IP_3 and DAG {Morgan and Baker, 1991}, however angiotensin II may directly attach to a receptor on the nucleus {Dzau et al. 1988}.

The biological expression of the sympathetic activity depends mainly on the effectiveness of the adrenergic receptors to receive and transfer the neuronal signal to the effector cell. This information is transferred in the cardiac tissue through α- and ß-adrenergic receptors, which mediate hypertrophic sympathetic effect as well as the other effects (e.g.inotropic and chronotropic effects). The ß-adrenergic effects ($ß_1$ or $ß_2$) are mediated through activation of adenylate cyclase {Brodde, 1988} leading to the formation of cAMP with an increase in intracellular Ca^{2+} levels and an activation of PKA {Krebs, 1989}. The elevated intracellular levels of all these signals, as discussed earlier, may play

a role in the development of cardiac hypertrophy.

Stimulation of α_1-adrenergic receptors generates a wide variety of intracellular signals. Previous classifications for α_1-adrenoceptors have coupled the α_{1b}-adrenoceptor subtype to the formation of inositol phosphates and release of intracellular Ca^{2+} from intracellular sources; whereas, the α_{1a}-adrenoceptors have been coupled to Ca^{2+} influx from extracellular sources {Minneman, 1988}. More recent data demonstrated a positive correlation between α_{1a}-adrenoceptor stimulation and inositol phosphate accumulation {Wilson and Minneman, 1990; del Balzo et al. 1990; Knowlton et al. 1993}. Wilson and Minneman {1990} showed different patterns of inositolphosphate formation in response to α_{1a} (in rat renal cells which contain 60% α_{1a} receptor subtype) and α_{1b} (rat hepatocytes) receptor activation. Omitting Ca^{2+} from the incubation buffer did not affect NE-stimulated [^{3}H]InsP accumulation in hepatocytes but reduced the effect of NE in renal cells by 60%. The authors suggested that α_{1b} subtype may selectively activate a Ca^{2+}-insensitive phospholipase C, while activation of α_{1a} subtype may involve a Ca^{2+} influx-stimulated hydrolysis of PI (rather than PIP_2). The influx of Ca^{2+} could be at least partly via voltage-operated channels {Michel et al. 1994}. Increased levels of both Ca^{2+} or IP_3 may have a significant role in hypertrophic processes.

(C) Regulation of Gene Expression

The increased levels or activity of the intracellular signals can lead to the activation of several transcription factors that can promote gene expression. Enhanced gene expression includes sequences coding for (but not limited to) proto-oncogenes. The

proteins encoded by these proto-oncogenes have very different structure. For example, some exhibit G-protein-like structures, such as the ras family or have been shown to be functioning as protein kinases, such as the src or raf oncogenes, or as nuclear proteins that interact with DNA to induce transcription, such as c-fos, c-jun, and c-myc {Morgan and Baker, 1991}. Some of these proto-oncogenes such as c-fos, c-myc, and c-ras are known to be involved in cardiovascular hypertrophy {Kolbeck et al. 1993}. Infusion of NE, increasing afterload, or increasing preload in isolated working rat heart induced a fivefold, a threefold, and a twofold increase of c-fos mRNA respectively after 30 min of the application of the trophic stimulus {Kolbeck et al. 1993}. Mechanical loading may directly regulate gene transcription without the participation of humoral factors {Komuro et al. 1990}. The products of these proto-oncogenes can further regulate protein synthesis by enhancing the rate of a normal protein synthesis or promoting a new protein synthesis resulting in myocyte hypertrophy {Morgan and Baker, 1991}.

Part 2. Functional Responses to α_1- or β-Adrenoceptor Stimulation.

(A) Inotropic Responses

Stimulation of α_{1a}- or α_{1b}-adrenoceptor subtype produces positive inotropic effects {Hanft and Gross, 1989; Michel et al. 1994}. This positive inotropy is not mediated by an increase in adenyl cyclase activity {Rabinowitz et al. 1975; Schwartz and Malik, 1991} or inhibition of Na^+ / K^+ pump {Williamson et al. 1993} rather, it could be explained by influx of extracellular Ca^{2+} {Minneman, 1988} by action potential prolongation {Fedida and Buuchard, 1992} or via voltage-operated channels {Michel et al. 1994}, an increase

in 1,4,5-IP_3 and 1,3,4,5-IP_4 {Schmitz et al. 1989}, or an increase in myofilament calcium sensitivity {Schmitz et al. 1989; Puceat et al. 1990}. The positive inotropic effect produced by stimulation of α_{1b}-adrenoceptor may be mediated through an increase in IP_3 and IP_4 {Minneman, 1988; Wilson and Minneman, 1990}, while the α_{1a}-adrenoceptor subtype mediated positive inotropy may be linked to influx of Ca^{2+} {Minneman, 1988; Tsujimoto et al. 1989} at least partly via voltage-channels {Michel et al. 1994} as well as stimulation of IP_3 formation {Wilson and Minneman, 1990; Del Balzo et al. 1990; Knowlton et al. 1993}.

Several studies have reported that β_1- and β_2-adrenergic receptors are present in a ratio of 70:30 in the ventricular myocardium {Brodde, 1988; Borea et al. 1992; Brodde, 1991}. Stimulation of cardiac β-adrenoceptors produce positive inotropic effect through coupling to adenylate cyclase and subsequent increases in the level of cAMP {Brodde, 1988} and in the intracellular levels of Ca^{2+} {Krebs, 1989}.

Figure 1. represents possible pathways for both cell hypertrophy and contraction through stimulation of α_1- or β-adrenoceptors. Stimulation of α_1-adrenoceptors stimulate PLC which increases the level of IP_3 and DAG with subsequent increases in the levels of Ca^{2+} and PKC. PKC can phosphorylate several transcriptional factors that may direct protein synthesis. Stimulation of ß-adrenoceptors increase the level of intracellular Ca^{2+} as well as PKA. The elevated level of intracellular Ca^{2+} (due to α_1- or ß-adrenergic stimulation) may also activate different kinases which in term may activate several transcription factors that can regulate protein synthesis.

In the myocardium contractility is triggered by Ca^{2+}-induced Ca^{2+} release from

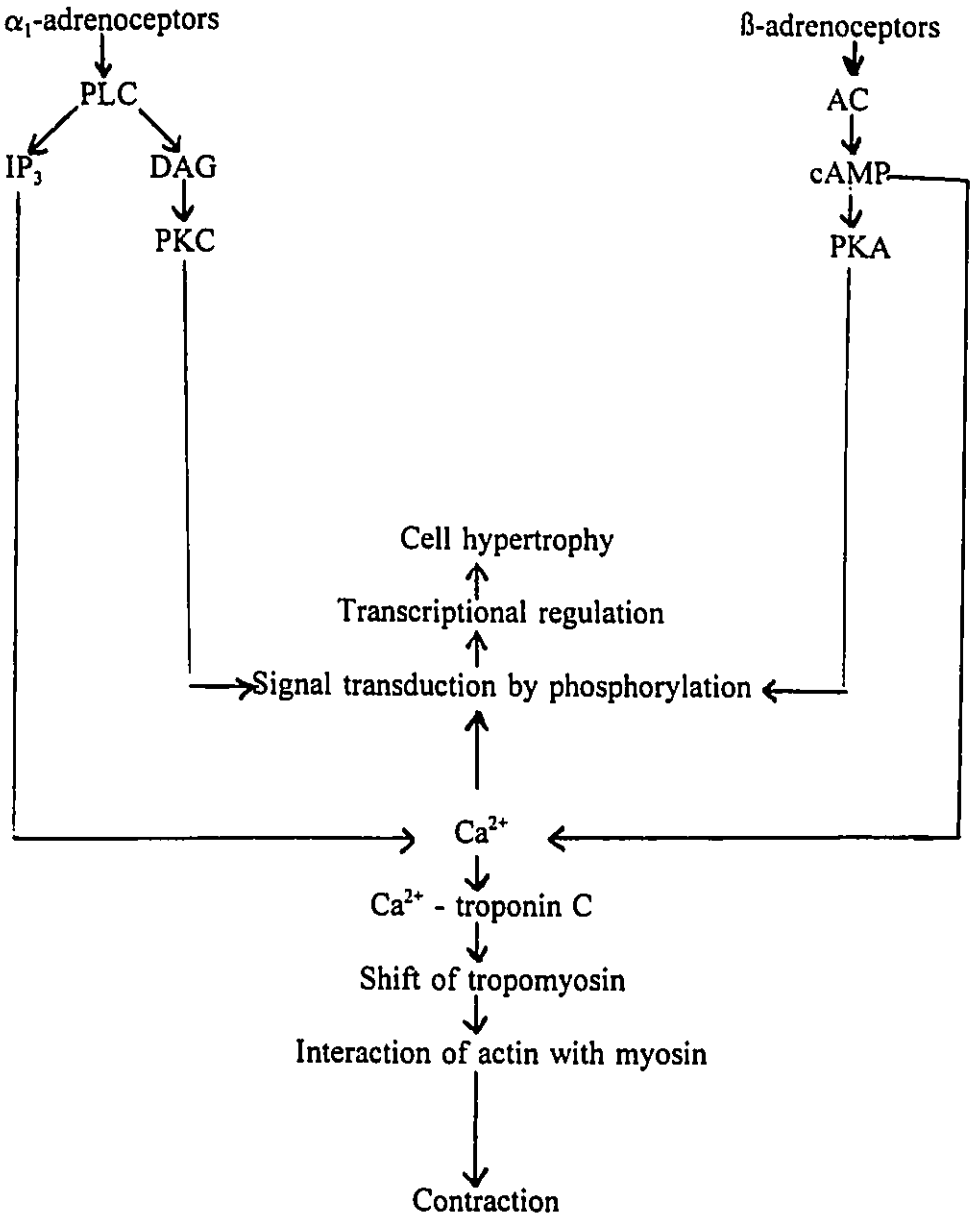


Figure 1.

Diagrammatic representation for the possible pathways for cell hypertrophy and contraction linked to α_1- or ß-adrenergic receptors.

sarcoplasmic reticulum. Tropomyosin at rest is located in the groove that runs along both sides of the double-stranded F-actin polymer in the thin filament of the sarcomere. Contraction is initiated when Ca^{2+} binds to troponin C causing tropomyosin to shift toward the centre of the groove thus allowing physical and chemical interactions to develop between the myosin cross-bridges of the thick filament and the active sites of actin in the thin filament {Katz, 1977}.

From this diagram it is apparent that measurement of rates of protein synthesis induced by α_1- or ß-adrenergic stimulation in cardiac myocytes or in isolated rat hearts is the most optimal approach to investigate the role of adrenergic receptors in cardiac hypertrophy. Measurement of functional responses of the isolated hearts have been investigated since both pathways are initiated through stimulation of the same receptors.

(B) Chronotropic Responses.

In contrast with many other positive inotropic agents, α_1-adrenoceptor agonists produce positive inotropic effect without tachycardia {Terzic et al. 1993}. Autoradiography demonstrated the presence of α_1-adrenoceptors in the conducting system of the rat heart {Saito et al. 1994}. α_1-adrenergic agonists may increase or decrease the automaticity of isolated Purkinje fibres depending on the stage of development and on the specific subset of fibres {Terzic et al. 1993}. 50% of adult canine Purkinje fibres manifested a decrease, and 50% manifested an increase in automaticity in response to α_1-adrenergic stimulation with NE. In contrasts, most neonatal Purkinje fibres showed an increase in automaticity in response to the same concentration of NE {del Balzo et al. 1990}. In another study, phenylephrine induced a significant increase in automatic beating rate in neonate rat ventricle, however it significantly decreased the rate in adult rat ventricle {Drugge et al. 1985; Han et al. 1989}. The chronotropic effect of α_1-adrenergic stimulation depends on maturation of cardiac sympathetic innervation {Drugge et al. 1985} and the presence of a pertussis toxin (PT)-sensitive G protein {Han et al. 1989}. Addition of sympathetic neurons to cultured neonatal myocardial cells converted the response to the α_{1a}-agonist phenylephrine from an increase to a decrease in automaticity {Drugge et al. 1985}. Neonatal heart is poorly innervated by cardiac sympathetic nerves {Lipp and Rudolph, 1982}, therefore, the rapid postnatal development of innervation might modulate the chronotropic response to α_1-adrenergic stimulation {Reder et al. 1984}. PT treatment also modulates the chronotropic responses to α_1-adrenergic stimulation. Inter-ventricular septa prepared from neonatal rat hearts showed

an increase in beating rate in response to phenylephrine in both control and PT-treated groups. Septa from adult rat hearts (controls) showed a significant decrease in beating rate in response to phenylephrine whereas, PT-treated adult septa exhibited an increase in rate {Han et al. 1989}. del Balzo et al. {1990} demonstrated that the α_{1a}-adrenoceptor subtype is linked to positive chronotropy via a PT-insensitive G protein, while the α_{1b}-subtype is linked to a decrease in automaticity via a PT-sensitive G protein. The ratio of α_{1a}-/α_{1b}-adrenoceptors in the neonate rats is 35/65 {Knowlton et al. 1993} and in the adult rat heart it is 20/80 {Michel et al. 1994}. Since the newborn rat heart contains a high proportion of α_{1a}-adrenoceptors (linked to PT-insensitive G protein) and it is poorly innervated by cardiac sympathetic nerves, an explanation for the ontogenic changes in the α_1-adrenergic effect on the chronotropic response from excitation (in newborn) to inhibition (in adult) {Drugge et al. 1985} can be provided. α_{1b}-adrenoceptor-mediated stimulation of Na^+/K^+ pump current in adult rat heart {Williamson et al. 1993} may link the suppression of automaticity to this subtype of α_1-adrenergic receptors {Terzic et al. 1993}, while inositol phosphate accumulation may account for the increase in automaticity triggered by α_{1a}-adrenoceptor stimulation. {del Balzo et al. 1990}. It is worth to mention that the density of α_1-adrenergic receptors varies with species. Rat and rabbit myocardia possess a high density of α_1-adrenergic-binding sites when compared with other species {Steinfath et al. 1992}.

Part 3. Dietary Sodium and Left Ventricular Hypertrophy.

The role of a high sodium intake in aggravating hypertension has been extensively demonstrated {Sullivan et al. 1980; Toal and Leenen 1983; Mervaala et al. 1992; De Simone et al. 1993; Colhoun et al. 1994}. Recent investigations have suggested a role for sodium as a potential modulator of myocardial hypertrophy independent of increased pressure load. Several studies have been conducted in hypertensive humans { Schmieder et al. 1988; Du Cailar et al. 1990; Heimann et al. 1991; Du Cailar et al. 1992}, and animals {Pfeffer et al. 1984; Sen and Young 1986; Leenen and Yuan 1992} as well as normotensive subjects {Du Cailar et al. 1992} and rats {Meggs et al. 1988; Kihara et al. 1985; Fields et al. 1991; Yuan and Leenen, 1991; Mervaala et al. 1992} and several mechanisms have been postulated for the trophic effect of high sodium diet {Yuan and Leenen, 1991; Heimann et al. 1991; Harmsen and Leenen, 1992}. Analysis of the data of these studies allowed the postulation of a hypothesis that sodium may modulate hypertrophic responses in myocardial tissues through an enhanced sensitivity to adrenergic stimulation. This hypothesis was examined in the present investigation.

(A) Human studies.

A-I Hypertensive patients: In hypertensive patients dietary sodium as assessed by 24-h urinary sodium excretion, was a strong predictor of structural cardiac adaptation in patients with essential hypertension {Schmieder et al. 1988; Du Cailar et al. 1990; Du Cailar et al. 1992}. This structural adaptation was independent of other variables, such as resting systolic or diastolic pressures, body mass index, age, or sex. Therefore they

concluded that dietary sodium is a BP-independent and strong determinant of the degree of myocardial hypertrophy. Assessment of salt-sensitivity in essential hypertensive patients submitted to a low-salt and a high-salt diet for 7 days revealed a higher LV weight in salt-sensitive than the salt-resistant patients {Heimann et al. 1991}. In these studies urinary sodium excretion (mmol/24 h) was positively correlated with most of left ventricular parameters such as posterior wall thickness, relative wall thickness, and LV mass.

A-II Normotensive subjects : Not much has been reported about the influence of high sodium intake on LV mass in normotensive subjects. The study of Du Cailar demonstrated a positive correlation between the 24-h urinary sodium excretion and LV mass index as assessed by echocardiography performed next day of the sample collection {Du Cailar et al. 1992}. In these normotensive subjects, urinary sodium excretion was also positively correlated with LV diastolic internal diameter, therefore the authors suggested that the increase in LV mass may be through a change in LV chamber size via modification of preload.

(B) <u>Animal studies.</u>

Rats have been by far the most commonly used experimental animals for dietary sodium studies. Rat models of human hypertension most frequently used for these purposes are: (1) the Dahl salt-sensitive (DS) and salt-resistant (DR) rats; (2) DOCA-salt (deoxycorticosterone acetate) loading in genetically normotensive rats; (3) the two-kidney

one clip (2K,1C) model of Goldblatt renal hypertension; and (4) the Okamoto-Aoki spontaneous hypertensive rats (SHR) or its stroke-prone variant (SHRSP) exposed to either salt-loaded food or to DOCA-salt treatment {Folkow and Ely, 1987}. Wistar-Kyoto (WKY), Wistar, and DR rats are the most common strains of normotensive rats being used in such studies.

B-I Hypertensive animals: DS rats kept on 0.4%, 4%, or 8% NaCl diet showed greater LV weights than those of the DR rats by 14%, 32%, and 54% respectively. In this animal model, there was a positive correlation between elevation in mean arterial pressure, LVH and the sodium content of the diet of these rats {Pfeffer et al. 1984}. Same results were demonstrated in the 1K,1C model of renal hypertension {De Simone et al. 1993}, while in the 2K,1C model of renal hypertension low sodium diet for 4 weeks decreased and high sodium diet increased heart weight despite lack of a significant effect on blood pressure {Lindpainter and Sen, 1985 ; Wilczynski et al. 1992; De Simone et al. 1993}. SHR kept on 2% NaCl for 12 weeks showed a significant increase in LV wall thickness with no significant increase in mean arterial pressure or LV mass {Leenen and Yuan, 1992}. These studies indicate that dietary sodium-induced LVH in these animal models are likely due , at least in part, to the role of sodium in influencing blood pressure but may also reflect additional nonhemodynamic effects of sodium on the heart.

B-II Normotensive animals : WKY rats kept on 1% NaCl as drinking water for 7 months (from 3 to 10 month of age) showed an increase of about 15% in heart weight compared

with control although there was no difference between the salt treated and control groups in BP or body weight {Kihara et al. 1985}. Similar results were obtained by other groups {Meggs et al. 1988; Fields et al. 1991; Yuan and Leenen, 1991; Mervaala et al. 1992}. Fields et al. {1991} also reported that the increase in LV weight in young rats was associated with an increase in wall thickness but not in internal diameter, a characteristic of concentric hypertrophy. Moreover, the trophic effect of dietary sodium was found to be strain, age, sodium content {Yuan and Leenen, 1991}, and time dependent {Yuan and Leenen, 1991; Fields et al. 1991}. Young WKY rats kept on high sodium diet (1,370 μmol N^+/g food) from 4 to 8 week of age increased LV weight by 25%, Wistar rats increased cardiac weight by 14%, while in DR rats no increase in cardiac weight occurred despite of the similarity in age and duration of high salt intake. The increase in LV weight was found to be only 10% when 9 weeks old Wistar rats kept on high salt diet for 4 weeks and LV weight was unaffected when the high sodium intake was started at 26 weeks of age. Also, the young 4-weeks old WKY rats increased LV mass in a dose-related fashion in response to increased sodium intake (342 μmol Na^+/g food or 1,370 μmol Na^+/g food), whereas 10-weeks old WKY rats did not show a response despite a longer exposure. 1% saline in drinking water for 3 weeks induced 10% LVH in young Wistar rats which increased to 19% after 6 weeks of treatment {Fields et al. 1991} suggesting a positive correlation between duration of treatment and the degree of resultant hypertrophy. The source of the animals as well as diet may also affect the degree of sodium-induced cardiac hypertrophy. 10 weeks old WKY rats did not develop cardiac hypertrophy despite of long exposure to high sodium intake (Yuan and Leenen, 1991},

however 9 weeks old WKY rats obtained from a different source and fed on diet from a different source too, developed 22% hypertrophy after 8 weeks of high sodium intake {Mervaala et al. 1992}. In most of the studies, no significant increase in right ventricle weight has been demonstrated {Kihara et al. 1985; Fields et al. 1991; Yuan and Leenen, 1991} in sodium-induced cardiac hypertrophy. The results of these studies show that dietary sodium can exert a clear trophic effect on the myocardium. The pathogenic mechanism linking high sodium diet to cardiac hypertrophy remains unknown. Several possible mechanisms could be involved in this trophic effect of dietary sodium.

Part 4. Possible Mechanisms of Dietary Sodium-Induced Left Ventricular Hypertrophy in Normotensive rats.

Dietary sodium affects several systems which could explain its trophic effects on the heart.

(A) Hemodynamic changes.

As discussed earlier, increased pressure or volume overload could increase protein synthesis and causes hypertrophy. However, no significant changes in blood pressure preceding high sodium diet-induced cardiac hypertrophy have been reported in normotensive rats kept for periods ranging from 4 weeks to 7 months on high sodium diet {Kihara et al. 1985; Meggs et al. 1988; Fields et al. 1991; Yuan and Leenen, 1991; De Simone et al. 1993}. The basal heart rate was also found to be unaltered in young, mature, or old age WKY rats placed on high sodium diet for 4-5 weeks {Huang and Leenen, 1992}. It must be realized, though that the hemodynamic variables were

measured during day time. It is well known that rats are mostly resting or asleep in daytime contrast to the nighttime when eating and drinking activities of the rats are at maximum. In WKY rats, high sodium intake for 2 weeks increased nighttime but not daytime mean arterial pressure by about 3 mmHg (105±1 versus 102±1 mmHg, WKY 8% versus WKY 1% during the 6 PM to midnight period) from the second day of high salt intake and continued throughout the experimental period with no net effect on 24-hour mean arterial pressure {Calhoun et al. 1994}. SHR showed a significant increases in both nighttime and daytime mean arterial pressure during high salt exposure {Ely et al. 1991; Calhoun et al. 1994}. The study of Ely et al. {1989} on SHR and WKY rats demonstrated that there is a short but significant increase in blood pressure (by about 25-30 mmHg) during each water drinking period. Rats kept on high salt diet drink up to 4 times more water compared with rats on normal diet { Fields et al. 1991; Yuan and Leenen, 1991}. Therefore, it is possible that the salt-induced increase in drinking which is mostly at nighttime, is responsible for the elevated nighttime mean arterial pressure. It is unlikely, however that this small increase in nighttime mean arterial pressure is responsible for cardiac hypertrophy induced by high sodium intake.

Cardiac volume overload also dose not appear to be responsible for sodium-induced cardiac hypertrophy. No increases in cardiac filling pressures or in cardiac output were associated with the development of left ventricular hypertrophy in WKY or Wistar rats kept on high sodium diet {Yuan and Leenen, 1991} or in Wistar rats receiving 0.9% saline {Fields et al. 1991}. Moreover, cardiac volume overload also causes right ventricular hypertrophy {Tsoporis et al. 1989}. In contrast, increases in right ventricular

weight by high sodium intake were not significant {Fields et al. 1991; Yuan and Leenen, 1991} and the hypertrophy caused by high sodium diet in normotensive rats represents concentric LV hypertrophy {Fields et al. 1991; De Simone et al. 1993} and not eccentric type which is associated with volume overload {Tsoporis et al. 1989}. Ely et al. {1989} demonstrated that, the high sodium-water intakes in high sodium treated SHR or WKY rats the increased volume was eliminated as urine within 20-30 min. Only the study of Du Cailar et al. {1992} in normotensive subjects suggests that the impact of sodium on LV mass in these subjects may be through a change in LV chamber size.

(B) Increased Sympathetic Activity.

It has been suggested that the trophic effects of high dietary sodium may involve changes in sympathetic neuroeffector mechanisms {Meggs et al. 1988}. Cardiac sympathetic activity may be evaluated by measuring catecholamine turnover rates. Catecholamine turnover is affected by sympathetic firing rate, presynaptic modulation, and reuptake and represents the amount of transmitter available to the receptors per unit time. Normotensive rats kept on high sodium diet for 6 or 8 weeks showed decreased myocardial NE concentration (ng/g) {Meggs et al. 1988; Mervaala et al. 1992}. 2K,1C renal hypertensive rats, regression of myocardial hypertrophy in hypertensive salt-restricted rats was accompanied by a significant increase in cardiac NE concentration (ng/g) and content (ng/heart) to values of normotensive controls {Lindpaintner and Sen, 1985}. The decreased myocardial levels of NE after high sodium intake might have resulted from increased release or decreased reuptake or both, of the neurotransmitter into

the sympathetic nerve endings. However, NE turnover rate was not increased in young rats after high sodium intake for 4 weeks {Yuan and Leenen, 1991}. It must be realized though, that NE turnover rate was measured during daytime which does not necessarily reflect the nighttime cardiac sympathetic activity when the rats are active and feeding. In addition, NE turnover rate was measured after 4 weeks of high salt intake. It is therefore possible that increased adrenergic transmitter release may have occurred during the initial days after increasing sodium intake, contributing early on to the trophic effect of dietary sodium. For this reason it is essential to measure cardiac catecholamine turnover rate during nighttime and during initial 1-2 weeks of high salt intake.

Effects of high salt loading on plasma NE concentration have been extensively demonstrated. In normotensive subjects high sodium intake significantly decreased and low sodium diet caused a significant increase in plasma NE concentration {Luft et al. 1979; Fraser et al. 1981; Rankin et al. 1981; Egan et al. 1991}. Chronic sodium depletion in dogs for 3 weeks resulted in a 76% rise in plasma NE concentration {Brosnihan et al. 1981}. In WKY rats, Dietz et al. {1982} reported that plasma NE levels tended to be lower in sodium loaded WKY rats as compared to their controls, other studies reported that there is no change in plasma NE after high sodium intake {Ely et al. 1989; Fields et al. 1991}.

(C) Increased Sensitivity for Adrenergic Stimulation.

Dietary sodium may increase the sensitivity of the heart to catecholamines by affecting the density (B_{max}) or affinity (K_d) of α_1- or ß-adrenergic receptors (effects at the

receptor levels), or by enhancing responsiveness to adrenergic receptor stimulation (post-receptor events). Normotensive Wistar rats kept on high sodium diet showed an increased density but reduced affinity of myocardial α_1-adrenoceptors {Meggs et al. 1988}. The authors suggest that the reduced affinity of the receptors for radiolabelled antagonist ^{125}IBE 2254 may be due to an alteration in the recognition unit possibly because of the synthesis of a variant form of the receptor or a change in receptor conformation induced by hypertrophic process. A significant increase in cardiac ß-adrenergic receptors number was also observed in normotensive rats after high sodium intake {Gallo et al. 1990}. Upregulation of α_1-adrenoceptors after high sodium intake in normotensive WKY rats was accompanied by decreased myocardial NE concentration {Meggs et al. 1988}. High sodium intake in normotensive subjects also produced significantly greater pressor response to NE infusion compared with controls {Rankin et al. 1981; Egan et al. 1991}. On the other hand chronic sodium depletion in dogs for 3 weeks significantly attenuated the pressor response to NE infusion {Brosnihan et al. 1981}. Fraser et al. {1981} found that, in normotensive male volunteers high sodium diet significantly increased the density of ß-adrenoceptor in white cells. This increase in ß-adrenoceptor density in white cells was reciprocally related to circulating catecholamine concentration and was directly correlated to cardiac sensitivity to isoproterenol. In another study the increased affinity of white cell ß-adrenoceptors after high sodium intake was directly correlated to ß-receptor-mediated vasodilatation and reciprocally related to circulating catecholamine concentration {Naslund et al. 1990}. These studies suggest a possible effect of high sodium diet at the level of adrenergic receptors.

It is also possible that high dietary sodium may produce its trophic effects by modulating post receptor events. Heart tissue isolated from unilaterally nephrectomized, DOCA-saline treated rats showed a significant increase in basal inositol phosphate production as well as when stimulated with NE compared with controls (given tap water to drink). The density of α_1-adrenoceptors in DOCA-saline treated rats was similar to, and the affinity was significantly lower than that of controls {Eid and De Champlain, 1988}. These results show a clear dissociation between the NE activation of the PI pathway and the number or affinity of α_{1a}-adrenoceptors in the heart, which suggests an increased responsiveness to the stimulation of α_1-adrenergic receptors via post receptor events. However, it should be pointed out that this study was conducted on hypertensive rats which may not reflect the mechanisms in normotensive rats.

(D) <u>Activation of certain Growth factors.</u>

It is possible that high sodium diet may stimulate certain growth factors such as angiotensin II (AII) which in term ,as discussed earlier, can stimulate cardiac growth. In normotensive subjects, 1 week of low salt diet (20 mmol sodium/day) increased, and 1 week of high salt diet (300 mmol sodium/day) decreased plasma renin activity (PRA) respectively {Overlack et al. 1993}. Young Wistar rats kept on high sodium diet (4% NaCl) showed a decreased plasma renin activity {Buttrick et al. 1993; De Simone et al. 1993}. PRA was also inversely related to the content of sodium in diet in the 2K,1C and 1K,1C renal hypertension model {De Simone et al. 1993}. The results of these studies make this pathway (contribution of PRA) less likely. However, the contribution of

cardiac renin-angiotensin system (RAS) should not be excluded. In DS rats, 8% NaCl diet significantly reduced the expression of angiotensinogen (precursor of AII) mRNA in the LV of these rats {Peeler et al. 1991}. Angiotensin converting enzyme inhibitors (ACEI) failed to inhibit high sodium-induced cardiac hypertrophy in DS rats or SHR {Peeler et al. 1991; Harrap et al. 1993}. Therefore, it is apparent from these studies that the contribution of cardiac RAS to sodium induced LVH is less likely.

It is also unlikely that sodium induces LVH through stimulation of triiodothyronine (T_3) because of the different expression patterns of the ventricular isomysins V_3 and V_1 associated with each case. Under the influence of T_3 the rat myocardial content of V_1-myosin is elevated and the synthesis of V_3-myosin is depressed {Gustafson et al. 1986}. Low sodium diet increased V_1 and deceased V_3 in normal Wistar rats {Sen and Young, 1986}. Moreover, measurement of serum T_4 levels in young Wistar rats kept on 0% NaCl, 0.4% NaCl, or 4% NaCl diet for 6 weeks revealed identical values in all the three groups despite the elevation of α-to-ß-myosin heavy chain mRNA ratio in the low sodium diet group {Buttrick et al. 1993}. The data of these studies suggest an identical role of low sodium diet and thyroid hormones on myosin isozyme shift, probably by different mechanisms and this makes the association of T_3 with high sodium diet is less likely.

(E) Increased Myocardial Sodium Influx.

High sodium diet increased and low sodium diet decreased plasma concentration of Na^+ by about 2% {Sullivan et al. 1980 ; Brosnihan et al. 1981}. In cerebrospinal fluid, a transient increase in Na^+ concentration was observed in rats at day 1 and 3 of 8% NaCl

intake but not thereafter {Mozaffari et al. 1990}. It is possible that high sodium diet might increase sodium concentration in a similar way in the interstitium of the heart which could result in an increased sodium influx. If so, then an enhanced protein synthesis would be expected as a result of increased intracellular levels of sodium { Kent et al. 1989} and/or increased intracellular pH { Fuller et al. 1990} caused by increased H^+ efflux. However, it is unlikely that a 2% increase in extracellular sodium is sufficient to enhance the Na^+ / H^+ exchange to stimulate protein synthesis, since the activity of this exchange is mainly determined by its affinity for intracellular H^+ {Soltoff and Cantley 1988}. Therefore, it is apparent that even if a transient increase in extracellular sodium is possible, it might not be sufficient to stimulate Na^+ / H^+ exchange, which makes the contribution of this mechanism to the sodium induced LVH less likely too.

HYPOTHESIS

In the present study we hypothesized that, the trophic effect of high dietary sodium may be mediated by sodium-induced increases in the density and/or affinity of cardiac α_1- or β-adrenoceptors. If so, then increased cardiac responses to adrenergic stimulation would be expected after high sodium intake. To test this hypothesis, in the present investigation we tested the effects of high sodium diet on the functional responses of isolated rat hearts to α_1- or ß-adrenergic stimulation as well as its effect on the functional responses of isolated hearts to α_{1a}- or α_{1b}-adrenoceptor subtype stimulation.

OBJECTIVES

1- To assess whether high sodium diet enhances the responses of the myocardium to α- or ß-adrenergic stimulation *in vitro*. The responses that have been evaluated are: inotropy (as expressed by developed pressure), chronotropy, and coronary flow.

2- To assess whether high sodium diet alters the responses of the myocardium for the above mentioned parameters to α_{1a}- or α_{1b}-adrenoceptors (subtypes of α_1-adrenergic receptors) stimulation.

MATERIALS AND METHODS

(A) Materials.

High sodium diet containing 1,370 μmmol Na^+ / g food (or 8% NaCl) and control diet containing 101 μmol Na^+ / g food (or 0.6% NaCl) were obtained from Teklad (Madison, WI). Sodium pentobarbital (65 mg/ml) was obtained from M. T. C. Pharmaceuticals (Canada Packers Inc. Cambridge, Ontario). Heparin 100 u/ml was prepared from a stock solution of heparin sodium 10,000 U.S.P units/ml obtained from Organon Teknika, Toronto, Canada (manufactured by Ayerst Lab). 0.9% NaCl was used as a diluent.

Methoxamine ampoules containing 20 mg/ml methoxamine hydrochloride (Burroughs Wellcome Inc. Kirkland, Que., Canada) was used to prepare the different concentrations of methoxamine. Isoproterenol ampoules containing 0.2 mg/ml isoproterenol hydrochloride U.S.P. (Sabex) was used to prepare different concentrations of isoproterenol. Propranolol ampoules containing 1 mg/ml propranolol hydrochloride (ICI) was used to prepare the required concentration. Krebs-Henseleit buffer was used as a diluent for preparing different concentrations of the agonists and antagonists used. Chloroethylclonidine (CEC) and urapidil were purchased from Research Biochemicals (Natick, MA, U.S.A.).

(B) Buffers.

All the experiments were performed using a modified Krebs-Henseleit (K-H) buffer of the following composition (all in mmol/l): NaCl 125, KCl 4.7, $CaCl_2$ 1.35,

$NaHCO_3$ 20, NaH_2PO_4 4, $MgCl_2$ 1.0, D-Glucose 10. All these chemicals were purchased from Sigma Chemical Co (St. Louis, MO). The buffer was equilibrated with a 5% CO_2 / 95% O_2 gas mixture to achieve a pH of 7.35 - 7.45.

(C) General Methods.

WKY rats at 4 weeks of age (Taconic Farms, Germantown, NY) were used in the study. The rats were housed two per cage on a 12-h light-dark cycle and were given tap water ad libitum. Depending on the experimental protocol to be conducted, rats were kept on a control (normal) or high sodium diet (1,370 μmol Na^+ /g food or 8% NaCl) for 1, 2, or 6 weeks. Other constituents of the diet remained the same. Body weight were monitored once a week.

(D) Isolation and Perfusion of rat Hearts.

At the proper time, WKY rats on high sodium diet or respective controls were anaesthetized with sodium pentobarbital i. p. (0.1 ml/100g B. W.) several minutes prior to performing laparotomy, after which, sodium heparin (0.1 ml/100g B. W.) was injected into the inferior vena cava. Heparin was allowed to circulate in the blood stream for 1 minute, the inferior vena cava was cut, thoracic cavity opened and the heart was cooled immediately with cold saline and crushed ice until stopped beating. A cannula was placed into the aorta, the heart was excised and was placed in a water-jacketed chamber at 37° C attached to the Langendorff perfusion apparatus (see Fig. 2). The pulmonary artery was cut and the aorta was perfused retrogradely at 75 mmHg with the modified Krebs-

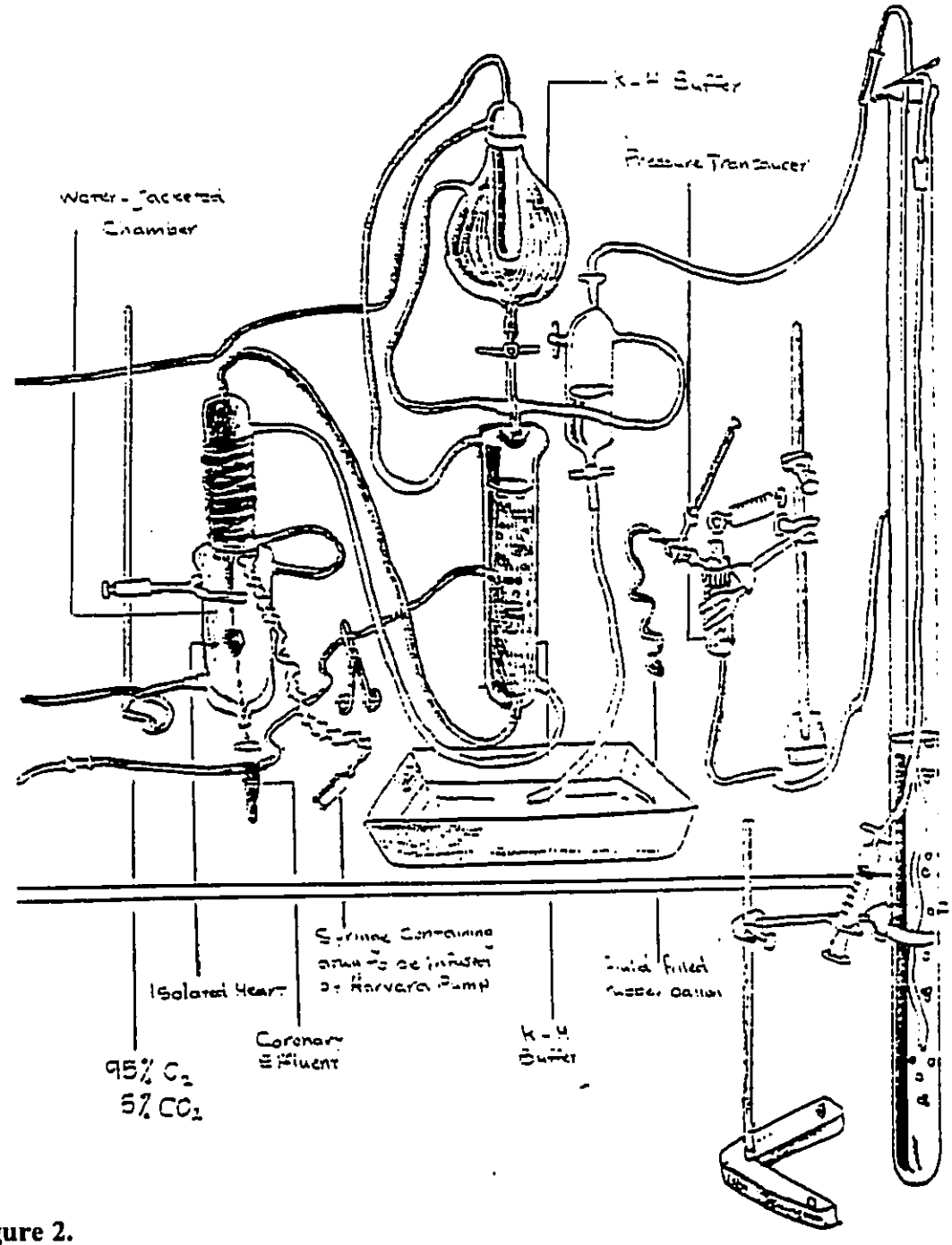


Figure 2.

Experimental set-up.

Henseleit buffer. A fluid-filled balloon, connected via a fluid-filled catheter to a pressure transducer (Statham Gould, USA) was placed in the left ventricle to measure the left ventricular contractile force as systolic and diastolic pressures. In preliminary experiments on rats at 6 weeks of age (n = 5) the pressure - volume relationship was established by gradually increasing the volume of the fluid in the rubber balloon inserted into the left ventricle of each isolated rat heart. The initial volume of the balloon was 50 μml, the recorded systolic pressures for the different hearts were 50, 27, 10, 36, and 32 mmHg. Systolic pressures at the maximum volume (V_{max} = 280 μml) were 165, 120, 92, 110, and 140 mmHg respectively. In all subsequent experiments the initial volume of the balloon was adjusted such that a value around 65 mmHg of systolic pressure was obtained, then the volume of the fluid in the balloon was increased or decreased until a stable left ventricular pressure recording for about 10 min. was obtained, after which the isolated hearts were allowed for further stabilization for another 20 min. and the baseline recording was obtained before the start of the drug infusion. Signals from the pressure transducer were digitized and analyzed by a computer where systolic (P-sys) and diastolic (P-dia) pressures as well as heart rate (HR) can directly be recorded from the monitor of the computer. Developed pressure (P-dev) was calculated as (P-sys - P-dia). Coronary flow (CF) was measured manually by collecting the coronary effluent dripping from the heart over a period of 1 min. and then was normalized per gram heart dry weight (g HDW).

(E) Experimental Protocols.

E-I Assessment of α_1- and ß-Adrenergic Stimulation in Isolated rat Hearts.

Two groups of hearts from WKY rats on a control diet (at 6 weeks of age) were used:

Group 1: α_1-adrenergic stimulation was evaluated by infusing the α_1-agonist methoxamine (MET) in incremental concentrations of 10^{-7}, 10^{-6}, and 10^{-5} M in the isolated rat hearts.

Group 2: ß-adrenergic stimulation was evaluated by infusing incremental concentrations (10^{-9}, 10^{-8}, and 10^{-7}, M) of the non-selective ß-agonist isoproterenol (ISO) to the isolated hearts.

Different concentrations of methoxamine or isoproterenol were infused successively to the system at the level of the aortic cannula by a Harvard Pump (Harvard Apparatus Co. Natick, MA) at a flow rates of 0.1, 0.2, and 0.5 ml/min for each concentration and over a period of 3 - 5 min for each of the 9 concentrations finally achieved. These concentrations of the drug infused at a constant velocity into aortic cannula were not the concentrations that were used in the dose-response curves. The real final concentration for each infused concentration (Methoxamine or Isoproterenol) was calculated as following:

Real concentration = pump speed * infused concentration / CF (ml/min).

Measurements of systolic pressure, diastolic pressure, heart rate, and coronary flow were made at 120 - 150 sec infusion of each of the 9 concentrations of methoxamine or isoproterenol finally achieved. Normalized coronary flow was calculated as: coronary

flow (ml/min) / Heart Dry Weight (HDW) g.

E-II Assessment of possible ß-Mediated effects for Methoxamine.

To evaluate whether MET-induced effects are purely α_1-adrenoceptor mediated or there is a contribution from ß-adrenoceptor stimulation, incremental concentrations (10^{-7} to 10^{-5} M) of methoxamine were infused with or without the non-selective ß-adrenoceptor blocker propranolol (10^{-6} M) into 2 groups of hearts isolated from rats an a control diet at 6 weeks of age as following:

Group 1: This group was treated as group 1 in *E-I*. The protocol can be summarized as following: 20-30 min stabilization period----> infusion of incremental concentrations of methoxamine.

Group 2: After 20-30 min stabilization, the non-selective ß-blocker propranolol (10^{-6} M) was infused for 5 minutes before and continued throughout the infusion of incremental concentrations (10^{-7} to 10^{-5} M) of methoxamine.

E-III Assessment of the Possibility for testing α_1 and ß Stimulation on the Same Isolated rat Heart.

In order to test whether α and ß stimulation could be performed on the same heart, 3 groups of hearts isolated from control rats at 6 weeks of age were subjected to different treatments to assess if prior α stimulation induced by methoxamine affects the responses of these hearts to ß-stimulation induced by isoproterenol. Since performing α stimulation before ß stimulation requires about 90 min, it became also important to know if the

differences (if any) between direct ß stimulation (groups 1) and ß stimulation after α stimulation (group 2) is due to methoxamine pre-treatment (group 2) or time (group 3).

Group 1: After 20-30 min stabilization, incremental concentrations (10^{-9}, 10^{-8}, 10^{-7} M) of isoproterenol were infused over a period of 30 minutes (9 concentrations, each infused for 3-5 minutes).

Group 2: After 20-30 min stabilization, incremental concentrations (10^{-7}, 10^{-6}, 10^{-5} M) of methoxamine were infused into the isolated hearts over 30 minutes. This was followed by a second stabilization period (recovery) for 20-30 minutes at the end of which incremental concentrations (10^{-9}, 10^{-8}, 10^{-7} M) of isoproterenol were infused (over 30 min) to the same isolated heart.

Group 3: In this group, the isolated hearts were perfused in drug free buffer perfusion state for 90 minutes. This was followed by the infusion of incremental concentrations (10^{-9}, 10^{-8}, 10^{-7} M) of isoproterenol over 30 minutes.

E-IV Assessment of the effects of High Sodium Diet on the Responses of Isolated rat Hearts to Adrenergic Stimulation.

E-IV-i Assessment of the effects of High Sodium Diet on α_1- or ß-Adrenergic Stimulation.

The protocol (E-III, Group 2) has been used to assess the effects of high sodium intake for 1, 2, or 6 weeks on the responses of isolated rat hearts to α_1- or ß-adrenergic stimulation. The effects of α_1 (as tested by methoxamine) or ß (as tested by isoproterenol) stimulation on developed pressure, heart rate, and coronary flow were evaluated in each salt treated group and compared with its respective control to identify

any significant alteration in these parameters induced by the high sodium diet.

E-IV-ii *Assessment of the effect of α_{1a}-Adrenoceptor Blockade on the Responses of Isolated rat Hearts to the α_1-Agonist Methoxamine.*

Hearts from rats on a control or high sodium diet for 2 weeks were allowed to stabilize for 30 min and the first baseline measurements were established. MET at a concentration of 10^{-5} M (high dose) was infused for 4-5 min and responses were evaluated. This was followed by a second stabilization period of 15-20 min at the end of which the α_{1a} blocker urapidil (Ura) at a concentration of 10^{-5} M was infused for 5 min. from another pump. This was followed by a stepwise, simultaneous infusion of three incremental concentrations low, medium, and high (10^{-7}, 10^{-6}, and 10^{-5} M) of methoxamine for a period of 4-5 min for each of the three concentrations. Effects on P-dev, HR, and CF were evaluated at the end of the second stabilization period (second baseline), Ura infusion, and each of the simultaneous Ura-MET infusion in both dietary groups.

E-IV-iii *Assessment of the effects of α_{1b}-Adrenoceptor Blockade on the Responses of Isolated rat Hearts to Methoxamine.*

Isolated hearts from rats on a control or high sodium diet for 2 weeks were treated as in *E-IV-ii*, except that the irreversible α_{1b}-adrenoceptor blocker chloroethylclonidine (CEC) at a concentration of $0.25*10^{-5}$ M for a period of 10 min after second baseline establishment was used instead of urapidil. To establish if α_{1b}-adrenoceptors were irreversibly blocked by CEC, hearts were allowed in drug free buffer perfusion state for

10-15 min after the last MET+CEC treatment. Methoxamine (10^{-5} M) was infused for 4-5 min and responses were evaluated in both dietary groups.

(F) Cardiac weight.

At the end of each experiment the heart was gently removed, the atria and the big vessels were trimmed, and the wet weight of the heart was recorded. The heart was then dried in an oven for 72 h. at 40° C and was weighed again for the dry weight determination.

(G) Statistical analysis.

Values were expressed as mean with the standard error of mean. The statistical significance of differences in body weight, heart dry weight, heart dry weight/body weight ratio, maximum response to a specific treatment was determined by Student's *t*-test for unpaired groups (salt vs. control). Differences within each group before and after treatment were assessed using Paired *t*-test. The dose response to graded doses of methoxamine or isoproterenol were evaluated by ANOVA and Duncan's test. EC_{50} was evaluated by estimating the dose of the agonist required to elicit 50% of the maximal response. This was done by the graphpad program. Differences between groups were assessed using *t*-test. Possible differences between regression lines at the linear part of curves were evaluated as described by Smith and Choi {1982}. A *P* level below 0.05 was considered statistically significant.

RESULTS

Part 1. Adrenergic Stimulation in Isolated rat Hearts.

Methoxamine, significantly increased developed pressure (P-dev) by about 130 mmHg (Fig. 3, *panel A*) in a concentration-related manner beginning at 10^{-7} M and reaching a peak at 10^{-5} M . This increase in P-dev is due to a significant, dose dependent increase in systolic pressure (Fig. 3, *panel B*) [from 65±4 to 192±16 mmHg]. Methoxamine had no significant effect on diastolic pressure (P-dia) (Fig. 3, *panel C*), heart rate (HR) (Fig. 3, *panel D*) or coronary flow (CF) (Fig. 3, *panel E*).

The ß agonist isoproterenol produced a dose-dependent, significant increase in P-dev up to 160 mmHg (Fig. 3, *panel A*) at 10^{-7} M. This increase in developed pressure is the net result of an increase in P-sys (Fig. 3, *panel B*), and a significant, dose-dependent decrease in P-dia (Fig. 3, *panel C*). It also increased HR and CF in a dose-dependent fashion. An increase of about 130 bpm (Fig. 3, *panel D*) in HR was observed, while the CF increased from 55±4 to 152±5 ml/min/g HDW (Fig. 3, *panel E*).

To explore if methoxamine-induced effects are purely α_1-adrenoceptor mediated or there is a contribution of ß-adrenoceptors, the non-selective ß-blocker propranolol was simultaneously infused with MET (Figure 4). ß-adrenoceptor blockade with 10^{-6} M propranolol did not alter the positive inotropic effects as expressed by developed pressure of methoxamine (*panel A*). ß blockade also had no effect on the small methoxamine-induced changes in HR (*panel B*) and CF (*panel C*).

Figure 3.

Effects of the α_1-adrenoceptor agonist methoxamine (10^{-7} to 10^{-5} M) and the ß-adrenoceptor agonist isoproterenol (10^{-9} to 10^{-7} M) on developed pressure (P-dev) {*panel A*}, systolic pressure (P-sys) {*panel B*}, diastolic pressure (P-dia) {*panel C*}, heart rate (HR) {*panel D*}, and coronary flow (CF) {*panel E*} of hearts isolated from WKY rats at 6 week of age. Each curve represents the mean ± SEM (n = 5/group). #$p < 0.05$ vs. before treatment within each group.

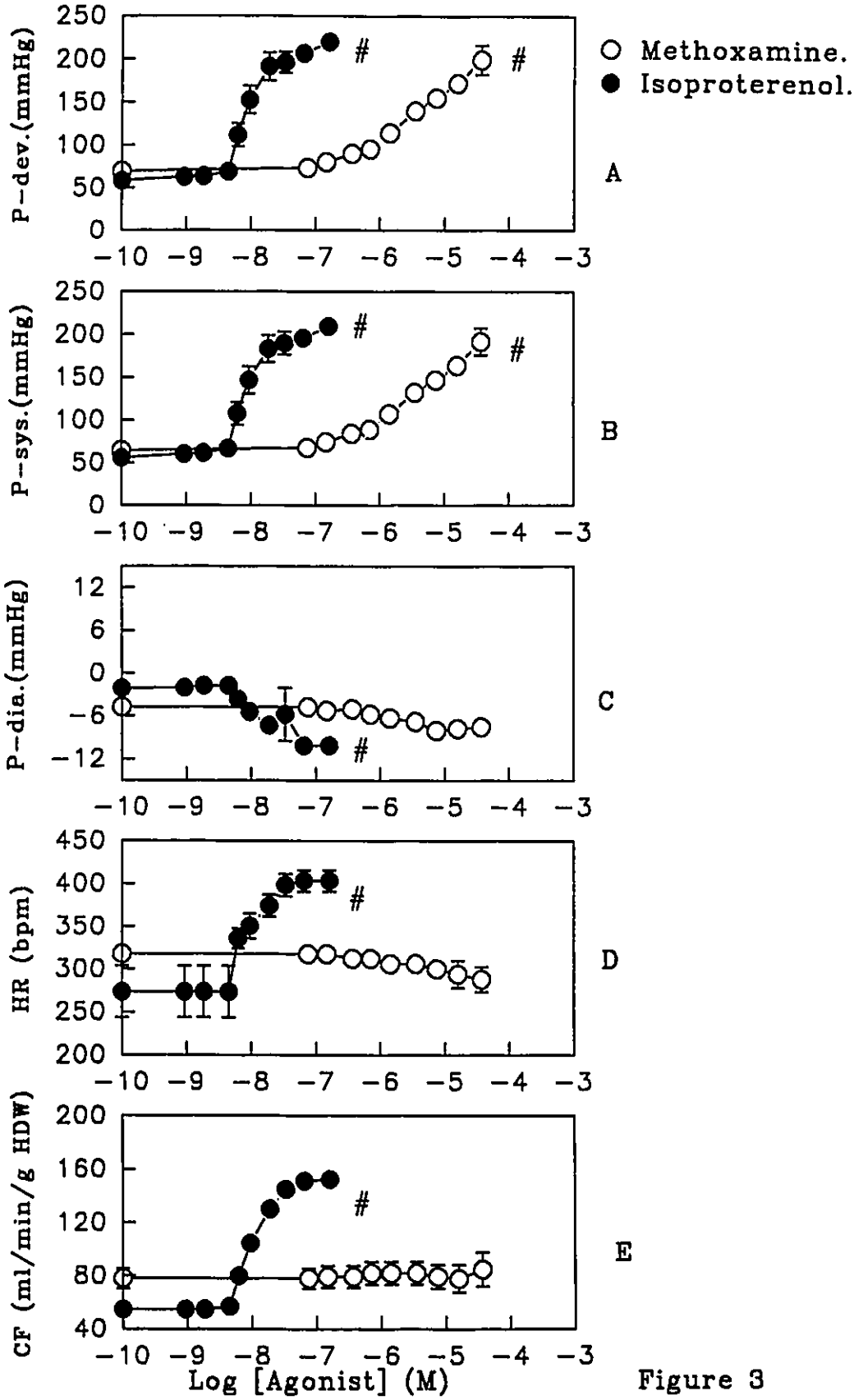

Methoxamine.
Isoproterenol.
P-dev.(mmHg)
P-sys.(mmHg)
P-dia.(mmHg)
HR (bpm)
CF (ml/min/g HDW)
Log [Agonist] (M)
A
B
C
D
E
#

Figure 3

Figure 4.

The effect of the nonselective ß-adrenoceptor antagonist propranolol (10^{-6} M) on methoxamine (10^{-7} to 10^{-5} M)-induced changes in developed pressure (P_{-dev}) {*panel A*}, heart rate (HR) {*panel B*}, and coronary flow (CF) {*panel C*} of hearts isolated from rats at 6 week of age. Each curve represents mean ± SEM (n = 5/group). #$p < 0.05$ vs. before treatment within each group.

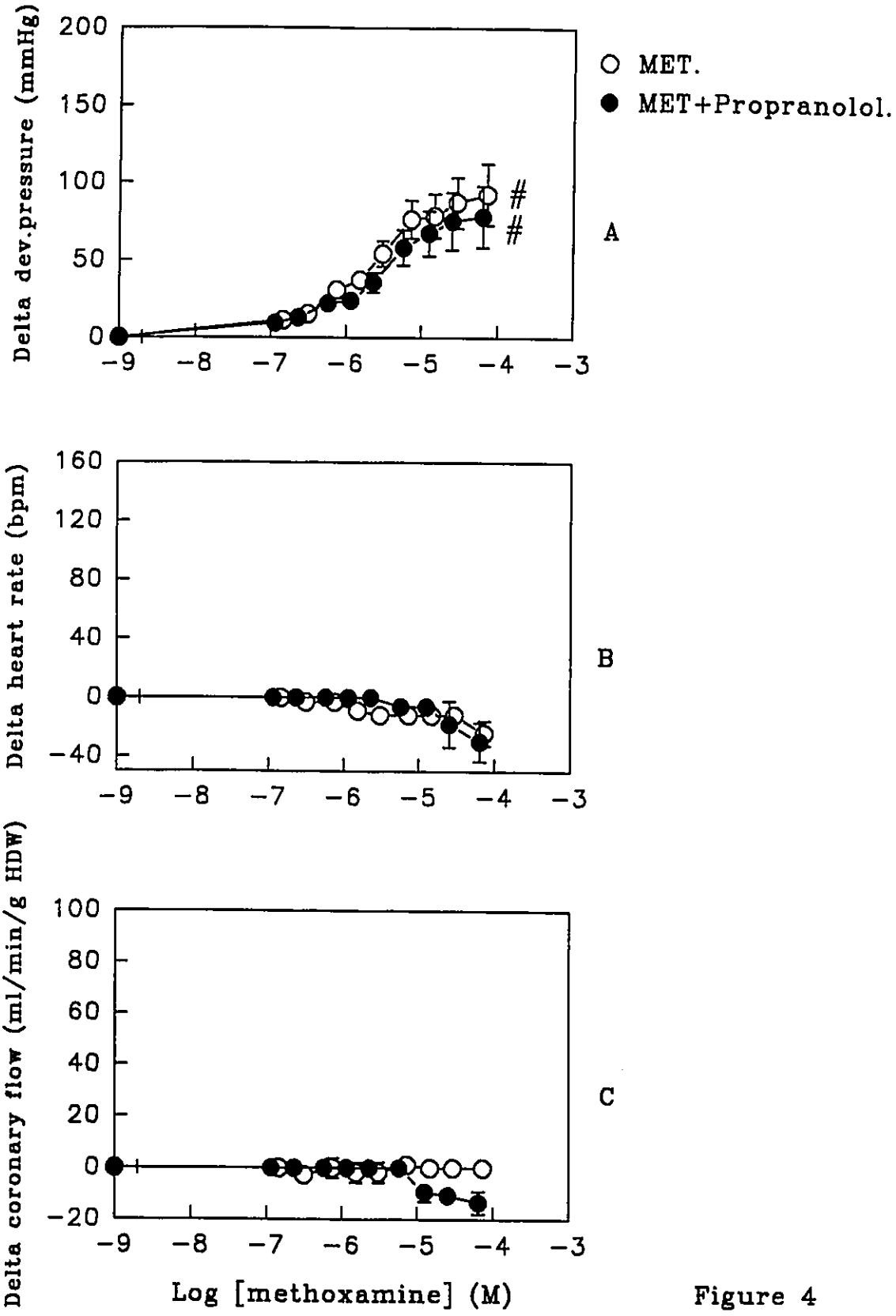

Delta dev.pressure (mmHg)
200
150
100
50
0
−9 −8 −7 −6 −5 −4 −3
#
#
A
MET.
MET+Propranolol.
Delta heart rate (bpm)
160
120
80
40
0
−40
−9 −8 −7 −6 −5 −4 −3
B
Delta coronary flow (ml/min/g HDW)
100
80
60
40
20
0
−20
−9 −8 −7 −6 −5 −4 −3
C
Log [methoxamine] (M)

Figure 4

In order to be cost-effective, the experimental protocol included both α_1- and ß-adrenoceptor stimulation on the same isolated heart. Therefore direct ß stimulation (after 30 min stabilization), ß stimulation following α stimulation in the same heart, and ß stimulation after 90 minutes stabilization were conducted in three groups of isolated hearts (see experimental protocol *E-III*). Direct ß stimulation of the isolated hearts with isoproterenol or preceding it with MET-induced α stimulation produced identical increases in developed pressure and HR (Fig. 5, *panels A* and *B*) at all the doses used. Both treatments also induced an identical maximal increase in coronary flow of about 100 ml/min/g HDW [Direct: from 57±3 to 150±4, After α stimulation: from 59±8 to 168±9 ml/min/g HDW]. Hearts treated with methoxamine prior to isoproterenol showed a greater increase in coronary flow at a given concentration of isoproterenol compared with hearts subjected to direct ß-stimulation (Fig. 5, *panel C*).

Infusion of isoproterenol after 30 or 90 min of stabilization (Fig. 6) produced identical increases in P-dev (*panel A*), HR (*panel B*) and CF (*panel C*) of isolated hearts by about 150 mmHg, 140 bpm and 90 ml/min/g HDW respectively. No significant differences in ß-adrenoceptor mediated responses of the isolated hearts subjected to different treatments were found.

Figure 5.

The effect of methoxamine (10^{-7} to 10^{-5} M) pre-treatment on isoproterenol (10^{-9} to 10^{-7} M)-induced changes in developed pressure (P_{dev}) {*panel A*}, heart rate (HR) {*panel B*}, and coronary flow (CF) {*panel C*} of hearts isolated from rats at 6 week of age. Each curve represents the mean ± SEM (n = 5-8/group). #$p < 0.05$ vs. before treatment within each group. *$p < 0.05$ vs. ISO after 30 min. stabilization.

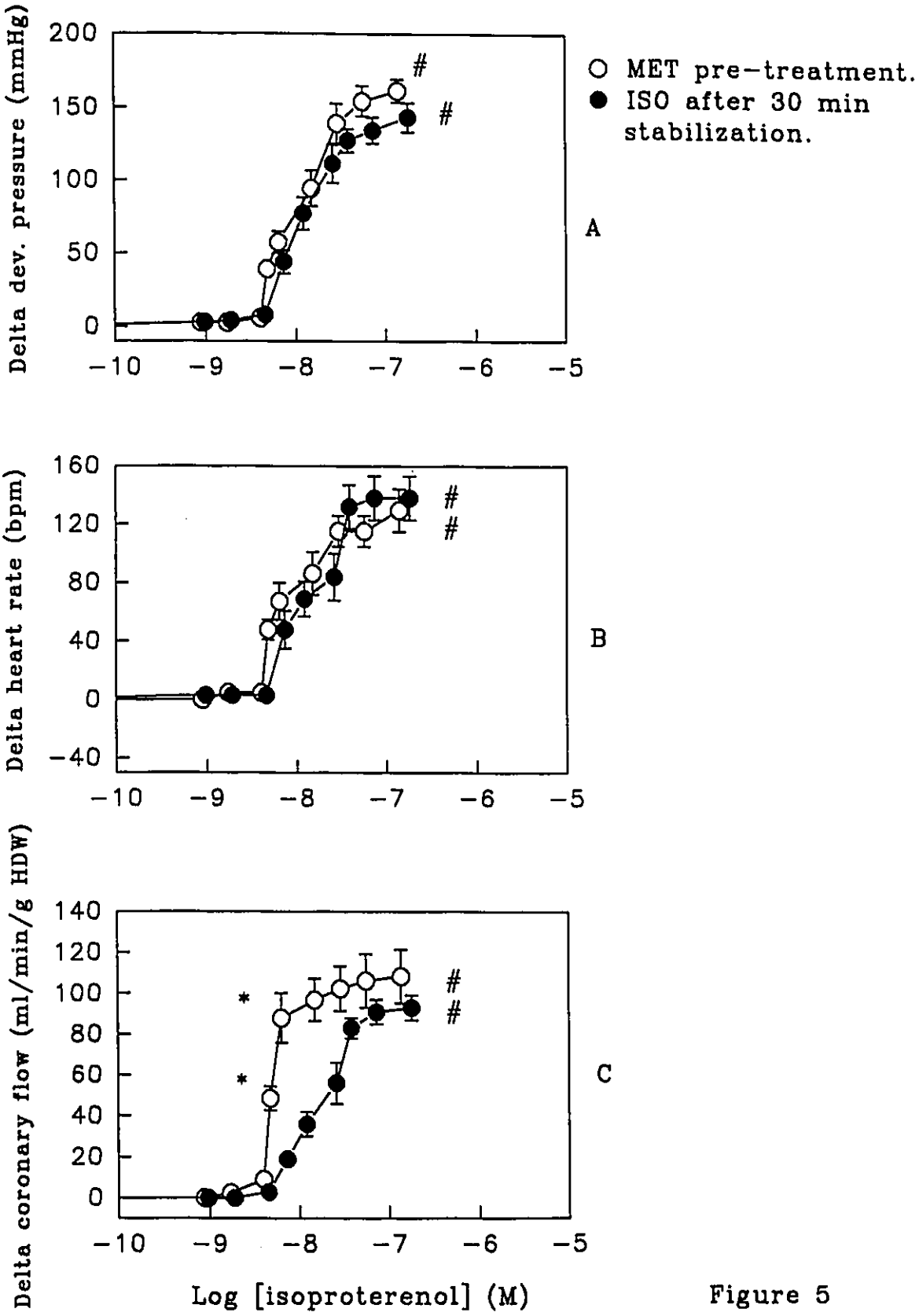

Delta dev. pressure (mmHg)
200
150
100
50
0
−10
−9
−8
−7
−6
−5
#
#
A
MET pre−treatment.
ISO after 30 min stabilization.
Delta heart rate (bpm)
160
120
80
40
0
−40
#
#
B
Delta coronary flow (ml/min/g HDW)
140
120
100
80
60
40
20
0
*
*
#
#
C
Log [isoproterenol] (M)

Figure 5

Figure 6.

The effect of 90 min vs. 30 min stabilization on isoproterenol (10^{-9} to 10^{-7} M)-induced changes on developed pressure (P_{dev}) {*panel A*}, heart rate (HR) {*panel B*}, and coronary flow (CF) {*panel C*} of hearts isolated from rats at 6 week of age. Each curve represents mean ± SEM (n = 3-8/group). #$p < 0.05$ vs. before treatment within each group.

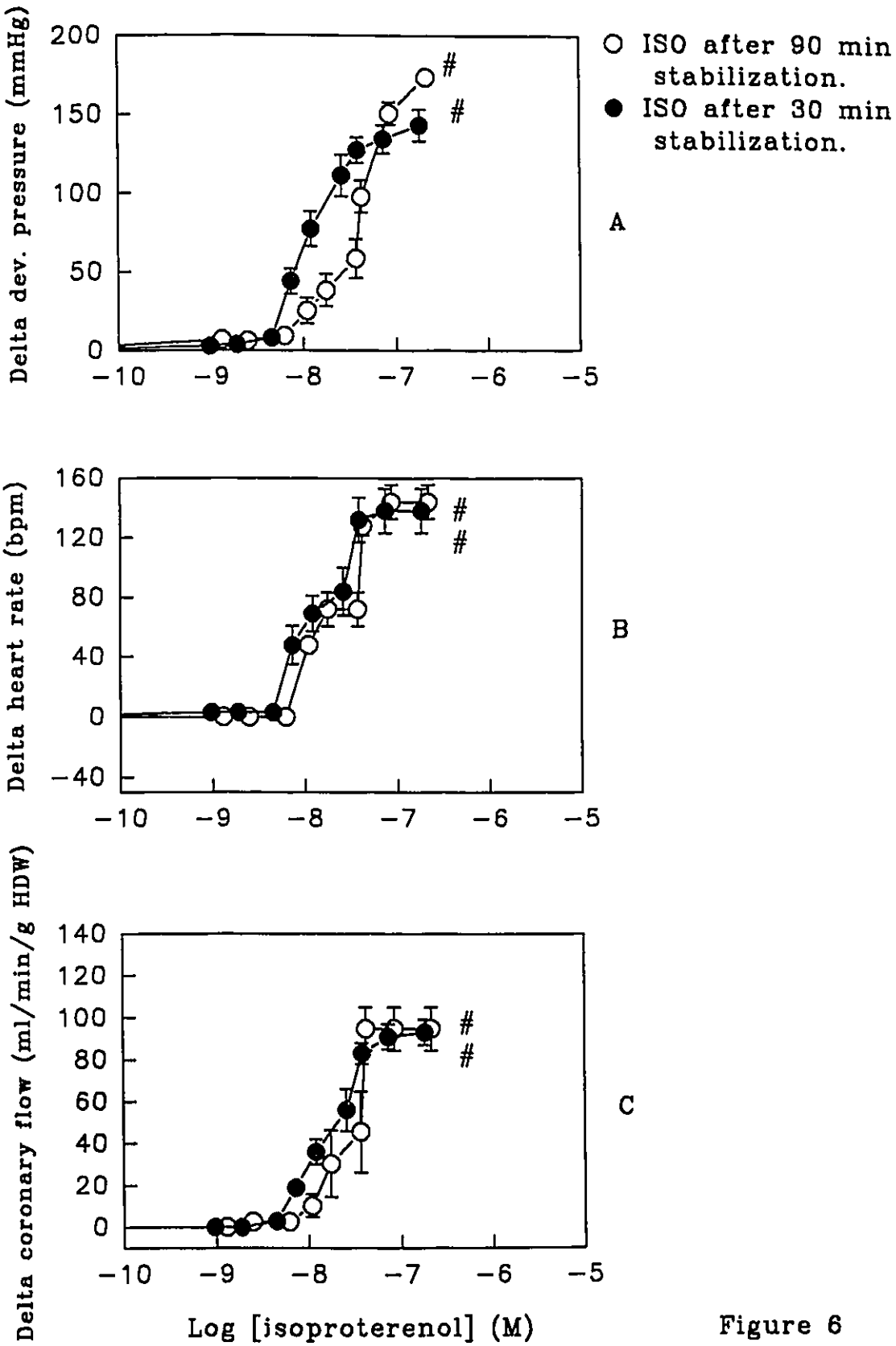

Delta dev. pressure (mmHg)
200
150
100
50
0
−10
−9
−8
−7
−6
−5
#
#
○ ISO after 90 min stabilization.
● ISO after 30 min stabilization.
A
Delta heart rate (bpm)
160
120
80
40
0
−40
#
#
B
Delta coronary flow (ml/min/g HDW)
140
120
100
80
60
40
20
0
#
#
C
Log [isoproterenol] (M)

Figure 6

Part 2. Effects of High Sodium Diet on Body Weight, Heart Dry Weight, and Heart Dry Weight / Body Weight Ratio in Young WKY rats.

Rats kept on a high sodium diet showed significantly less increase in body weight after 3 days of high sodium intake, thereafter all animals showed a normal pattern of growth. The body weight was slightly but significantly lower in high sodium treated rats at 10 weeks of age (Fig. 7, *panel A*). A significant increase in heart dry weight was found in all groups of hearts from rats kept on high sodium diet for 1 week and longer compared with their respective controls (Fig. 7, *panel B*). Heart dry weight /body weight ratio for rats kept on high sodium diet was significantly and time-dependently increased after 1 week (+9%), 2 weeks (+12%) and 6 weeks (+15%) compared with their respective controls (Fig. 7, *panel C*).

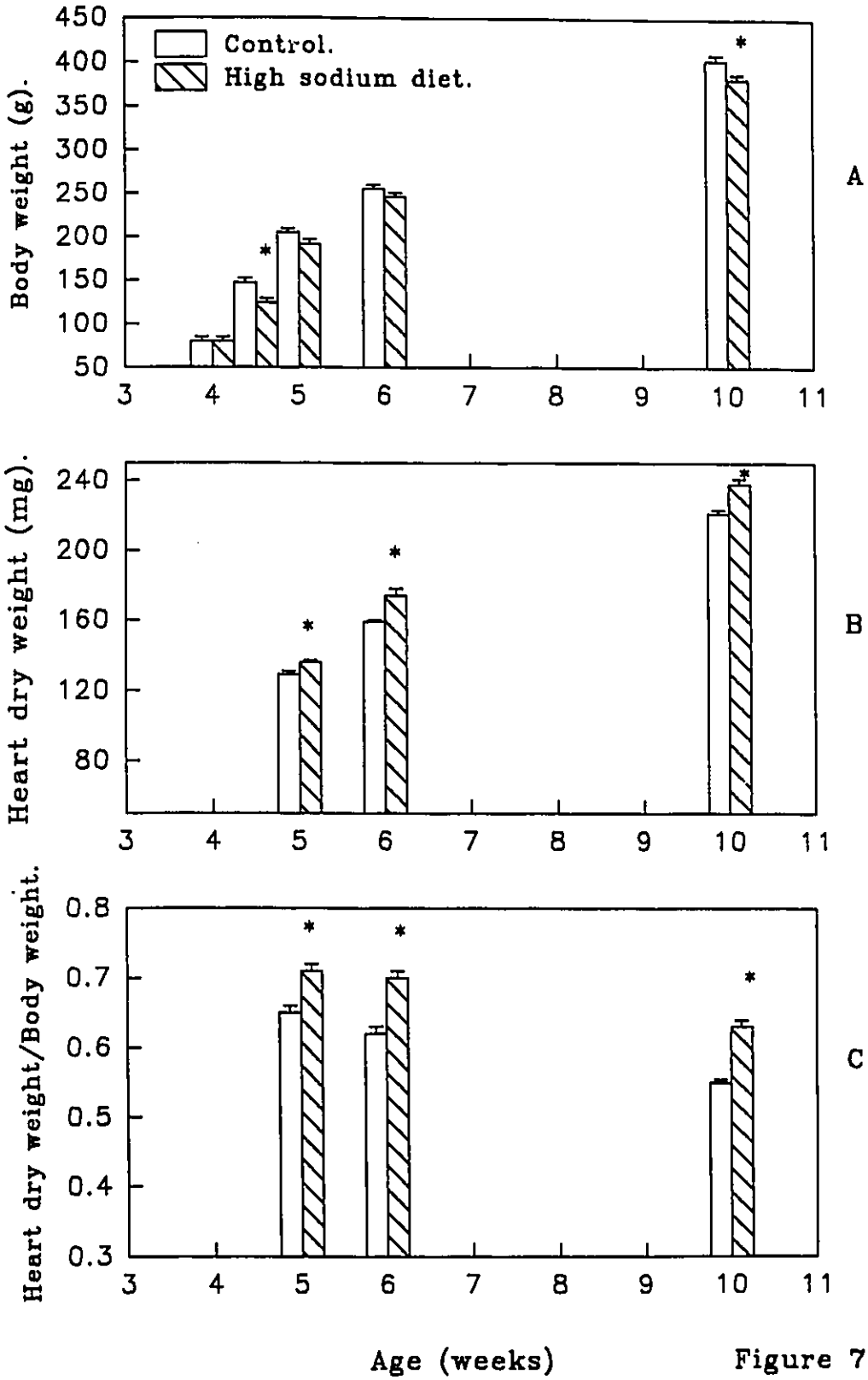

Control.
High sodium diet.
Body weight (g).
450
400
350
300
250
200
150
100
50
3
4
5
6
7
8
9
10
11
A
Heart dry weight (mg).
240
200
160
120
80
B
Heart dry weight/Body weight.
0.8
0.7
0.6
0.5
0.4
0.3
C
Age (weeks)

Figure 7

Figure 7.

The effect of high sodium diet (8% NaCl) on body weight {*panel A*}, heart dry weight (HDW) {*panel B*},and heart dry weight / body weight ratio {*panel C*} of young WKY rats after 3 days (from 4 to 4.5 wk of age, only body weight), 1 week (from 4 to 5 wk of age), 2 weeks (from 4 to 6 wk of age), and 6 weeks (from 4 to 10 wk of age) of high sodium intake. Bars represent mean ± SEM (n = 5-10/group). *$p < 0.05$ vs. respective control.

Part 3. Effects of High Sodium Diet on α_1- and ß-Adrenergic Stimulation in Isolated rat Hearts.

(A) α_1-Adrenergic Stimulation.

The α_1-agonist methoxamine, significantly and dose-dependently increased P-dev of hearts from 5, 6, or 10 weeks old WKY rats on a control or high sodium diet (Fig. 8, *panels A, B, and C*). No difference between high sodium treated groups and their respective controls was observed.

Isolated hearts from most groups showed small non significant decreases in HR in response to incremental concentrations of MET (Fig. 9). A decrease of about 25-30 bpm was observed in hearts isolated from 5 or 10 weeks old rats on a control or high sodium diet (Fig. 9, *panels A and C*). Hearts isolated from rats at 6 weeks of age showed a greater decrease in HR that achieved a final significant decrease of about 45-50 bpm (Fig. 9, *panel B*) at the highest concentration of methoxamine. No difference between hearts from rats fed on high sodium diet and their respective controls was observed.

Methoxamine, induced a small increase (10-20 ml/min/g HDW) in CF of all groups of isolated hearts (Fig. 10). Hearts isolated from rats on high sodium diet for 1 week (S_1) showed a higher response for a given concentration of methoxamine than control hearts (EC_{50}; $S_1 = 1.4* 0^{-6} \pm 6.7* 0^{-8}$ M , $C_1 = 3.3*10^{-6} \pm 1*10^{-6}$ M) without significantly affecting the maximum response (Fig. 10, *panel A*). Otherwise, no differences were observed between the two diet groups.

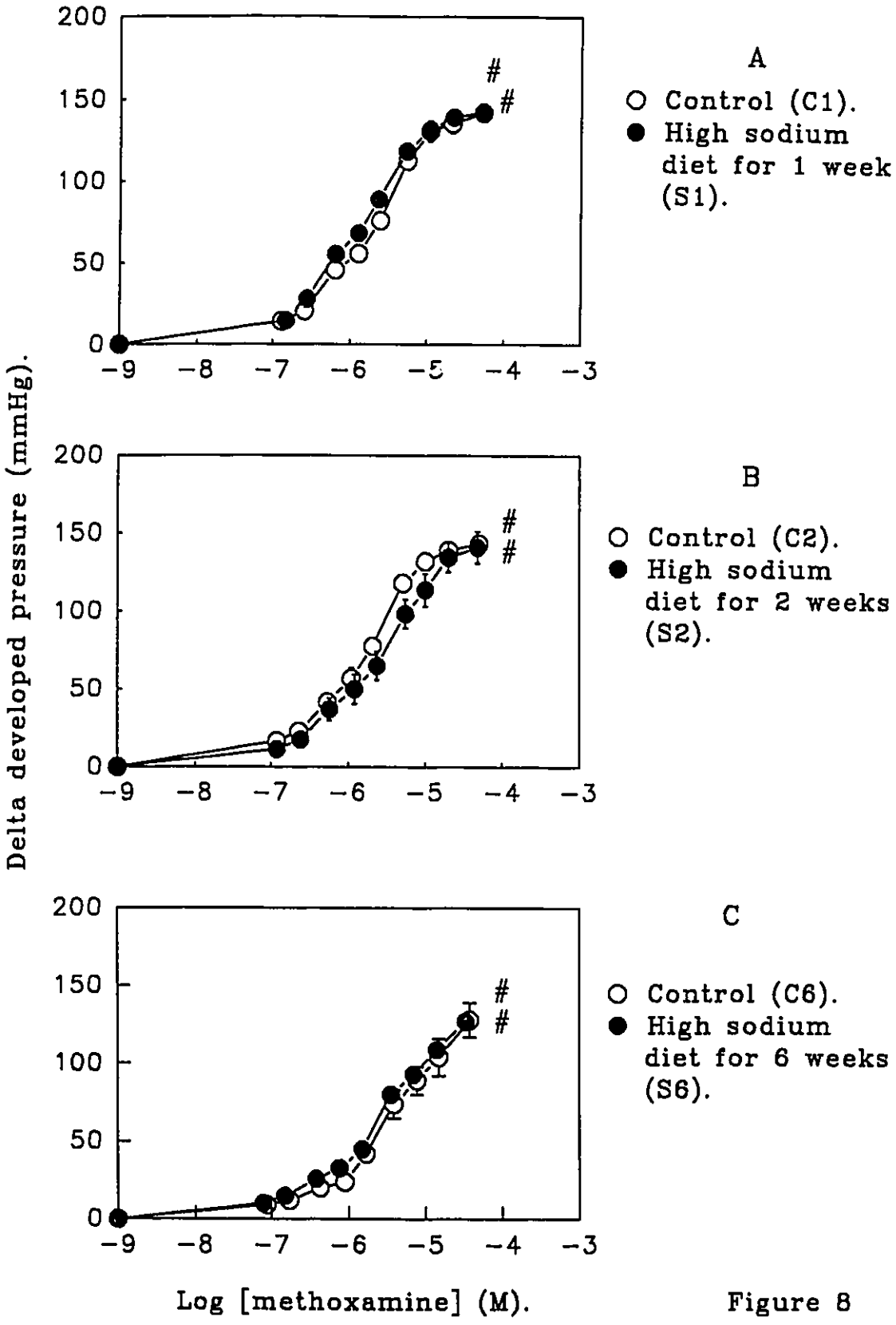

A
Control (C1).
High sodium diet for 1 week (S1).
B
Control (C2).
High sodium diet for 2 weeks (S2).
C
Control (C6).
High sodium diet for 6 weeks (S6).
Delta developed pressure (mmHg).
Log [methoxamine] (M).
200
150
100
50
0
−9
−8
−7
−6
−5
−4
−3
#
#

Figure 8

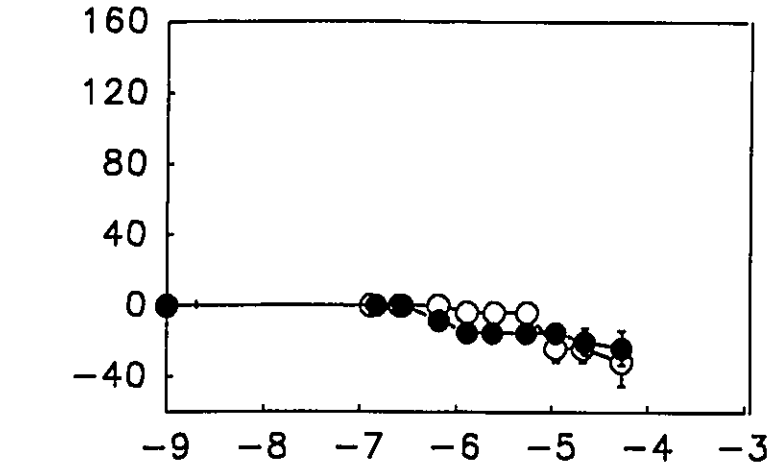
Figure 8

The effect of the α_1-adrenoceptor agonist methoxamine (10^{-7} to 10^{-5} M) on developed pressure (P_{-dev}) of hearts isolated from rats on a control (C_1, C_2, or C_6) or high sodium diet, after 1 week (S_1) {*panel A*}, 2 weeks (S_2) {*panel B*}, or 6 weeks (S_6) {*panel C*} of high sodium intake. Each curve represents mean ± SEM (n = 5-8/group). #$p < 0.05$ vs. before treatment within each group. The baseline values were:

$C_1 = 85\pm3$, $S_1 = 90\pm5$

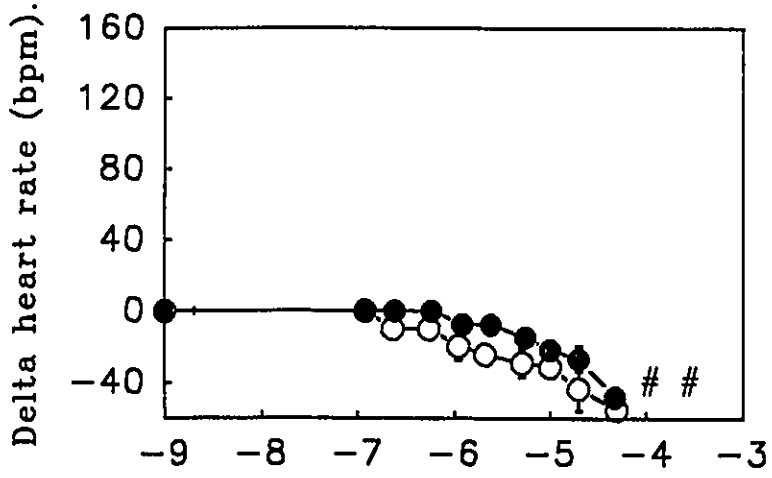
$C_2 = 88\pm3$, $S_2 = 86\pm3$

$C_6 = 75\pm5$, $S_6 = 78\pm4$



A

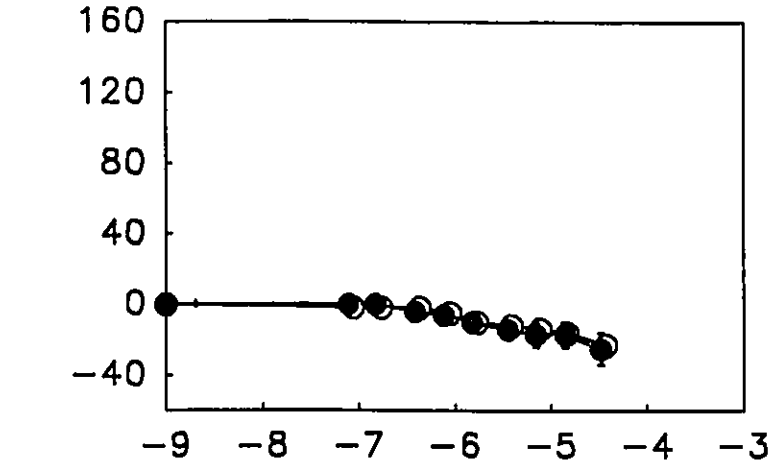
○ Control (C1).
● High sodium diet for 1 week (S1).



Delta heart rate (bpm).

B

○ Control (C2).
● High sodium diet for 2 weeks (S2).



C

○ Control (C6).
● High sodium diet for 6 weeks (S6).

Log [methoxamine] (M).

Figure 9

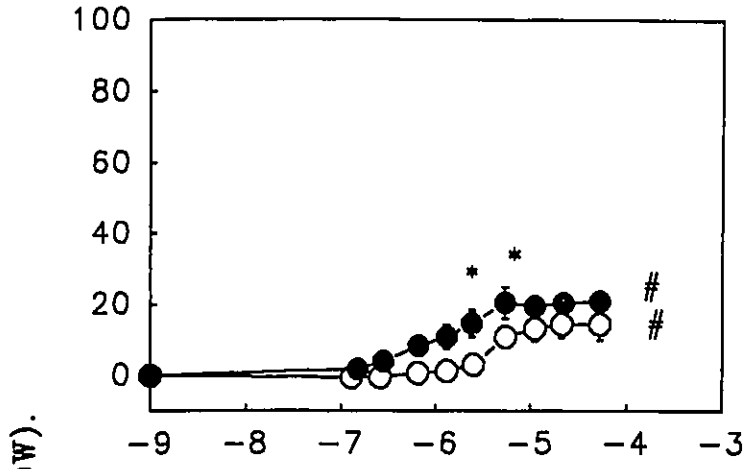
Figure 9.

The effect of the α_1-adrenoceptor agonist methoxamine (10^{-7} to 10^{-5} M) on heart rate (HR) of hearts isolated from rats on a control (C_1, C_2, or C_6) or high sodium diet, after 1 week (S_1) {*panel A*}, 2 weeks (S_2) {*panel B*}, or 6 weeks (S_6) {*panel C*} of high sodium intake. Each curve represents mean ± SEM (n = 5-8/group). #$p < 0.05$ vs. before treatment within each group. The baseline values were:

C_1 = 280±22, S_1 = 280±18

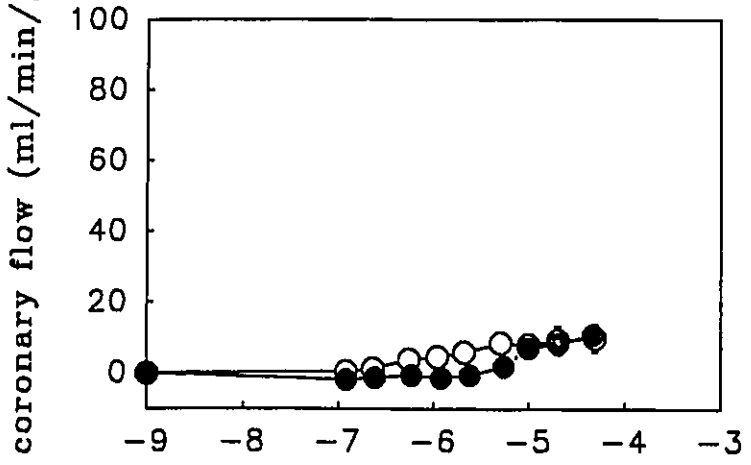
C_2 = 298±27, S_2 = 312±15

C_6 = 288±10, S_6 = 288±7



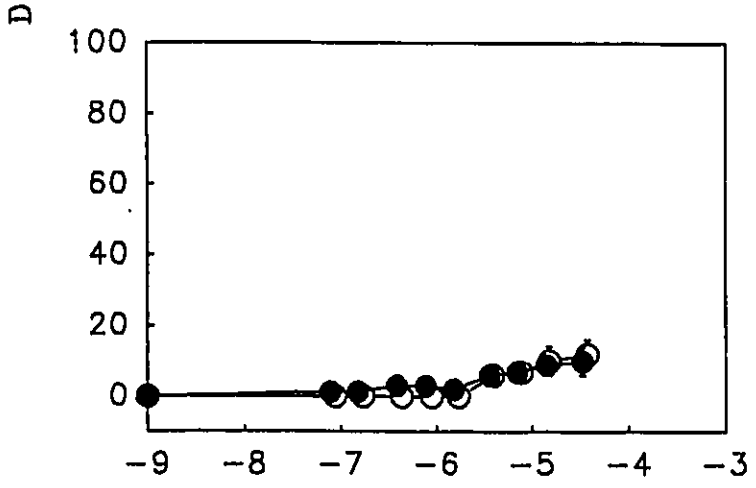
A

○ Control (C1).
● High sodium diet for 1 week. (S1).



B

○ Control (C2).
● High sodium diet for 2 weeks (S2).



C

○ Control (C6).
● High sodium diet for 6 weeks (S6).

Delta coronary flow (ml/min/g HDW).

Log [methoxamine] (M)

Figure 10

Figure 10.

The effect of the α_1-adrenoceptor agonist methoxamine (10^{-7} to 10^{-5} M) on coronary flow (CF) of hearts isolated from rats on a control (C_1, C_2, or C_6) or high sodium diet, after 1 week (S_1) {*panel A*}, 2 weeks (S_2) {*panel B*}, or 6 weeks (S_6) {*panel C*} of high sodium intake. Each curve represents mean ± SEM (n = 5-8/group). #$p < 0.05$ vs. before treatment within each group. *$p < 0.05$ vs respective control. The baseline values were:

$C_1 = 55\pm3$, $S_1 = 45\pm8$

$C_2 = 55\pm4$, $S_2 = 55\pm4$

$C_6 = 54\pm3$, $S_6 = 54\pm3$

(B) ß-Adrenergic Stimulation.

The ß-agonist isoproterenol increased developed pressure of hearts isolated from rats on a control or high sodium diet in a concentration-related fashion (Fig. 11, *panels A, B, and C*). No differences between hearts isolated from rats on high sodium diet and their respective controls were observed.

Infusion of isoproterenol also significantly increased HR of the isolated hearts at all ages (Fig. 12). An increase of 110-150 bpm was observed in hearts isolated from rats on a control or high sodium diet for 1, 2, or 6 weeks respectively (*panels A, B, and C*). Hearts isolated from rats on high sodium diet for 1 week showed a higher response at a given concentration of ISO compared with control hearts (EC_{50} , $S_1 = 1.9*10^{-8} \pm 1.5*10^{-9}$ M, $C_1 = 4.3*10^{-8} \pm 8.8*10^{-9}$ M) (Fig. 12, *panel A*) without a significant effect on maximum responses. No other differences between hearts isolated from high sodium treated rats and their respective controls were found.

The potent vasodilator isoproterenol, induced a dose-dependent increase of 50-70 ml/min/g HDW in CF of hearts isolated from rats at different ages and diets (Fig. 13). At the high concentrations of isoproterenol (around 10^{-7} M), hearts isolated from rats on high sodium diet for 6 weeks showed a smaller increases in coronary flow than control hearts. The EC_{50} values (EC_{50} , $S_6 = 1.1*10^{-8} \pm 1*10^{-9}$ M, $C_6 = 1.2*10^{-8} \pm 1.7*10^{-9}$ M) as well as maximum response (Fig. 13, *panel C*) were not significantly different. No other differences between the two dietary groups were observed.

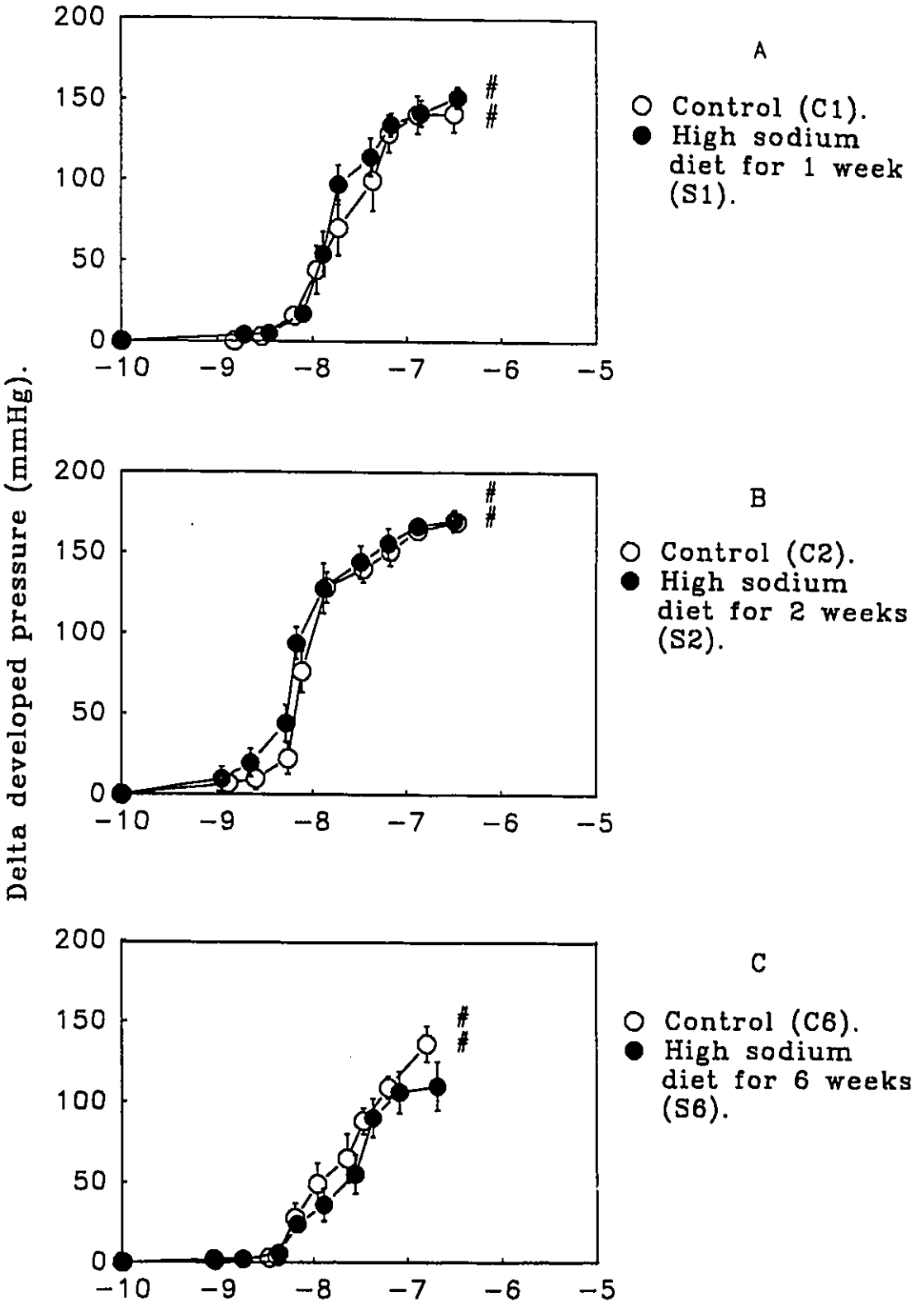

A
Control (C1).
High sodium diet for 1 week (S1).
B
Control (C2).
High sodium diet for 2 weeks (S2).
C
Control (C6).
High sodium diet for 6 weeks (S6).
Delta developed pressure (mmHg).
Log [isoproterenol] (M).
200
150
100
50
0
-10
-9
-8
-7
-6
-5
#
#

Figure 11

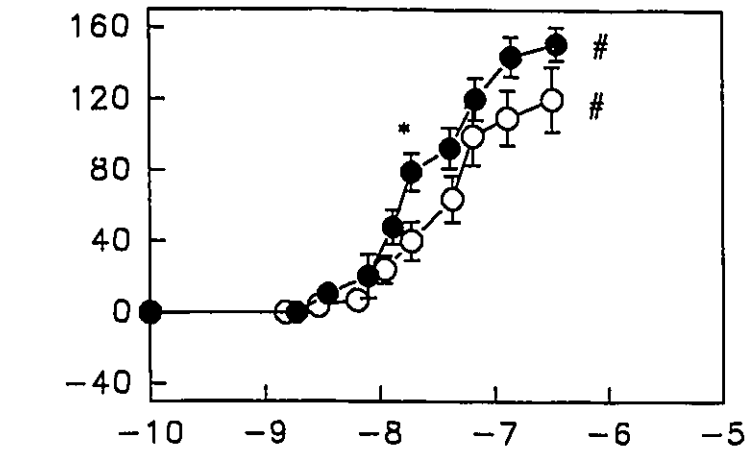
Figure 11.

The effect of the ß-adrenoceptor agonist isoproterenol (10^{-9} to 10^{-7} M) on developed pressure (P_{dev}) of hearts isolated from rats on a control (C_1, C_2, or C_6) or high sodium diet, after 1 week (S_1) {*panel A*}, 2 weeks (S_2) {*panel B*}, or 6 weeks (S_6) {*panel C*} of high sodium intake. Each curve represents mean ± SEM (n = 5-8/group). #$p < 0.05$ vs. before treatment within each group. The baseline values were:

$C_1 = 65\pm6$, $S_1 = 75\pm2$

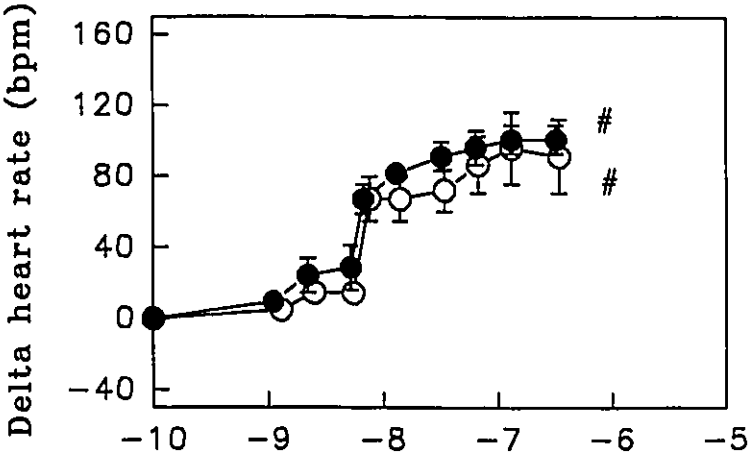
$C_2 = 76\pm3$, $S_2 = 73\pm3$

$C_6 = 65\pm4$ $S_6 = 65\pm6$



A

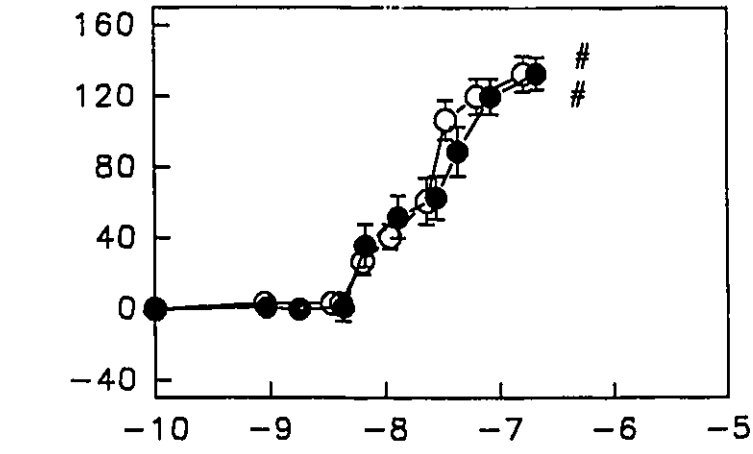
○ Contro (C1).
● High sodium diet for 1 week (S1).



Delta heart rate (bpm).

B

○ Control (C2).
● High sodium diet for 2 weeks (S2).



C

○ Control (C6).
● High sodium diet for 6 weeks (S6).

Log [isoproterenol] (M).

Figure 12

Figure 12.

The effect of the ß-adrenoceptor agonist isoproterenol (10^{-9} to 10^{-7} M) on heart rate (HR) of hearts isolated from rats on a control (C_1, C_2, or C_6) or high sodium diet, after 1 week (S_1) {*panel A*}, 2 weeks (S_2) {*panel B*}, or 6 weeks (S_6) {*panel C*} of high sodium intake. Each curve represents mean ± SEM (n = 5-8/group). #$p < 0.05$ vs. before treatment within each group. *$p < 0.05$ vs respective control. The baseline values were:

C_1 = 284±12, S_1 = 280±22

C_2 = 278±15, S_2 = 293±13

C_6 = 270±11, S_6 = 270±9

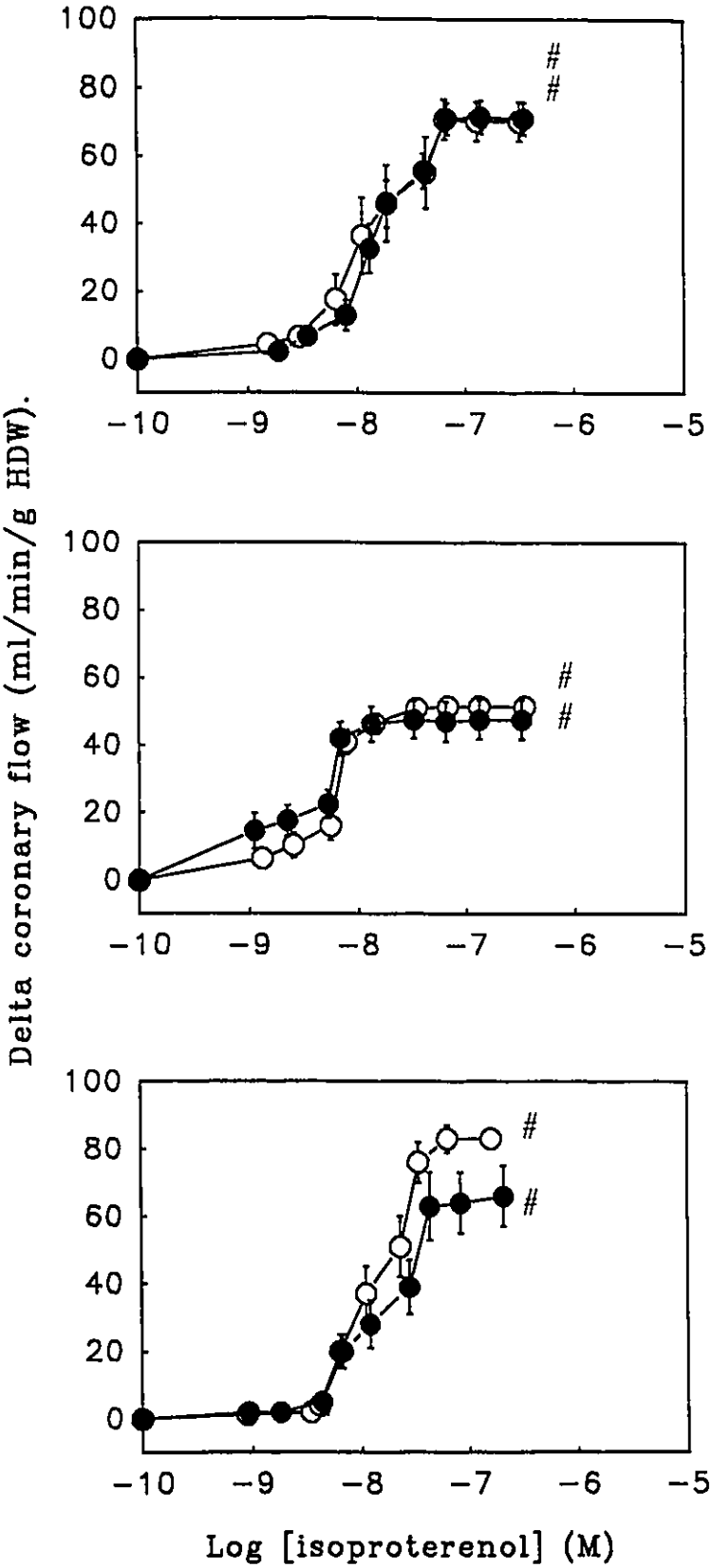

A
B
C
100
80
60
40
20
0
−10
−9
−8
−7
−6
−5
#
#
Delta coronary flow (ml/min/g HDW).
Log [isoproterenol] (M)
○ Control (C1).
● High sodium diet for 1 week (S1).
○ Control (C2).
● High sodium diet for 2 weeks (S2).
○ Control (C6).
● High sodium diet for 6 weeks (S6).

Figure 13

Figure 13

The effect of the ß-adrenoceptor agonist isoproterenol (10^{-9} to 10^{-7} M) on coronary flow (CF) of hearts isolated from rats on a control (C_1, C_2 or C_6) or high sodium diet, after 1 week (S_1) {*panel A*}, 2 weeks (S_2) {*panel B*} or 6 weeks (S_6) {*panel C*} of high sodium intake. Each curve represents mean ± SEM (n = 5-8/group). #p < 0.05 vs. before treatment within each group. The baseline values were:

C_1 = 47±3, S_1 = 40±2

C_2 = 43±1, S_2 = 48±6

C_6 = 50±2, S_6 = 45±3

Part 4. High Sodium Diet and α_1-Adrenergic Stimulation of Isolated rat Hearts in the presence of α_{1a}- or α_{1b}-Adrenoceptor Subtype Blocker.

To investigate the effect of high sodium diet on the responses of the isolated rat hearts to α_{1b}- or α_{1a}-adrenoceptor stimulation, three successive, incremental concentrations (low [10^{-7}], medium [10^{-6}], and high [10^{-5}] M) of MET were infused with the α_{1a}-adrenoceptor blocker Ura or the irreversible α_{1b}-adrenoceptor blocker chloroethylclonidine (CEC) into hearts isolated from rats on a control or high sodium diet for 2 weeks. In the presence of Ura, low, medium, or high doses of MET elicited small but significant increases in developed pressure of hearts isolated from rats on a control or high sodium diet (Control: 6±2, 8±2 and 15±2 mmHg. Salt: 5±1, 7±1, and 15±0) (Fig. 14, *panel A*). The high dose (10^{-5} M) of MET without Ura (Fig. 15*)* increased developed pressure of hearts from control rats by 98 mmHg and that of hearts from salt treated rats by 112 mmHg. These results indicate that, in the presence of Ura there is about 85% inhibition in MET-induced (at the high dose of MET) increases in developed pressure, similar for both dietary groups.

Following CEC ($0.25*10^{-5}$ M) treatment, MET elicited similar significant increases in developed pressure of hearts from control or high sodium treated rats (Control: 8±1, 22±4, and 56±6 mmHg. Salt: 7±1, 21±2, and 60±5 mmHg) in response to low, medium, and high doses respectively (Fig. 16, *panel A*). Without CEC, MET at the high dose produced similar increases in developed pressure of hearts from both dietary groups (Control 147±10; Salt 146±4 mmHg) (Fig. 17, *panel B*). These results suggest that CEC treatment produces about 60% inhibition in MET-induced increases (at the high dose of

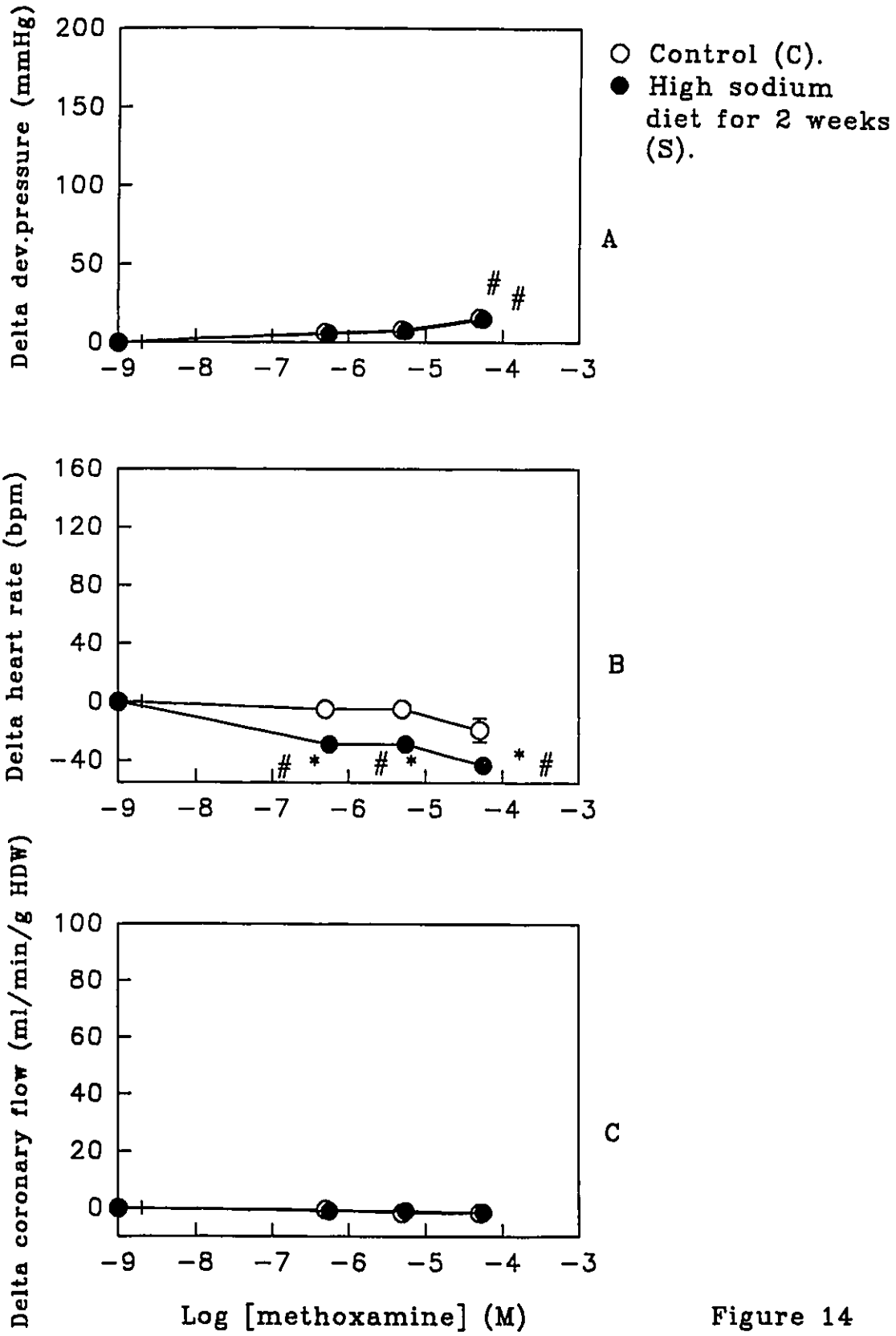
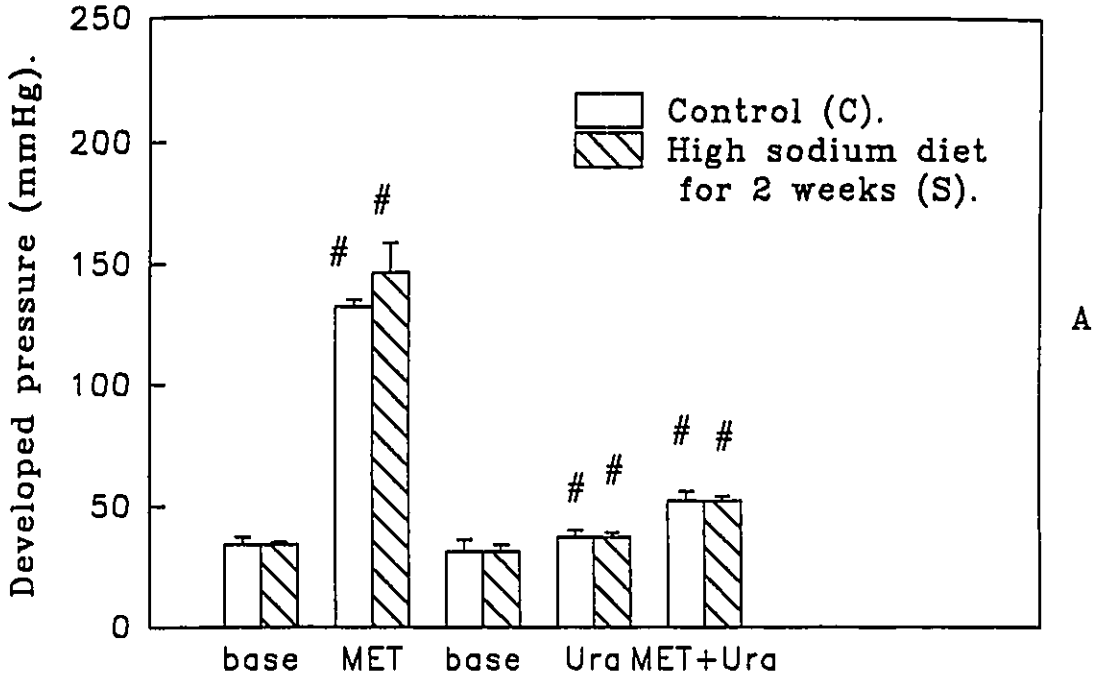

Delta dev.pressure (mmHg)
200
150
100
50
0
−9 −8 −7 −6 −5 −4 −3
# #
A
○ Control (C).
● High sodium diet for 2 weeks (S).
Delta heart rate (bpm)
160
120
80
40
0
−40
* # * *
B
Delta coronary flow (ml/min/g HDW)
100
80
60
40
20
0
C
Log [methoxamine] (M)

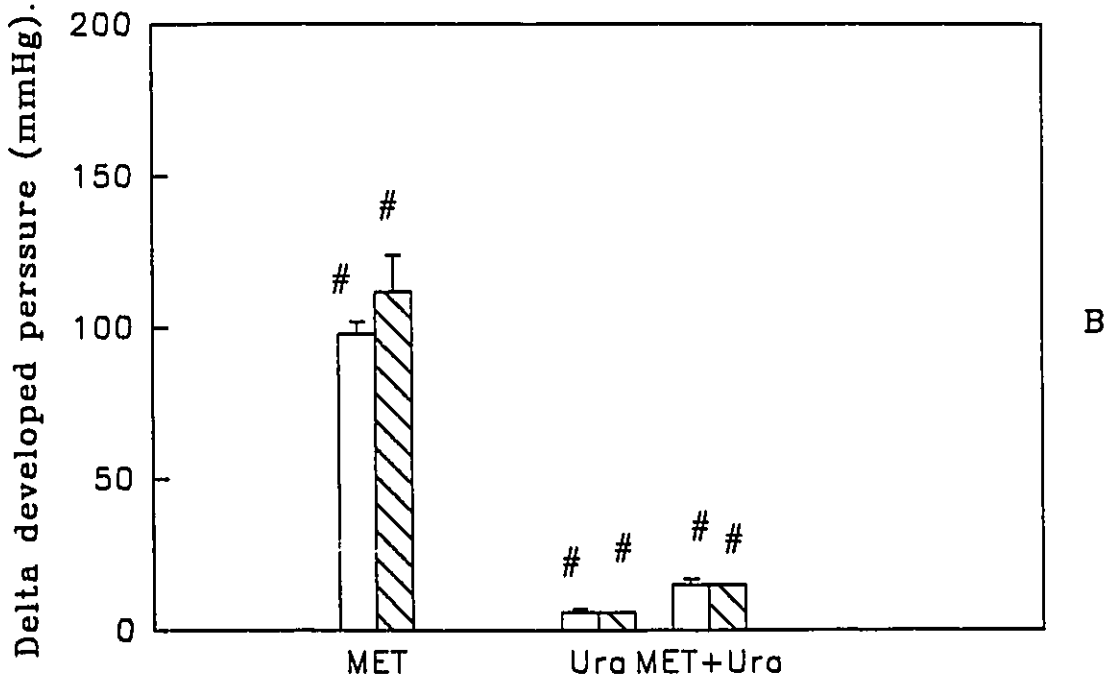
Figure 14

Figure 14.

The effects of the low (10^{-7} M), medium (10^{-6} M), and high (10^{-5} M) concentrations of the α_1-adrenoceptor agonist methoxamine in the presence of (10^{-5} M) urapidil (α_{1a}-adrenoceptor blocker) on changes in (Delta) developed pressure {*panel A*}, heart rate {*panel B*} and coronary flow {*panel C*} of hearts isolated from WKY rats on a control or high sodium diet for 2 weeks (from 4 to 6 wk of age). Each curve represents mean ± SEM (n = 5/group). #$p < 0.05$ vs. before treatment within each group. *$p < 0.05$ vs. control.


Developed pressure (mmHg).
250
200
150
100
50
0
Control (C).
High sodium diet
for 2 weeks (S).
#
base
MET
base
Ura
MET+Ura

A


Delta developed perssure (mmHg).
200
150
100
50
0
#
MET
Ura
MET+Ura

B

Figure 15

Figure 15.

The effect of the α_{1a}-adrenoceptor blocker urapidil (Ura, 10^{-5} M) on methoxamine (MET, 10^{-5} M)-induced changes in developed pressure (P_{-dev}) of hearts isolated from WKY rats on a control (C) or high sodium (S) diet for 2 weeks (From 4 to 6 wk of age). *Panel A* represents the original data, *panel B* represents the specific treatment-induced change in (Delta) developed pressure. Bars represent mean ± SEM (n = 5/group). #$p < 0.05$ vs. before treatment within each group.

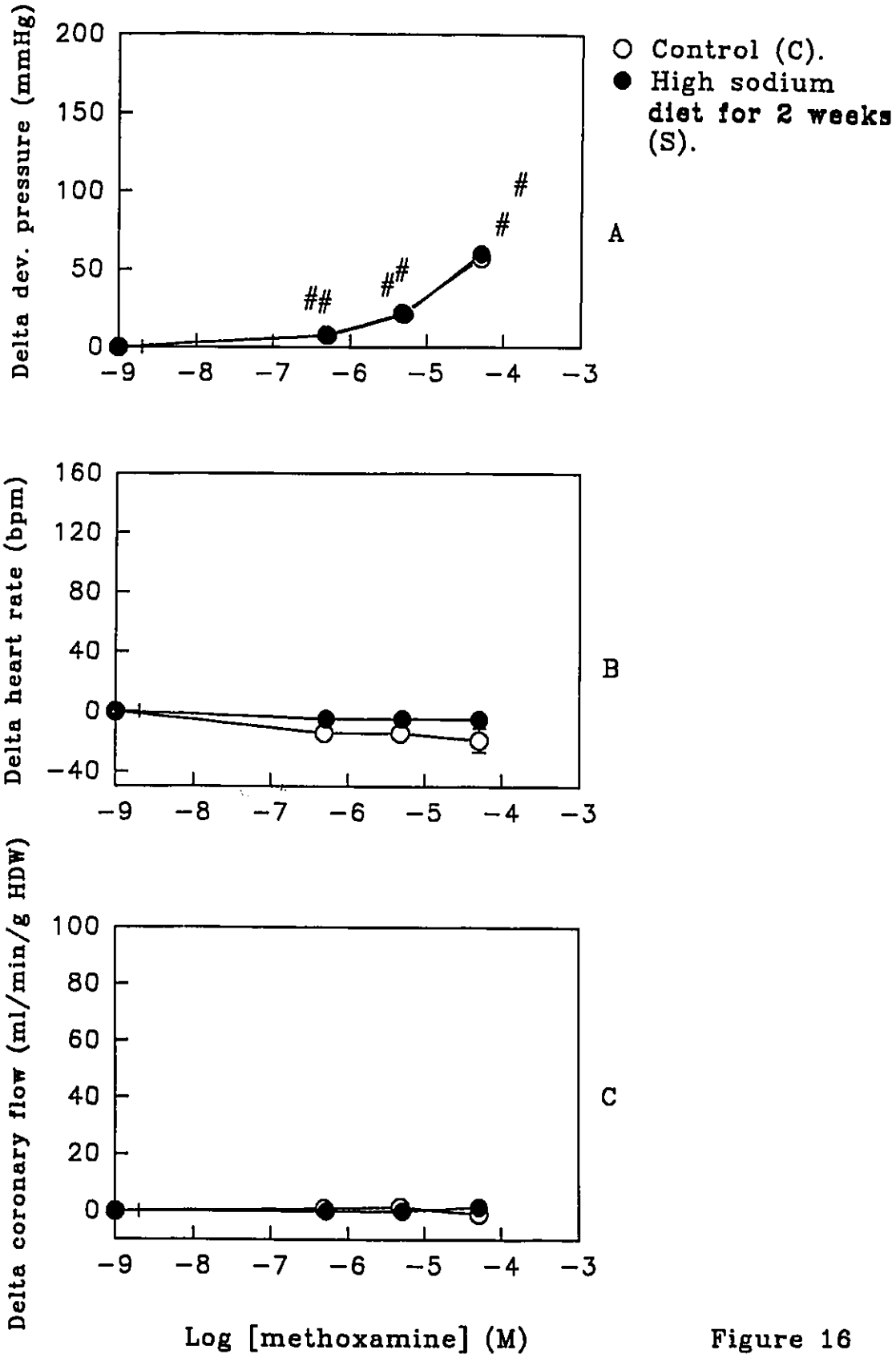

Delta dev. pressure (mmHg)
200
150
100
50
0
-9
-8
-7
-6
-5
-4
-3
##
#
#
#
A
Control (C).
High sodium diet for 2 weeks (S).
Delta heart rate (bpm)
160
120
80
40
0
-40
B
Delta coronary flow (ml/min/g HDW)
100
80
60
40
20
0
C
Log [methoxamine] (M)

Figure 16

Figure 16.

The effects of the low (10^{-7} M), medium (10^{-6} M), and high (10^{-5} M) concentrations of the α_1-adrenoceptor agonist methoxamine in the presence of ($0.25*10^{-5}$ M) CEC (α_{1b}-adrenoceptor blocker) on changes in (Delta) developed pressure {*panel A*}, heart rate {*panel B*} and coronary flow {*panel C*} of hearts isolated from WKY rats on a control or high sodium diet for 2 weeks (from 4 to 6 wk of age). Each curve represents mean ± SEM (n = 5/group). #$p < 0.05$ vs. before treatment within each group.

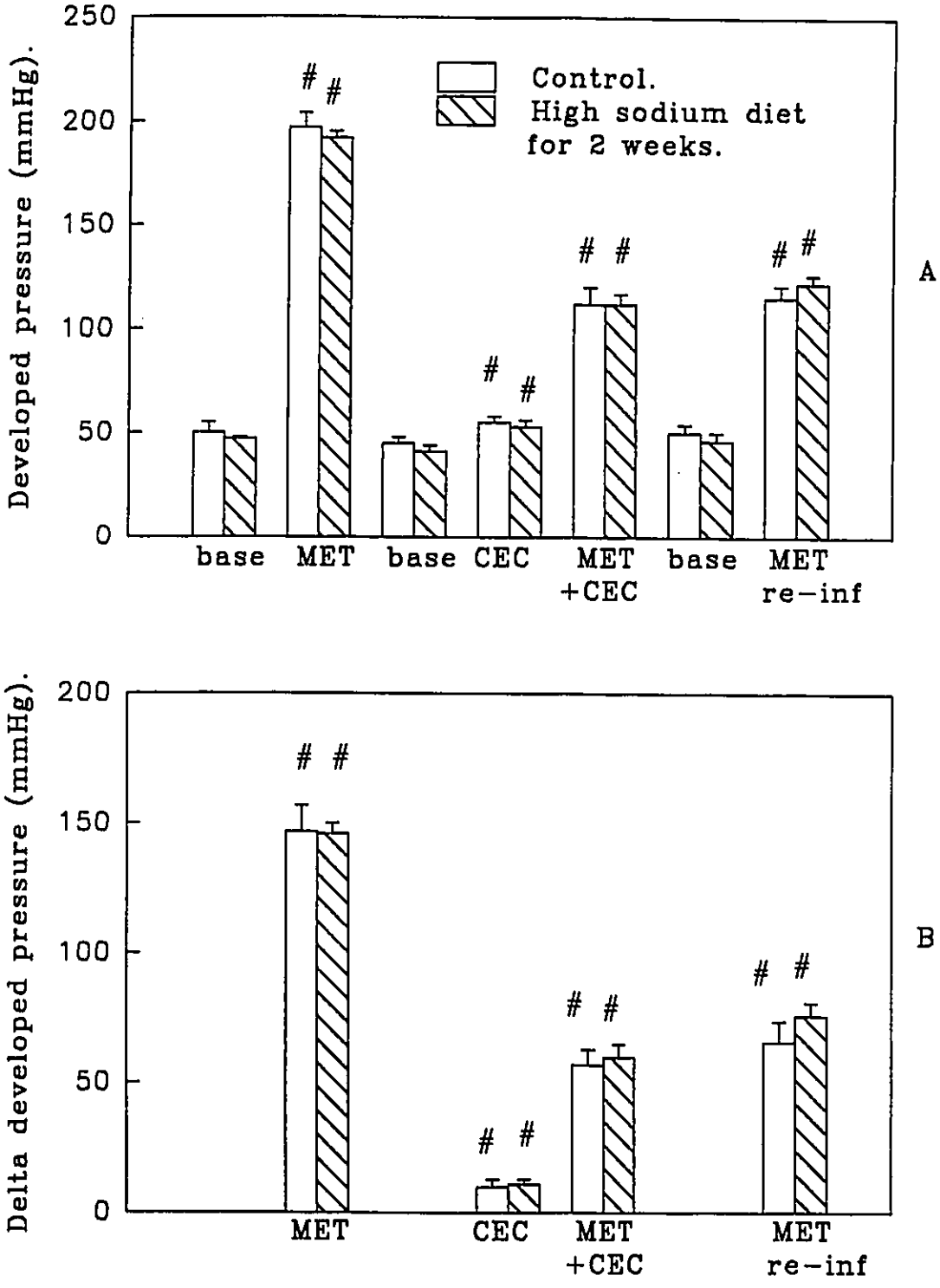

Developed pressure (mmHg).
250
200
150
100
50
0
Control.
High sodium diet
for 2 weeks.
# #
base
MET
base
CEC
MET
+CEC
base
MET
re-inf
A
Delta developed pressure (mmHg).
200
150
100
50
0
MET
CEC
MET
+CEC
MET
re-inf
B

Figure 17

Figure 17.

The effect of the α_{1b}-adrenoceptor blocker chloroethylclonidine (CEC, $0.25*10^{-5}$ M) on methoxamine (MET, 10^{-5} M)-induced changes in developed pressure (P_{-dev}) during simultaneous infusion (MET+CEC) or after 10-15 min of drug free buffer perfusion (MET re-inf) of hearts isolated from WKY rats on a control (C) or high sodium (S) diet for 2 weeks (From 4 to 6 wk of age). *Panel A* represents the original data, *panel B* represents the specific treatment-induced change in (Delta) developed pressure. Bars represent mean ± SEM (n = 5/group). #$p < 0.05$ vs. before treatment within each group.

MET) in developed pressure similar in hearts from control or high sodium treated rats. No significant differences in inotropic responses to the different doses of MET in the presence of α_{1a}- or α_{1b}-adrenoceptor blockade were found between hearts from salt treated rats and their respective controls.

10^{-5} M MET without Ura, produced a small but significant decrease of -22 - -25 bpm in the heart rate of hearts from control rats (Fig. 18, *panel B*). In the presence of Ura, MET at low, medium or high concentration produced small non significant decreases of -5, -5, and -19 bpm respectively in the heart rate of hearts from control rats (Fig. 14, *panel B*). In hearts isolated from rats on high sodium diet for 2 weeks, high concentration of MET produced a non-significant decrease of -14 bpm in the heart rate. However, following Ura treatment, methoxamine at low, medium or high concentration produced a significant decrease in HR by -29, -29, and -44 bpm (Fig. 14 *and* 18, *panel B*). Analysis of the data obtained revealed a significant difference between hearts isolated from rats on high sodium diet and their respective controls at all the concentrations of MET following the α_{1a}-adrenoceptor blocker urapidil for the chronotropic effects.

In the presence of CEC, low, medium or high dose of MET produced a non significant decrease of -12 - -15 bpm in heart rate of hearts from control rats at all the concentrations of MET. In hearts from salt treated rats MET after CEC treatment produced smaller non significant decreases of about -5 bpm in heart rate at all of the three concentrations of MET (Fig. 16, *panel B*). The decrease of -5 bpm in heart rate of hearts from salt treated rats was not significantly different from the decrease of -14 bpm observed in the heart rate of control hearts. MET at the high dose in the absence of CEC

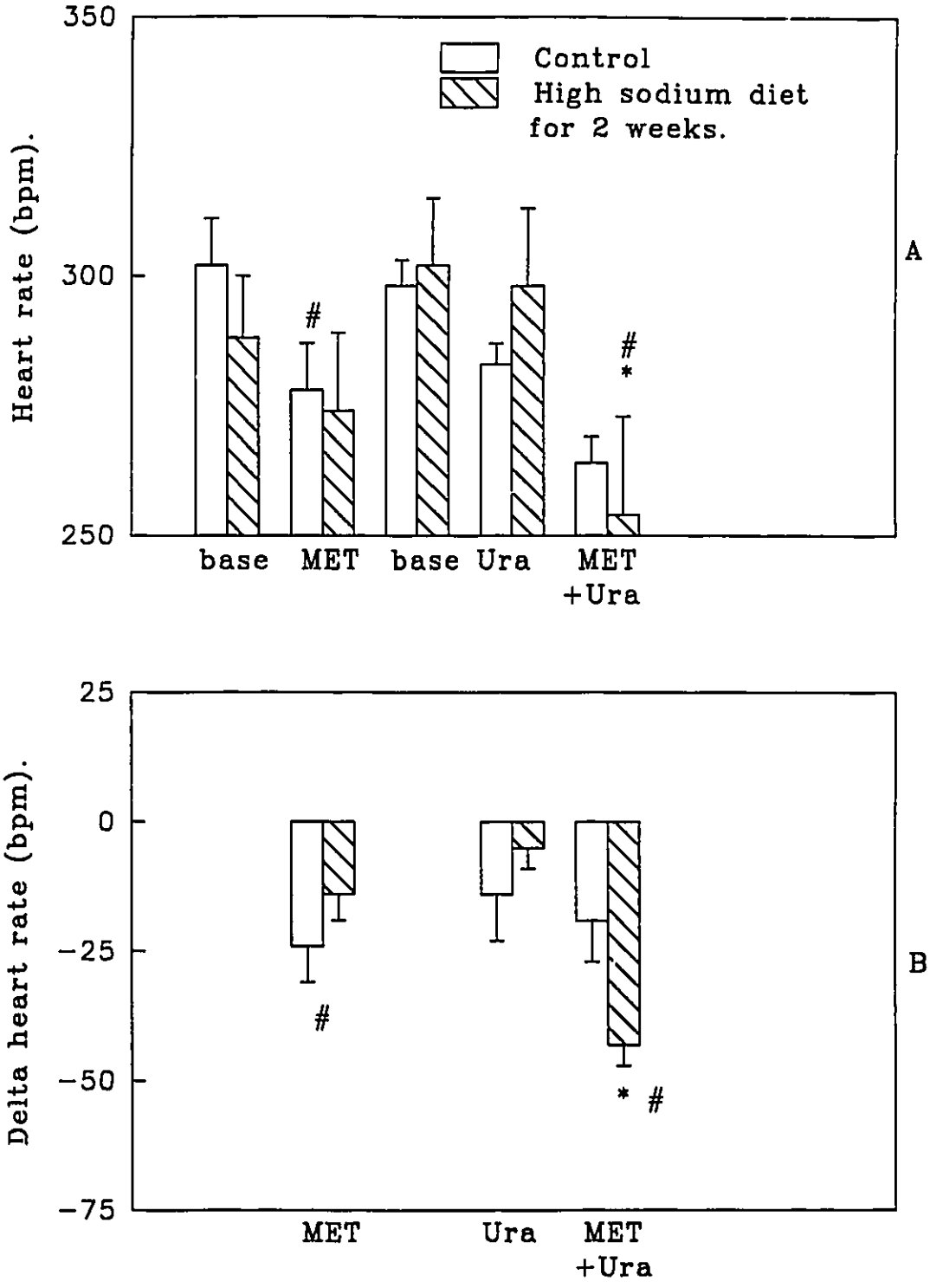

350
300
250
Heart rate (bpm).
Control
High sodium diet
for 2 weeks.
A
#
#
*
base
MET
base
Ura
MET
+Ura
25
0
−25
−50
−75
Delta heart rate (bpm).
B
#
* #
MET
Ura
MET
+Ura

Figure 18

Figure 18.

The effect of the α_{1a}-adrenoceptor blocker urapidil (Ura, 10^{-5} M) on methoxamine (MET, 10^{-5} M)-induced changes in heart rate (HR) of hearts isolated from WKY rats on a control (C) or high sodium (S) diet for 2 weeks (From 4 to 6 wk of age). *Panel A* represents the original data, *panel B* represents the specific treatment-induced change in (Delta) heart rate. Bars represent mean ± SEM (n = 5/group). #$p < 0.05$ vs. before treatment within each group. *$p < 0.05$ vs. control.

produced non significant decreases of -24 and -19 bpm respectively in heart rate of hearts from control or high sodium treated rats (Fig. 19, *panel B*).

In both dietary groups, both Ura or CEC treatment prevented the small methoxamine-induced increases in CF at any concentration of MET (Figs. *14 panel C; 20; 16 panel C; and 21).* No significant differences in CF response to MET following Ura or CEC treatment between hearts isolated from rats on high sodium diet for 2 weeks and their respective controls were found.

The α_{1a}-adrenoceptor blocker urapidil caused a small (6 mmHg) but significant increase in P-dev of the isolated hearts from both dietary groups. It had no significant effect on heart rate or coronary flow of both groups. CEC, the α_{1b}-adrenoceptor irreversible blocker also caused a small (about 10 mmHg) but significant increase in developed pressure. However it produced a decrease of about 35 bpm in heart rate of the isolated hearts from both dietary groups (Fig. 19, *panel B*). It also significantly reduced the coronary flow in all the groups of isolated hearts (Fig. 21, *panel B*).

To test whether CEC blockade was irreversible, high concentration of MET was infused into the isolated hearts after a drug free buffer perfusion for 10-15 min from the last MET+CEC treatment. The increase in P-dev of isolated hearts from control or high sodium treated rats induced by MET after washout of CEC (Fig. 17) was significantly less than that induced by the same concentration of MET at the beginning of the experiment, and was similar to that induced by methoxamine before washout of CEC. Heart rate (Fig. 19) and coronary flow (Fig. 21) responses of hearts from both dietary groups to methoxamine after washout of CEC was not different from that of before the washout.

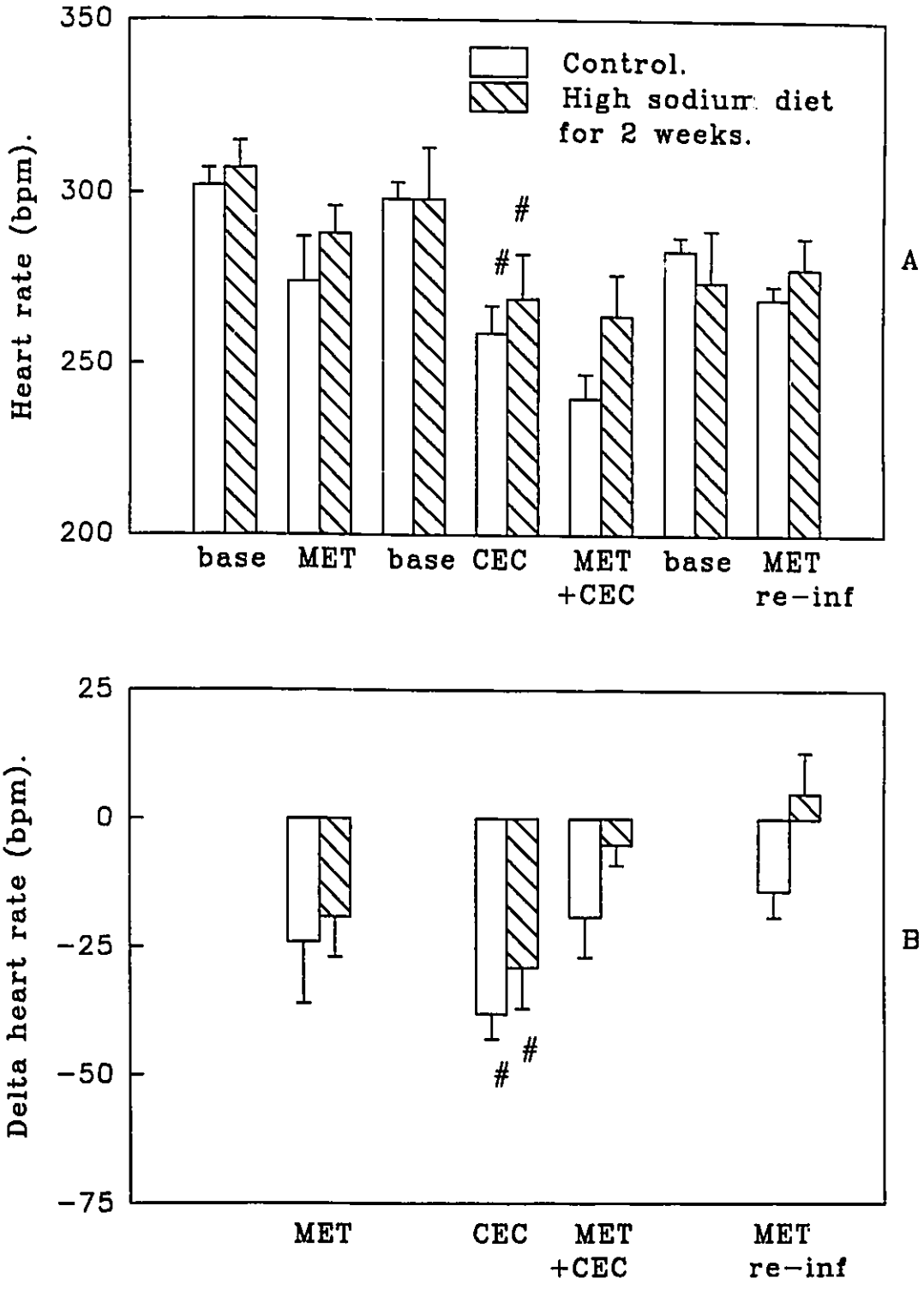
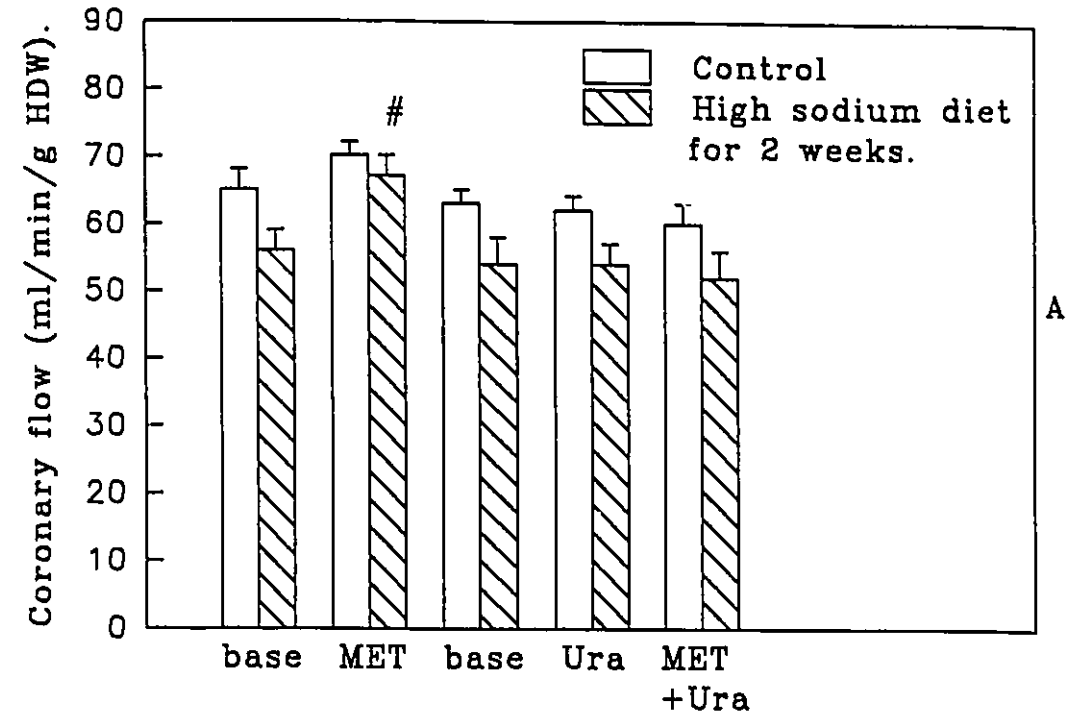

350
300
250
200
Heart rate (bpm).
Control.
High sodium diet
for 2 weeks.
#
#
A
base
MET
base
CEC
MET
+CEC
base
MET
re-inf
25
0
-25
-50
-75
Delta heart rate (bpm).
#
#
B
MET
CEC
MET
+CEC
MET
re-inf

Figure 19

Figure 19.

The effect of the α_{1b}-adrenoceptor blocker chloroethylclonidine (CEC, $0.25*10^{-5}$ M) on methoxamine (MET, 10^{-5} M)-induced changes in heart rate (HR) during simultaneous infusion (MET+CEC) or after 10-15 min of drug free buffer perfusion (MET re-inf) of hearts isolated from WKY rats on a control (C) or high sodium (S) diet for 2 weeks (From 4 to 6 wk of age). *Panel A* represents the original data, *panel B* represents the specific treatment-induced change in (Delta) heart rate. Bars represent mean ± SEM (n = 5/group). #$p < 0.05$ vs. before treatment within each group.


Coronary flow (ml/min/g HDW).
90
80
70
60
50
40
30
20
10
0
#
Control
High sodium diet
for 2 weeks.
base
MET
base
Ura
MET
+Ura
A

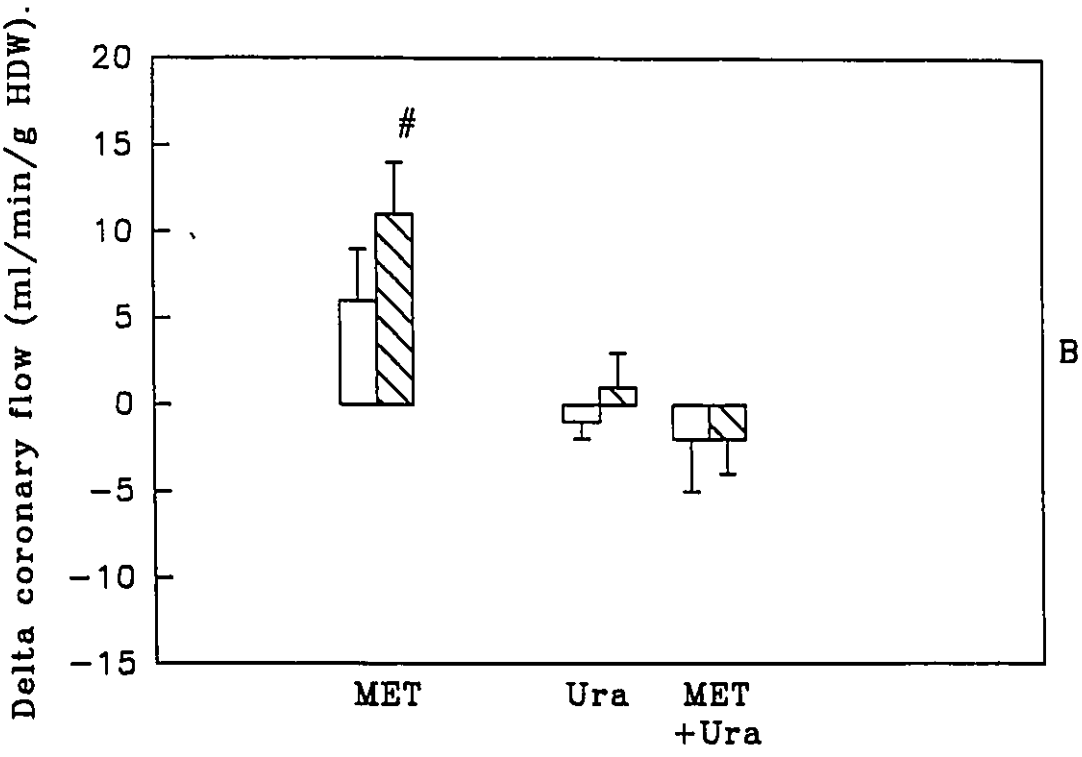

Delta coronary flow (ml/min/g HDW).
20
15
10
5
0
−5
−10
−15
#
MET
Ura
MET
+Ura
B

Figure 20

Figure 20.

The effect of the α_{1a}-adrenoceptor subtype blocker urapidil (Ura, 10^{-5} M) on methoxamine (MET, 10^{-5} M)-induced changes in coronary flow (CF) of hearts isolated from WKY rats on a control (C) or high sodium (S) diet for 2 weeks (From 4 to 6 wk of age). *Panel A* represents the original data, *panel B* represents the specific treatment-induced change in (Delta) coronary flow. Bars represent mean ± SEM (n = 5/group). #$p < 0.05$ vs. before treatment within each group.

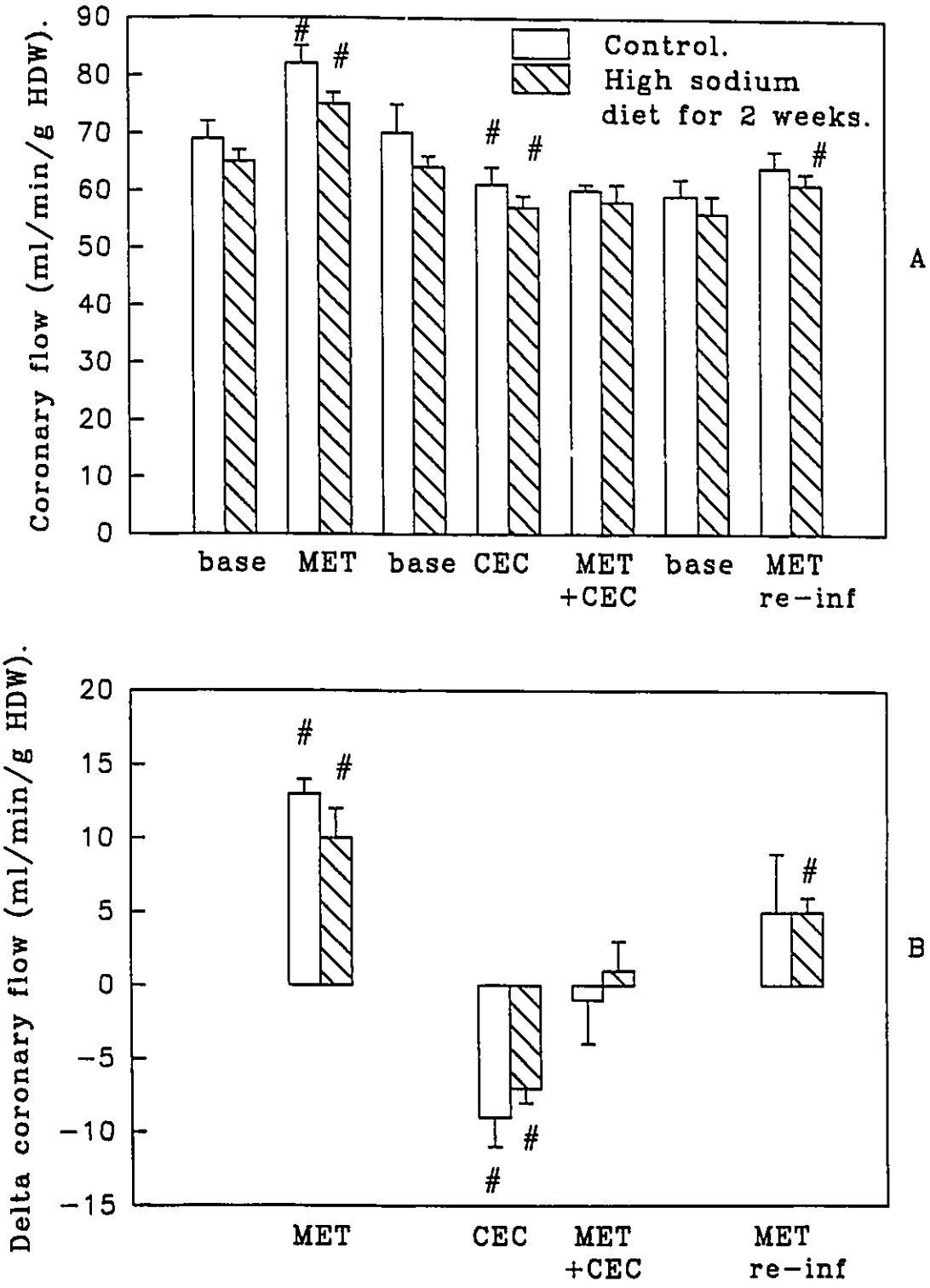

Coronary flow (ml/min/g HDW).
90
80
70
60
50
40
30
20
10
0
Control.
High sodium
diet for 2 weeks.
#
base
MET
base
CEC
MET
+CEC
base
MET
re-inf
A
Delta coronary flow (ml/min/g HDW).
20
15
10
5
0
-5
-10
-15
MET
CEC
MET
+CEC
MET
re-inf
B

Figure 21

Figure 21.

The effect of the α_{1b}-adrenoceptor blocker chloroethylclonidine (CEC, $0.25*10^{-5}$ M) on methoxamine (MET, 10^{-5} M)-induced changes in coronary flow (CF) during simultaneous infusion (MET+CEC) or after 10-15 min of drug free buffer perfusion (MET re-inf) of hearts isolated from WKY rats on a control (C) or high sodium (S) diet for 2 weeks (From 4 to 6 wk of age). *Panel A* represents the original data, *panel B* represents the specific treatment-induced change in (Delta) coronary flow. Bars represent mean ± SEM (n = 5/group). #$p < 0.05$ vs. before treatment within each group.

DISCUSSION

Part 1. Adrenergic Stimulation in Isolated rat Hearts.

In the present study the Langendorff heart has been used to evaluate the sensitivity of hearts isolated from rats on a control or high sodium diet to α_1- or ß-adrenergic stimulation. The advantages of this technique are mainly the technical ease by which variety of parameters can be measured. This approach also minimizes the factors and variations which can occur in the in vivo studies and which could influence cardiac responses.

Limitations of the study may be summarized in the following points:

1- A pressure-volume curve was not constructed for each isolated heart. In preliminary experiments pressure-volume curves were constructed for a group of isolated hearts (see isolation and perfusion of rat hearts), the data obtained in these experiments were considered in subsequent experiments. A similar method has been used by others {Ayob and Tarazi 1984}. However, it is possible that the chosen systolic pressure and subsequently the filling volume of the left ventricle for each individual heart may not be sufficient to allow the heart to operate at the proper portion of the ascending limb of Frank-Starling curve. This may affect the initial sarcomere length and thereby the inotropic responses induced by a drug, and this may differ between diet groups.

2- The different concentrations of the drugs were infused at a constant velocity into the aortic infusion cannula in a non-constant flow system. This is consistent with the method used by others {Ayob and Tarazi 1984, Vleeming et al. 1991}. However, although the real final concentration was calculated for each infused concentration, changes in the

coronary flow induced by the drugs (gross increase in coronary flow in case of isoproterenol) affected the value of the calculated concentration as well as the shape of the curves. It is also worth to mention that during infusion of isoproterenol the responses observed were not stable. The observed values for systolic pressure and heart rate were sometimes quickly changing. This problem was observed only with isoproterenol but not with methoxamine probably because of the gross increases in coronary flow associated with the use of high doses of isoproterenol with the subsequent changes in the concentration. Therefore, it is possible that 1 or 2 responses in the dose-response curves for isoproterenol may not represent the exact response. A better method for drug administration such as the use of a perfusion system which enables switching of perfusion solutions from different reservoirs with concentrations previously determined prevents this problem.

3- The experimental protocol was designated to evaluate chronotropic as well as inotropic responses in response to α or ß stimulation in isolated rat hearts on different diets. The inotropic effects were tested in spontaneously beating hearts {Ayob and Tarazi 1984}. However, because of force-frequency relationship of the mammalian heart the inotropic response may be influenced by a concomitant current chronotropic effect. Methoxamine had no significant effects on heart rate, therefor the inotropic responses were not affected by the concomitant chronotropic responses. However because of the enhanced chronotropic responses to the incremental doses of isoproterenol a possible effect of the chronotropic responses on the inotropic responses elicited by this drug may be expected.

(A) α_1-Adrenergic Stimulation in Isolated rat Hearts.

In the present study, in perfused hearts from young WKY rats the α_1-agonist methoxamine caused dose-dependent increases in left ventricular pressure with no significant effects on heart rate or coronary flow. These effects of methoxamine are mediated through stimulation of α_1-adrenoceptors. The inotropic, chronotropic, or coronary flow responses evoked by incremental doses of methoxamine were not affected by the non-selective ß blocker propranolol (Fig. 4). These results suggest the absence of ß-mediated effects for methoxamine {Rabinowitz, 1975; Vleeming et al. 1991}. Vleeming et al. { 1991} found that in isolated rat hearts the increase in left ventricular pressure and coronary flow evoked by isoproterenol but not of methoxamine were almost completely antagonized by 0.3 μM propranolol. The positive inotropic effect of methoxamine is consistent with prior studies in various cardiac preparation from several species {Rabinwitz et al. 1975; Tsujimoto et al. 1989; Sedaa et al. 1989; Vleeming et al. 1991; Fedida and Bouchard, 1992; Wiener and Thalody, 1993}. Kronenberg et al. {1989} observed a negative inotropic effect in rabbit isolated heart at high concentration of methoxamine (higher than the range of concentrations used in the present study), but not at the low concentrations. This negative inotropic effect may be explained by mechanisms other than α-adrenergic stimulation which might be activated by large concentrations of methoxamine such as reduction in phase O depolarization {Imai, 1961} with subsequent reduction in calcium entry and contractility {Imia et al. 1961}, or ß-adrenergic blocking effect {Imia et al. 1961}. It is also possible that the negative inotropic effect of methoxamine may be due to myocardial ischemia. Intracoronary methoxamine increased

coronary vascular resistance, decreased left ventricular ejection fraction and produced ST segment changes suggestive of myocardial ischemia {Maturi et al. 1986}.

Methoxamine (10^{-5} M) produced a maximum increase of about 130 mmHg in developed pressure of the isolated hearts which was almost equivalent to isoproterenol-induced (160 mmHg) increase in this response. However, the time required by each concentration of MET to produce the steady state effect was about 120 sec which was considerably longer than for ISO (30-40 sec). In isometrically contracting cat papillary muscles, methoxamine 10^{-4} M or isoproterenol 10^{-5} M increased the isometric contraction force (f) by +42±5 and +36±7 respectively {Rabinowitz. 1975}. Vleeming et al.{1991} reported a 60% increase in left ventricular pressure of the isolated rat hearts in response to the infusion of methoxamine (10^{-9} to 10^{-6} M) compared with 100% increase induced by isoproterenol (10^{-12} to 10^{-9} M).

The α_1-agonist methoxamine had a tendency to decrease the heart rate which became significant in some groups at higher concentrations of methoxamine (Fig. 9, *panel B*; Fig. 18, *panel B*). Stimulation of adult canine Purkinje fibres with NE resulted in a decrease in automaticity of 50% of the fibres, while the other 50% manifested an increase in automaticity {del Balzo et al. 1990}. NE has similar affinity to α_{1a}- and α_{1b}-adrenoceptors {Lomasney et al. 1991}. Since the α_{1a}-adrenoceptors are linked to positive chronotropy via PT-insensitive G protein and the α_{1b}-adrenoceptors are linked to negative chronotropy via a PT-sensitive G protein {del Balzo et al. 1990}, the degree of the decrease in heart rate in response to α_1-adrenergic stimulation in adult rat heart depends on the relative proportions of α_{1b}- and α_{1a}-adrenoceptors, as well as the affinity of the α_1-

agonist used to the α_{1a}- and α_{1b}-adrenoceptors. In the present study the decrease in heart rate induced by methoxamine was mostly non significant. The α_1-agonist methoxamine used in this study has about 15-fold more affinity to α_{1a}-adrenoceptors {Lomasenv et al. 1991} linked to positive chronotropy {del Balzo et al. 1990} compared to α_{1b}-adrenoceptors. The fifteen fold higher affinity of methoxamine to α_{1a}-adrenoceptors linked to positive chronotropy my counteract the α_{1b}-mediated negative chronotropy although the latter constitute about 80% of the total α_1-adrenoceptors {Michel et al. 1994}.

Despite the reported vasoconstrictor effect of methoxamine {Kronenberg et al. 1989; Elhawary and Pang, 1994}, we did not find a decrease in coronary flow in any group of isolated hearts used in the present study (Fig. 3, *panel E*; Fig. 4, *panel C*; Fig. 10, *panels A, B, and C*). The reason for the small methoxamine-induced increase in coronary flow is not obvious. However it could be induced by metabolic vasodilatation that overrules methoxamine-induced-vasoconstriction. In the present study, HPLC analysis of a sample of the perfusate collected at the last minute of methoxamine (10^{-5} M) infusion revealed a significant increase in adenosine concentration from approximately 0.10 μmol/l to 0.22 μmol/l (see Fig. 22). This is in agreement with methoxamine-induced release of adenosine reported by others {Sedaa et al. 1989; Wiener and Thalody, 1993}. The strong vasodilator adenosine {Hartmann and Schrader, 1992} may counteract methoxamine-induced vasoconstriction.

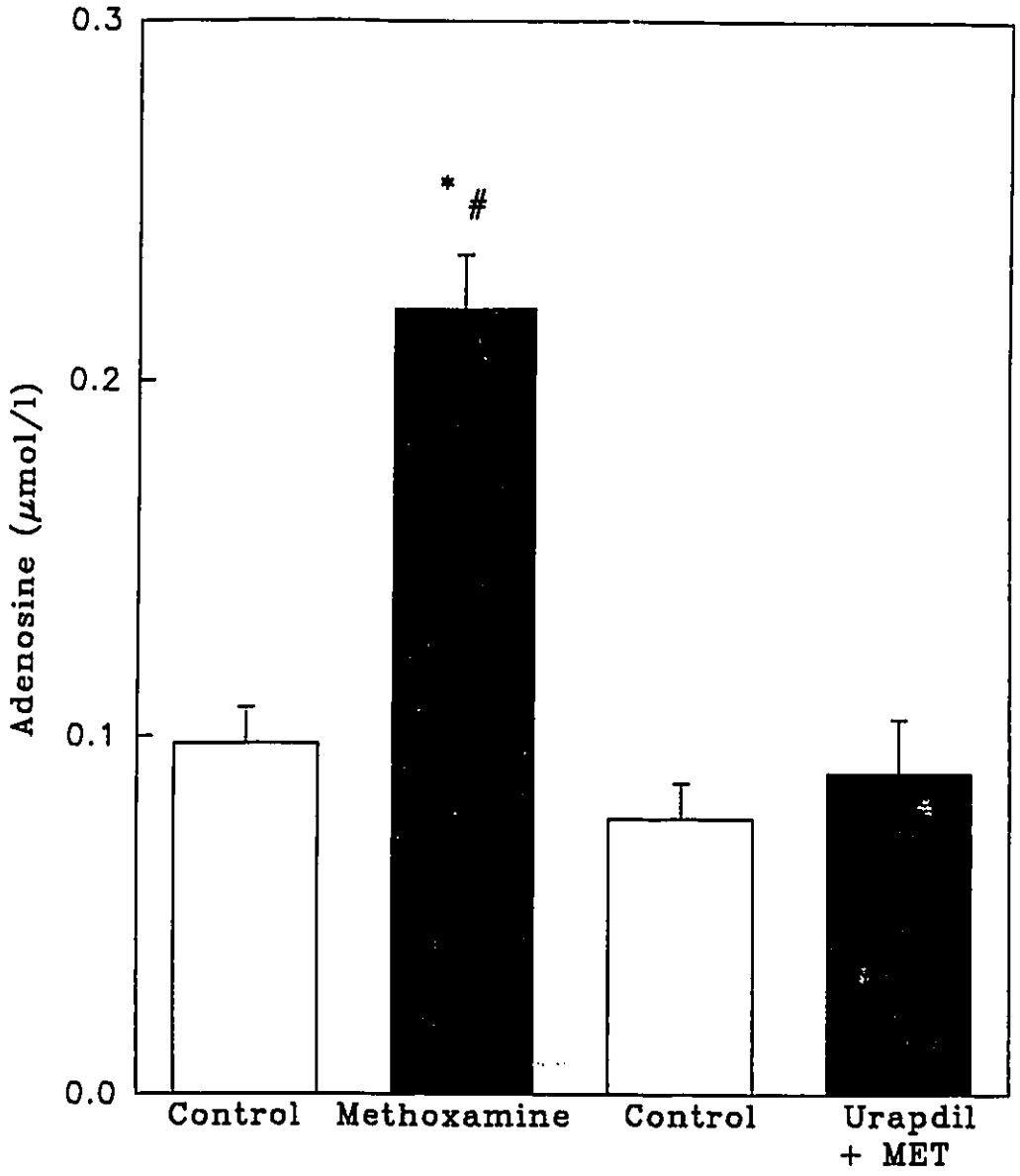


Figure 22.

The effect of methoxamine (10^{-5} M) on the release of adenosine in isolated rat hearts. Bars represent mean ± SEM (n = 5/group). #$p < 0.05$ vs. before treatment within the group, *$p < 0.05$ vs. Ura+MET treatment.

The α_{1a}-adrenoceptor blocker urapidil abolished the methoxamine-induced-increase in developed pressure of control hearts by about 85% (Fig. 15, *panel B*) suggesting very high contribution of α_{1a}-adrenoceptors to methoxamine-induced inotropic effect. On the other hand, CEC a selective α_{1b} blocker, inhibited 60% of methoxamine-induced-increase in developed pressure indicating somewhat less contribution from α_{1b}-adrenoceptors to methoxamine-induced inotropic effect. The concentrations of α_{1a}- or α_{1b}-adrenoceptor blockers used for inactivation of these subtypes in rat ventricular strips are in the micromolar range {Michel et al. 1994; Han et al. 1987} which are consistent with the concentrations used in the present study that have been selected in preliminary experiments. Hanft and Gross {1989} reported that urapidil and some of its derivatives which are substituted at the 5-position of the uracil moiety can discriminate between α_{1a}- and α_{1b}-adrenoceptors but the order of selectivity to α_{1a}-adrenoceptors was: 5-methyl-urapidil (has approximately 70 fold selectivity for α_{1a}-subtype) > 5-formyl-urapidil > 5-acetyl-urapidil > urapidil. In ventricular myocytes isolated from adult rat heart, high concentrations of 5-methyl-urapidil (0.1 μM) exhibited substantial affinity also to α_{1b}-adrenoceptor subtype {Wang et al. 1991}.

CEC irreversibly blocks the α_{1b}-adrenoceptors {Han et al. 1987; del Balzo et al. 1990; Lomasney et al. 1991; Vargas et al. 1993}. Consistent with these studies we found that the percent reduction in maximal inotropic effect for the high dose of methoxamine determined in the presence of CEC (60%) and after washout of the antagonist (56%) were not significantly different, suggesting an irreversible blockade of the receptors by CEC. Studies using CEC have shown that the α_{1a}-adrenoceptors are less sensitive to inactivation

by the micromolar concentrations of CEC. 15% of α_{1r}-adrenoceptors may be inactivated by these concentrations of CEC compared to the 70% inactivation of the α_{1b}-adrenoceptors {Lomasney et al. 1991}. Taking together, our results suggest that methoxamine interacts with both α_{1a}- and α_{1b}-adrenoceptors. This is in agreement with {Tsujimoto et al. 1989; Elhawary et al. 1994}. In the present investigation, the urapidil study may suggest that a high proportion of methoxamine-induced increase in developed pressure of the isolated hearts may be mediated via stimulation of α_{1a}-adrenoceptors and only 15% of this response is elicited via stimulation of the α_{1b}-adrenoceptors in response to the high dose of MET (Fig. 15, *panel B*). However, in rat heart the α_{1b}-adrenoceptors represent about 80% of total α_1-adrenoceptors {Michel et al. 1994}. In the present study the more likely explanations for the 85% inhibition of MET-induced increase in developed pressure by urapidil may be the high affinity of methoxamine to rat α_{1a}-adrenoceptors (K_i = 110 000 nM) compared to α_{1b}-adrenoceptors (K_i = 1610 000 nM) and / or a possible blockade of some α_{1b}-adrenoceptors by Ura resulting in diminished α_{1b}-mediated effects of MET following urapidil treatment. The CEC blockade study supports the latter explanation. This study suggests that the α_{1a}-mediated inotropic effect represents about 40% of methoxamine-induced increase in developed pressure (Fig. 17 ,*panel B*) which is more consistent with the 20% ratio of this (α_{1a}) subtype in rat heart {Michel et al. 1994} and the high affinity of methoxamine to α_{1a}-adrenoceptors {Lomaseny et al. 1991}. Overall, these results suggest that: 1) methoxamine-induced positive inotropic effects in rat heart may be mediated by α_{1a}- and α_{1b}-adrenoceptors. 2) although the ratio of α_{1a}- /α_{1b}-adrenoceptors in adult rat heart is 20:80, the higher affinity of methoxamine to α_{1a}-

adrenoceptors may result in a higher component of α_{1a}-mediated inotropic effect.

3) urapidil may be not a very selective α_{1a}-adrenoceptor antagonist in rat myocardium.

Because the α_{1a}-adrenoceptors are linked to positive chronotropy {del Balzo et al. 1990}, we expected that combined with the α_{1a}-antagonist urapidil, methoxamine would induce a greater decrease in heart rate compared with that induced by methoxamine without urapidil. WB4101, another α_{1a}-blocker antagonized the NE-induced increase in automaticity in neonate and adult canine Purkinje fibres {del Balzo et al. 1990}. In the present study, urapidil treatment did not induce a greater decrease in heart rate of hearts from control rats (Fig. 18, *panel B*) suggesting a non-significant component of α_{1a}-adrenoceptor mediated chronotropic effect for increasing the heart rate. Therefore, it is possible that the blockade of α_{1a}-adrenoceptors which represent 20% in adult rat heart had no effect on methoxamine-induced effects on heart rate. A possible blockade of some α_{1b}-mediated negative chronotropy by urapidil may also be considered. Despite the coupling of α_{1b}-adrenoceptor subtype to negative chronotropy {Han et al. 1989, del Balzo et al. 1990}, we did not observe a significant increase in heart rate of control hearts after the blockade of this subtype by CEC. CEC pretreatment attenuated methoxamine-induced decrease in heart rate (Fig. 19, *panel B*), but it did not reach a significant increase in heart rate probably due to the partial α_{1b}-adrenergic receptor agonistic activity of CEC {del Balzo et al. 1990} as this alkylating agent without the simultaneous infusion of methoxamine produced a significant decrease of about 38 bpm in heart rate of the isolated hearts (Fig. 19, *panel B*).

(B) ß-Adrenergic Stimulation in Isolated rat Hearts.

Isoproterenol evoked dose-dependent increases in developed pressure, heart rate and coronary flow of the isolated rat hearts {Brodde, 1988 ; Chevalier et al. 1989; Vleeming et al. 1991; Borea et al. 1992}. The positive inotropic effect of isoproterenol is mediated at least in humans and sheep through stimulation of both $ß_1$ and $ß_2$-adrenoceptors {Brodde, 1988; Borea et al. 1992}. Rats are quite similar to human beings in the number, affinity and heterogeneity of the left ventricle ß-adrenergic receptors {Tracisio et al. 1984}.

Infusion of isoproterenol into isolated hearts dose-dependently decreased diastolic pressure (Fig.3, *panel C*). This ß-adrenergic cardiac relaxant effect is due to phosphorylation of phospholamban by cAMP-dependent protein kinase A (PKA) which significantly increases the Ca^{2+} uptake rate by sarcoplasmic reticulum {Arai et al. 1994}. Isoproterenol also dose-dependently increased heart rate {Brodde, 1991; Hartmann and Schrader, 1993} and coronary flow of the isolated rat hearts {Chevalier et al. 1989; Vleeming et al. 1991}. Tachycardia induced by isoproterenol may be mediated by both $ß_1$- and $ß_2$-adrenoceptor activation {Brodde, 1991}.

Part 2. Dietary Sodium-Induced Cardiac Hypertrophy.

Consistent with previous studies {Fields et al. 1991; Yuan and Leenen, 1991; Mervaala et al. 1992} results of the present study show that high sodium diet can exert a clear trophic effect on rat myocardium. Hypertrophy is apparent within 1 week of high sodium treatment. Heart dry weight and heart dry weight/body weight ratio were

significantly increased in high sodium treated rats (Fig. 7). The 9% increase in heart dry weight/body weight ratio in the young WKY rats after 1 week of high salt intake was mainly due to a significant increase in heart dry weight, as the body weight of these salt treated rats did not show a significant deviation from that of controls. Wistar rats kept on 1% saline treatment for 10 days did not show cardiac hypertrophy {Fields et al. 1991}. The difference between the results of that and the present study may be due to the strain of rats used. Wistar rats are less sensitive to high sodium diet compared to WKY rats used in the present study {Yuan and Leenen, 1991}, although, the use of salt-enriched diet or saline may also be important. To the best of my knowledge, in normotensive rats no other studies estimated high sodium induced cardiac hypertrophy after 1 week or closely related time of high sodium intake. Since the extent of the trophic effect of high sodium diet is strain-, time- and amount of sodium-dependent {Yuan and Leenen, 1991}, the differences between the degree of hypertrophy observed in the present study and what has been reported by the previous studies {Kihara et al. 1985; Meggs et al. 1988; Gallo et al. 1990; Fields et al. 1991; De Simone et al. 1993} can be explained. The study of Yuan and Leenen {1991} demonstrated 14% and 24% LVH in 4 weeks old WKY rats kept on high sodium (8% NaCl) for 2 and 4 weeks respectively. This is in consistence with the 12% and 15% hypertrophy observed in the present study (Fig. 7, *panel C*).

Part 3. Effects of High Sodium Diet on the Responses of Isolated rat Hearts to α_1-, Subtypes of α_1- or ß-Adrenergic Stimulation.

(A) High Sodium Diet and α_1-, α_{1a}-, and α_{1b}-Adrenergic Stimulation.

The results of the present study show that there are some differences in the coronary flow response of the isolated hearts from the two dietary groups after α_1-adrenergic stimulation (Fig. 10, *panel A*). However, no significant differences between the inotropic responses (P-dev) of hearts isolated from young WKY rats kept on high sodium intake for 1, 2, or 6 weeks and their respective controls in regard to α_1-adrenergic stimulation was observed. This is in contrast to our expectation, but may be explained by two possible factors: 1) A possible effect of dietary sodium or left ventricular hypertrophy on a specific subtype of α_1-adrenoceptor. Methoxamine has affinity to both (α_{1a} and α_{1b}) subtypes of α_1-adrenergic receptors {Tsujimoto et al. 1989; Lomaseny et al. 1991}. Therefore, it is possible that simultaneous stimulation of α_{1a}- and α_{1b}-adrenoceptors by methoxamine can not discriminate between the contribution of each subtype to methoxamine-induced effects on developed pressure, heart rate ,or coronary flow. 2) The α_{1b}-adrenoceptor subtype may have a tonic inhibitory effect on α_{1a}-adrenoceptors. Treatment of rat ventricular strips with CEC increased the relative contribution of α_{1a}-adrenoceptors from 26 to 89% {Michel et al. 1994}. Therefore, evaluation of responses to the subtypes of α_1-adrenoceptors may be more appropriate than simultaneous stimulation of both subtypes. To date no selective α_{1a}- or α_{1b}-adrenoceptor agonists neither absolutely subtype-selective antagonists have been identified. Epinephrine and phenylephrine have similar affinity to both subtypes of α_1-adrenoceptors.

Methoxamine has 15 fold higher affinity to α_{1a}-adrenoceptor subtype {Lomasney et al. 1990}. Recent studies have reported that α_{1a}-adrenoceptor mediated hypertrophic effects are linked to the stimulation of α_{1a} -adrenoceptors {Simpson et al. 1990; Knowlton et al. 1993}. The ratio of α_{1a} / α_{1b} in adult rat hearts is 20:80 {Michel et al. 1994}. Therefore, because of the high affinity of methoxamine to α_{1a}-adrenoceptors this α_1-agonist has been used. Moreover, unlike epinephrine and phenylephrine, methoxamine has no ß stimulant effect {Rabinowitz, 1975; Vleeming et al. 1991}.

In micromolar concentrations the irreversible α_{1b}-adrenoceptor subtype blocker CEC can inactivate 15% of α_{1a} and only about 70% of α_{1b}-adrenoceptors {Lomasney et al. 1990}. Urapidil and some of its derivatives have been used to inactivate α_{1a}-adrenoceptors {Hanft et al. 1989}. CEC has been used in almost all studies to evaluate the role of the α_{1b}-adrenoceptors {Han et al. 1987; del Balzo et al. 1990; Lomasney et al. 1991; Vargas et al. 1993}. It is apparent from these studies that although none of the blockers are absolutely subtype-selective, the blockade of one subtype may represent a useful tool to study the functional role of the other subtype. Accordingly, if the α_{1a}-adrenoceptors are upregulated in hearts from salt treated rats, then a greater increase in developed pressure and a possible positive chronotropic effect following CEC (α_{1b}-blockade) would be expected in hearts from salt treated rats compared with control hearts. On the other hand, if the α_{1b}-adrenoceptors are the upregulated subtype, a greater increase in developed pressure and a possible negative chronotropic effect in salt treated rats would be expected following α_{1a}-adrenoceptor blockade (Ura treatment) compared with control hearts. In the present study, the α_1-adrenoceptor subtype stimulation study revealed

identical increases in developed pressure of hearts isolated from rats on a control or high sodium diet to α_1-stimulation induced by MET in the presence of α_{1a} or α_{1b} blocker. In both dietary groups a 15% and a 40% response for high dose of methoxamine was observed when α_{1a}- or α_{1b}-adrenoceptors were blocked by urapidil or CEC respectively (Figs. 15 and 17). These results suggest that there is no change in α_{1a}- or α_{1b}-mediated inotropic responses to methoxamine following high sodium intake.

Since α_{1a}-adrenoceptors stimulation is linked to positive chronotropy through the IP_3 pathway and stimulation of α_{1b}-adrenoceptors produces negative chronotropy {del Balzo et al. 1990} via the stimulation of Na^+ / K^+ pump {Terzic et al. 1993}, differences associated with changes in subtype density or sensitivity may be more clear when evaluating chronotropic responses. Methoxamine has a tendency to decrease heart rate (Fig. 3, *panel D*; Fig. 9, *panel B*; Fig. 14, *panel B*; and Fig. 16, *panel B*) probably as a net result of stimulation of both α_{1a}- and α_{1b}-adrenoceptors {Lomasney et al. 1991, Tsujimoto et al. 1991}. In control hearts, combined with the α_{1a}-antagonist urapidil, MET at the high dose did not produce a greater decrease in heart rate compared with that induced by the same dose of MET without Ura. However, in hearts from salt treated rats the high dose of MET combined with Ura produced a significant decrease in heart rate by -44 bpm compared with the decrease induced by MET without Ura (-14 bpm) (Fig. 18, *panel B*). Moreover, combined with Ura, MET at low as well as medium dose also produced a significant decrease of -30 bpm in heart rate of hearts isolated from salt treated rats. This treatment had no significant effect (the decrease in HR was -5±4 bpm) on the heart rate of hearts from control rats (Fig. 14, *panel B*). In the presence of CEC,

it is apparent, that although there was no significant differences between hearts isolated from salt treated rats and their respective controls in heart rate responses to the different doses of methoxamine combined with CEC, the heart rate decreasing tendency of methoxamine when combined with CEC was more attenuated in hearts from salt treated rats compared with controls (-5±4 and -14±5 bpm, salt vs. control) at any concentration of MET combined with CEC (Fig. 16, *panel B*), as well as to MET (high dose)-induced decrease in heart rate in the absence of CEC (-19±8 and -5±4 bpm, MET without CEC vs. MET combined with CEC) (Fig. 19, *panel B*). These results suggests that there may be a significant (from Ura study) or higher (CEC study) component of α_{1a}-adrenoceptor mediated chronotropic effect in hearts from salt treated rats. It is also possible that the significant methoxamine-induced-decrease in heart rate of hearts from salt treated rats following urapidil treatment may be due to an upregulation of α_{1b}-adrenoceptors in this group. However, if the α_{1b}-adrenoceptors are upregulated then a greater, significant decrease in heart rate in response to α_1-stimulation (without any blocker) would also be expected in hearts from salt treated rats compared with control hearts as this (α_{1b}) subtype (linked to negative chronotropy) represents 80% of the total α_1-adrenoceptors in rat heart. In the present study no significant decrease in heart rate was observed in either dietary group following methoxamine infusion (Fig. 19, *panel B*). Because MET has high affinity to α_{1a}-adrenoceptors, the α_{1b}-mediated effects of MET may not be consistent with the proportion of this subtype in the myocardium. Therefore, it is possible that both subtypes may be upregulated.

(B) **High Sodium Diet and ß-Adrenergic Stimulation.**

Effects of ß-adrenergic stimulation in hearts isolated from rats on high sodium diet were only to a minor extent different from that in control hearts. The dose-response curve of heart rate for hearts isolated from rats after 1 week of high sodium intake showed that, at a given concentration of isoproterenol the increase in heart rate of hearts from salt treated rats was slightly more than that in the control hearts and it reached statistical significance at the EC_{50} (EC_{50}: Control = $4.3*10^{-8} \pm 8.8*10^{-9}$ M, Salt = $1.9*10^{-8} \pm 1.5*10^{-9}$ M) with no significant difference between the maximum responses of the two dietary groups (Fig. 12, *panel A*). Also, at the high doses of isoproterenol hearts isolated from rats on high sodium diet for 6 weeks showed smaller increases in coronary flow than control hearts (Fig. 13, *panel C*). However, neither the maximum responses nor the EC_{50} (Control = $1.2*10^{-8} \pm 1.7*10^{-9}$ M, Salt = $1.1*10^{-8} \pm 1*10^{-9}$ M) were significantly different between the groups.

Increased dietary sodium intake increased total number of ß-adrenergic receptors in normotensive rats {Gallo et al. 1990}. In young normotensive subjects, high sodium intake increased the density of white cell ß-adrenergic receptors {Fraser et al. 1981; Naslund et al. 1990}. In these subjects, high sodium intake also increased cardiac sensitivity to isoproterenol as assessed by the dose of isoproterenol required to increase the heart rate by 25 bpm {Fraser et al. 1981}. However, in hypertensive subjects and in older subjects low-sodium diet and not high sodium diet corrects the defects in both vascular and white cell ß-adrenergic responsiveness {Feldman, 1990; Naslund et al. 1990; Feldman, 1992}. These studies suggest a possible change in atrial ß-adrenergic receptor

sensitivity in normotensives after high sodium intake. Consistent with these findings, in the present study the slightly increased heart rate response exhibited by hearts isolated from rats after 1 week of high salt intake in response to ß-stimulation reflects the responses to atrial ß-adrenoceptors. Responses to ventricular ß-receptors stimulation were not affected.

Part 4. Relevance of Findings to Sodium Induced Left Ventricular Hypertrophy

The data obtained in the present study suggests that there may be some changes in cardiac α_{1a}- or α_{1b}- and ß-adrenoceptors after high sodium intake. However, it must be realized that the differences between control and high sodium treated rats were in chronotropic responses which mostly reflect the effects on SA node rather than the left ventricle, involved in sodium-induced cardiac hypertrophy. The inotropic responses mediated by the stimulation of ventricular α_{1a}-, α_{1b}-, or ß-adrenoceptors did not change in hearts isolated from rats on high sodium intake although these rats showed increased heart dry weight/body weight ratios within one week of high sodium intake. The unchanged contractile functional responses to ventricular α_1- or ß-adrenoceptor stimulation after high sodium diet suggest that changes in ventricular α_1- or ß-adrenoceptors at the level of receptor events unlikely play a role in dietary sodium induced left ventricular hypertrophy. High sodium diet produces concentric left ventricular hypertrophy {Fields et al. 1991; Yuan and Leenen, 1991}, while isoproterenol-induced cardiac hypertrophy is characterized by a greater hypertrophy of the right ventricle and a dilated left ventricle {Allard et al.

1990}. These data makes a possible role for ß-adrenoceptors in dietary sodium induced cardiac hypertrophy less likely.

For α_1-adrenoceptors, although hypertrophy and contractile responses may be mediated through stimulation of same receptors, the unchanged contractile responses by high sodium diet do not necessarily exclude the role of sympathetic nervous system in dietary sodium-induced left ventricular hypertrophy. It is still possible that the hypertrophic pathway may dissociate from contraction pathway at certain stages of the intracellular signalling pathway (see Fig. 1). Contractility is triggered by Ca^{2+} induced Ca^{2+} release from sarcoplasmic reticulum. An enhanced protein kinase activity may account for hypertrophic processes in high sodium treated rats. This may not be associated with a concomitant higher levels of intracellular Ca^{2+} sufficient to elicit a higher contractile response in sodium treated rats in response to α_1- or ß-adrenoceptors stimulation. The results also suggest that evaluation of functional responses alone in isolated hearts may not be sufficient enough to assess a possible role of the receptors involved in cardiac hypertrophy. The role of α_1-adrenoceptors in dietary sodium-induced left ventricular hypertrophy may better be investigated by measuring rates of protein synthesis in response to α_1-stimulation after high sodium diet.

CONCLUSIONS

1- Cardiac Hypertrophy.

The results of the present study indicate that high sodium diet induces cardiac hypertrophy in young WKY rats within 1 week of high sodium intake.

2- Adrenergic Stimulation in Isolated Rat Hearts.

α_1-adrenergic stimulation in isolated rat hearts produces positive inotropic effect via stimulation of both α_{1a}- and α_{1b}-adrenoceptors. It also caused small decreases in heart rate and produced small increases in coronary flow of the isolated rat hearts. ß-adrenergic stimulation of the isolated hearts produced positive inotropic and chronotropic effects and significantly increased coronary flow of the isolated rat hearts. α_1-adrenergic stimulation in isolated rat hearts is as efficient as ß-adrenergic stimulation for inotropic effects. The increase in developed pressure of the isolated rat hearts in response to α_1-adrenergic stimulation is mainly due to an increase in systolic pressure. In ß-adrenergic stimulation the increase in developed pressure is the result of an increase in systolic pressure and a decrease in diastolic pressure.

3- α_1-Adrenergic Receptor Subtype Blockers.

Urapidil itself had no significant effects on the isolated rat hearts. This α_{1a}-subtype blocker is not very selective to α_{1a}-adrenoceptors. CEC irreversibly blocked the α_{1b}-adrenoceptors but had significant effects on developed pressure, heart rate, and coronary flow of the isolated rat hearts.

4- High Sodium Diet and Adrenergic Stimulation in Isolated Rat Hearts.

Preliminary results from this study suggest that there are no changes in contractile functional responses to ventricular α_{1a}-, α_{1b}-, or ß-adrenoceptors stimulation in the isolated hypertrophied rat hearts after high sodium intake. A possible effect of high sodium diet on atrial α_{1a}-, α_{1b}-, or ß-adrenoceptors is suggestive.

5- Dietary Sodium Induced Left Ventricular Hypertrophy and Adrenergic Stimulation.

Results from this study suggest a less likely role for changes in adrenergic receptors number or affinities in dietary sodium induced left ventricular hypertrophy. One cannot exclude a possible dissociation between intracellular signals associated with dietary sodium induced left ventricular hypertrophy from those associated with contractility.

FUTURE EXPERIMENTS

The following experiments may be more useful in identifying a possible role for α_1-adrenoceptors in dietary sodium-induced cardiac hypertrophy.

1- Measurement of protein synthesis rate in response to α_1-adrenergic stimulation in isolated rat hearts after high sodium intake for different times.

2- The use of a selective α_{1a}-adrenoceptor blocker prior to initiating a high sodium diet in the young WKY rats to identify a possible prevention of cardiac hypertrophy compared with rats on a high sodium diet as well as controls.

3- Radioligand biding studies in rat ventricular membrane obtained from rats kept on high sodium diet for different times to identify a possible effect of sodium on the density or number of the α_1-adrenoceptor subtypes.

4- The use of neonate rats rather than the young rats in exploring high sodium induced cardiac hypertrophy as these rats have a higher number of α_{1a}-adrenoceptor subtype.

REFERENCES

1- Allard, M. F., De Venny, M. F., Doss, L. K., Grizzle, W. E., and Bishop, S. P. 1990; Alteration in Dietary Sodium Affect Isoproterenol-induced cardiac Hypertrophy. J. Mol. Cell. Cardiol. 22: 1135-1145.

2- Arai, M., Matsui, H., and Samy, M. P. 1994; Sarcoplasmic Reticulum Gene Expression in Cardiac Hypertrophy and Heart Failure. Circ. Res. 74 (4): 555-564.

3- Ayobe, M. H., and Tarazi, R. C. 1984; Reversal of Changes in Myocardial ß-Receptors and Inotropic Responsiveness with Regression of Cardiac Hypertrophy in Renal Hypertensive Rats (RHR). Circ. Res. 54: 125-134.

4- Baker, K. M., Chernin, M. I., Wixson, S. K., and Aceto, J. F. 1990; Renin-Angiotensin System Involvement in Pressure-Overload Cardiac Hypertrophy in Rats. Am. J. Physiol. 259 (Heart Circ. Physiol. 28): H328-H332.

5- Berridge, M. J. and Irvine, R. F. 1989; Inositol Phosphates and Cell Signalling. Nature, 341: 197-205.

6- Borea, P. A., Amerini, S.I., Cerbait, M. E., Ledda, F., Mantelli, L., K. Varani, and Mugelli, A. 1992; $ß_1$- and $ß_2$-adrenoceptors in Sheep Cardiac Ventricular Muscle. J. Mol. Cardiol. 24: 753-764.

7- Brodde, O. 1988; The Functional Importance of $Beta_1$ and $Beta_2$ Adrenoceptors in the Human Heart. Am. J. Cardiol. 62: 24C-29C.

8- Brodde, O. 1991; $ß_1$- and $ß_2$-Adrenoceptors in the Human Heart: Properties, Function, and Alterations in Chronic Heart Failure. Pharmacol. Rev. 43 (2): 203-239.

9- Brosnihan, K. B., Szilagyi, J. E., and Ferrario, C. M. 1981; Effect of Chronic Sodium Depletion on Cerebrospinal Fluid and Plasma Catecholamines. Hypertens. 3: 233-239.

10- Brown, J.E., McLeod, A. A., and Shand, D. G., and Shand D. G. 1986; In Support of Cardiac Chronotropic Beta$_2$ Adrenoceptors. Am. J. Cardiol. 57: 11F-16F.

11- Brown, L., Serina, C., Newling, R., and Fletcher, P. 1992; Cardiac Responses After Norepinephrine-Induced Ventricular Hypertrophy in Rats. J. Cardiovasc. Pharmacol. 20: 316-323.

12- Bustamante, J.O., Ruknudin, A. and Sachs, F. 1991; Stretch-Activated Channels in Heart Cells: Relevance to Cardiac Hypertrophy. J. Cardiovasc. Pharmacol. 17 (suppl. 2): S110-S113.

13- Buttrick, P. Kaplan, M. Camargo, M. J. F., deSimone, G., Laragh, J. H. Wallerson, D. and Devereux, R. B. 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.

14- Calhoun, D. A., Zhu, S., Wyss, J. M., Oparil, S. 1994; Diurnal Blood Pressure Variation and Dietary Salt in Spontaneously Hypertensive Rats. Hypertens. 24: 1-7.

15- Chevalier, B., Mansier, F., El Amarani, C. F., and Swynghedauw, B. 1989; ß-Adrenergic System Is Modified in Compensatory Pressure Cardiac Overload in Rats: Physiological and Biochemical Evidence. J. Cardiovas. Pharmacol. 13: 412-

420.

16- Chiba, M., Shida, M., Miyazaki, Y., Koga, Y., and Toshima, H. 1989; Role of Adrenergic Receptor Systems in Canine Left Ventricular Hypertrophy. J. Mol. Cardiol. 21 (suppl. V): 39-47.

17- Choi, S.C., and Smith, P.J. 1982; Simple Test to Compare Two Dependent Regression Lines. Technometrics, 24: 123-126.

18- Clark, W. A., Rudnick, S. J., LaPres, J. J., Andersen, L. C., LaPointe, M. C. 1993; Regulation of Hypertrophy and Atrophy in Cultured Adult Heart Cells. Circ. Res. 73: 1163-1176.

19- Cooper IV, G., Kent, R. L., and Mann, D. L. 1989; Load Induction of Cardiac Hypertrophy. J. Mol. Cell. Cardiol. 21 (Suppl. V): 11-30.

20- De la Bastie, D., Levitsky, D., Rappaport L., Mercadier, J., Marotte, F., Wisnesky, C., Brovkovich, V., Schwartz, K., and Lompre, A. 1990; Function of the Sarcoplasmic Reticulum and Expression of Its Ca^{2+}-ATPase Gene in Pressure Overload-Induced Cardiac Hypertrophy in Rats. Circ. Res. 66: 554-564.

21- De Simone, G., Devereux, R. B., Camargo, M. J. F., Wallerson, D. C., and Laragh, J. H. 1993; Influence of Sodium Intake on In Vivo Left Ventricular Anatomy in Experimental Renovascular Hypertension. Am. J. Physiol. 264 (Heart Circ. Physiol. 33): H2103-H2110.

22- del Balzo, U., Rosen, M. R., Malfatto G., Kaplan, L. M., and Steinberg, S. F. 1990; Specific α_1-Adrenergic Receptor Subtypes Modulate Catecholamine-Induced Increases and Decreases in Ventricular Automaticity. Circ. Res. 67: 1535-1551.

23- Dietz R., Schomig, A., Rascher, W., Strasser, R., Luth, J. B., Ganten, U., and Kubler, W. 1982; Contribution of the Sympathetic Nervous System to the Hypertensive Effect of a High Sodium Diet in Stroke-Prone Spontaneously Hypertensive rats. Hypertens. 4: 773-781.

24- Dostal, D. E., and Baker, K. M. 1992; Angiotensin II Stimulation of Left Ventricular Hypertrophy in Adult Rat Heart, Mediation by the AT_1 Receptor. Am. J. Hypertens. 5: 276-280.

25- Drugge, E. D., Rosen, M. R., and Robinson, R. B. 1985; Neuronal Regulation of the Development of the α-Adrenergic Chronotropic Response in the Rat Heart. Circ. Res. 57: 415-423.

26- Du Cailar, G., Ribstein, J., Grolleau, R., and Mimran, A. 1990; Influence of Sodium Intake on Left Ventricular Structure in Untreated Essential Hypertensives. J. Hypertens. 7 (Suppl. 6): S258-S259.

27- Du Cailar, G., Ribstein, J., Daures, J., and Mimran, A. 1992; Sodium and Left Ventricular Mass in Untreated Hypertensive and Normotensive Subjects. Am. J. Physiol. 263 (Heart Circ. Physiol. 32): H177-H181.

28- Dzau, V. J., Burt, D. W. and Pratt, R. E. 1988; Molecular Biology of the Renin-Angiotensin System. Am. J. Physiol. 225 (Renal Fluid Electrolyte Physiol. 24): F563-F573.

29- Egan, B. M., Weder, A. B., Petrin, J., and Hoffman, R. G. 1991; Neurohumoral and Metabolic Effects of Short Term Dietary NaCl Restriction in Men. Relationship to Salt - Sensitivity Status. Am. J. Hypertens. 4: 416-421.

30- Eid, H., and De Champlain, J. 1988; Increased Inositol Monophosphate Production in Cardiovascular Tissue of DOCA-Salt Hypertensive Rats. Hypertens. 12: 122-128.

31- Elhawary, A. M., and Pang, C. C. Y. 1994; α_{1b}-Adrenoceptors Mediate Renal Tubular Sodium and Water Reabsorption in the Rat. Br. J. Pharmacol. 111: 819-824.

32- Ely, D. E., Thoren, P., Wiegand, J., and Folkow, B. 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. Scan. 129: 81-91.

33- Ely, D., Folkow, B., and Paradise, N. F. 1989; Risks of Salt Depletion and Salt Excess. *in* Salt and Hypertension: Dietary Minerals, Volume, Homeostasis; cardiovascular Regulation. *edited by* R. Rettig, D. Ganten, and F. C. Luft. Springer-Verlag, Berlin. pp. 67-82.

34- Ely, D. L., Folkow, B., and Paradise, N. F. 1990; Risks Associated with Dietary Sodium Reduction in the Spontaneous Hypertensive Rat Model of Hypertension. Am. J. Hypertens. 3: 650-660.

35- Endoh, M., Takanashi, M., and Norota, I. 1992; Role of α_{1A} Adrenoceptor Subtype in Production of the Positive Inotropic Effect Mediated Via Myocardial α_1 Adrenoceptors in the Rabbit Papillary Muscle: Influence of Selective α_1 Subtype Antagonists WB 4101 and 5-Methylurapidil. Naur. n-Schmiedeberg's Arch Pharmacol. 345: 578-585.

36- Engelmann, G. L., Boehm, K. D., Haskell, J. F., Khairallah, P. A., and Ilan, J. 1989; Insulin-Like Growth Factors and Neonatal Cardiomyocyte Development: Ventricular Gene Expression and Membrane Receptor Variations in Normotensive and Hypertensive Rats. Mol. Cel. Endocrinol. 63: 1-14.

37- Fedida, D., and Bouchard, R. A. 1992; Mechanisms for the Positive Inotropic Effect of α_1-Adrenoceptor Stimulation in Rat Cardiac Myocytes. Circ. Res. 71: 673-688.

38- Feldman, R. D. 1990; Defective Venous Beta-Adrenergic Response in Borderline Hypertensive Subject is Corrected by a Low Sodium Diet. J. Clin. Invest. 85: 647-652.

39- Feldman, R. D. 1992; A Low-Sodium Diet Corrects the Defect in ß-Adrenergic Response in Older Subjects. Circ. 85: 612-618.

40- Fields, N. G., Yuan, B., and Leenen, F. H. H. 1991; Sodium-Induced Cardiac Hypertrophy. Cardiac Sympathetic Activity Versus Volume Load. Circ. Res. 68: 745-755.

41- Folkow, B., and Ely, D. L. 1987; Dietary Sodium Effects on Cardiovascular and Sympathetic Neuroeffector Functions as Studied in Various Rat Models. J. Hypertens. 5: 383-395.

42- Fraser, J., Nadeau, J., Robertson, D., and Wood, A. J. J. 1981; Regulation of Human Leukocyte Beta Receptors by Endogenous Catecholamines. J. Clin. Invest. 67: 1777-1784.

43- Fuller, S. J., Gaitanaki, C. J., and Sugden, P. H. 1990; Effects of Catecholamines on Protein Synthesis in Cardiac Myocytes and Perfused Hearts Isolated from Adult Rats. Biochem. J. 266: 727-736.

44- Fuller, S. J., Gaitanaki, C. J., Hatchett, R. J., and Sudgen, P. H. 1991; Acute α_1-Adrenergic Stimulation of Cardiac Protein Synthesis May Involve Increased Intracellular pH and Protein Kinase Activity. Biochem. J. 273: 347-353.

45- Gaitanaki, C. J., Sugden, P. H., and Fuller, S. J. 1990; Stimulation of Protein Synthesis by Raised Extracellular pH in Cardiac Myocytes and Perfused Hearts. FEBS Letters, 260 (1): 42-44.

46- Gallo, A., Taquini, C. M., Fontan, M., Campissi, R., Kuraja, I., Llambi, H. G., Taquini, A. 1990; Sodium Intake Modulates the Development of Cardiac Hypertrophy in Two-Kidney, One Clip Rats. Hypertens. 15(Suppl. I): 157-160.

47- Gans, J. H., and Cater, M. R. 1970; Norepinephrine Induced Cardiac Hypertrophy in Dogs. Life Sciences, 9(1): 731-740.

48- Gerova M., Holecyova, A., Kristek, F., Fizel', A., and Fizelova, A. 1993; Remodelling and Functional Alterations of the Rabbit Coronary Artery in Volume Overloaded Heart. Cardiovasc. Res. 27 (11): 2005-2010.

49- Gustafson, T. A., Markham, B. E., and Morkin, E. 1986; Effects of Thyroid Hormone on α-Actin and Myosin Heavy Chain Gene Expression in Cardiac and Skeletal Muscles of the Rat: Measurement of mRNA Content Using Synthetic Oligonucleotide Probes. Circ. Res. 59: 194-201.

50- Hammond, G. L., Wieben, E., and Markert, C. L. 1979; Molecular Signals for Initiating Protein Synthesis in Organ Hypertrophy. Medical Sciences, 76 (5): 2455-2459.

51- Han, C., Abel, P. W., and Minneman, K. P. 1987; Heterogeneity of α_1-Adrenergic Receptors Revealed by Chloroethylclonidine. Mol. Pharmacol. 32: 505-510.

52- Han, C., and Minneman, K. P. 1991; Interaction of Subtype-Selective Antagonists with α_1-Adrenergic Receptor Binding Sites in Rat Tissues. Mol. Pharmacol. 40: 531-538.

53- Han, H., Robinson, R. B., Bilezikian, J. P., and Steinberg, S. F. 1989; Developmental Changes in Guanine Nucleotide Regulatory Proteins in the Rat Myocardial α_1-Adrenergic Receptor Complex. Circ. Res. 65: 1763-1773.

54- Hanft, G., and Gross, G. 1989; Subclassification of α_1-Adrenoceptor Recognition Sites by Urapidil Derivatives and Other Selective Antagonists. Br. J. Pharmacol. 97: 691-700.

55- Hanft, G., Gross, G., Beckeringh, J. J., Korstanje, C. 1989; α_1-Adrenoceptors: the ability of various agonists and antagonists to discriminate between two distinct [^{3}H]prazosin binding sites. J. Pharm. Pharmacol. 41: 714-716.

56- Harmsen, E., and Leenen, F. H. H. 1992; Dietary Sodium Induced Cardiac Hypertrophy. Can. J. Physiol. Pharmacol. 70: 580-586.

57- Harmsen, E., Schoemaker, R., Yu, J., Ruzicka, M., and Leenen, F. H. H. 1994; Sensitivity to Ischaemic ATP Breakdown in Different Models of Cardiac Hypertrophy in Rats. J. Hypertens. 12: 49-57.

58- Harrap, S. B., Mitchell, G. A., Casley, D. J., Mirakian, C., and Doyle A. E. 1993; Angiotensin II, Sodium, and Cardiovascular Hypertrophy in Spontaneously Hypertensive Rats. Hypertens. 21 (1): 50-55.

59- Harsdorf, R., Lang, R., Fullerton, M., Smith, A. I., and Woodcock, E. A. 1988; Right Atrial Dilatation Increases Inositol-(1,4,5)Triphosphate Accumilation. FEB Letters, 233 (1): 201-205.

60- Harsdorf, R., Lang. R. E., Fullerton, M., and Woodcock, E. 1989; Myocardial Sretch Stimulates Phosphatidylinositol Turnover. Circ. Res. 65: 494-501.

61- Hartmann, M., and Schrader, J. 1993; Isoproterenol Antagonistic Effect of 2',5'-Dideoxyadenosine in the Isolated Perfused Guinea-Pig Heart. J. Mol. Cell. Cardiol. 25: 331-338.

62- Heimann, J. C., Drumond, S., Alves, A. T. R., Barbato, A. J. G., Dichtchekenian, V., and Marcondes, M. 1991; Left Ventricular Hypertrophy is More Marked in Salt-Sensitive than in Salt-Resistant Hypertensive Patients. J. cardiovasc. Pharmacol. 17 (Suppl. 2): S122-S124.

63- Huang, B. S., and Leenen, F. H. H. 1992; Dietary Na, Age, and Baroreflex Control of Heart Rate and Renal Sympathetic Nerve Activity in Rats. Am. J. Physiol. 262 (Heart Circ. Physiol. 31): H1441-H1448.

64- Ikeda, U., Tsuruya, Y., and Yaginuma, T. 1991; α_1-Adrenergic Stimulation is Coupled to Cardiac Myocytes Hypertrophy. Am. J. Physiol. 260 (Heart Circ. Physiol. 29): H953-H956.

65- Imai, S. 1961; The Effect of Methoxamine Upon The Membrane Resting and Action Potentials of the Guinea Pig's Ventricular Muscle Fibres. Jpn. J. Physiol. 11: 54-61.

66- Imai, S., Shigel, T., and Hashimoto, K. 1961; Cardiac Actions of Methoxamine (With Special Reference to Its Antagonistic Action to Epinephrine). Circ. Res. 9: 552-560.

67- Karim, M. A., Ferguson, A. G., Wakim, B. T., and Samarel, A. M. 1991; In Vivo Collagen Turnover During Development of Thyroxine-Induced Left Ventricular Hypertrophy. Am. J. Physiol. 260 (Cell Physiol. 29): C316-C326.

68- Kent, R. L., Hoober, J. K., and Cooper IV, G. 1989; Load Responsiveness of Protein Synthesis in Adult Mammalian Myocardium: Role of Cardiac Deformation Linked to Sodium Influx. Circ. Res. 64: 74-85.

69- Kent, R. L., Rozich, J. D., McCollam, P. L., McDermott, D. E.,Thacker, U. F., Menrick, D. R., McDermott, P. J., and Cooper IV, G. 1993; Rapid Expression of the Na^{+}-Ca^{2+} Exchnger in Response to Cardiac Pressure Overload. Am. J. Physiol. 265 (Heart Circ. Physiol. 34): H1024-H1029.

70- Kihara, M., Utagawa, N., Mano, M., Nara, Y., Horie, R., and Yamori, Y. 1985; Biochemical Aspects of Salt-Induced, Pressure-Independent Left Ventricular Hypertrophy in Rats. Heart and vessels, 1: 212-215.

71- Kinami, J., Tsuchihashi, H., Baba, S., Mano, F., Maruyama, K., and Nagatomo, T. 1992; α_1-Adrenoceptor Subtypes in the Rat Ventricular Muscle. J. Pharm. Pharmacol. 44: 97-100.

72- Knowlton, K. U., Michel, M. C., Itani, M., Shubeita, H. E., Ishihara, K., Brown, J. H., and Chien, K. R. 1993; The α_{1a}-Adrenergic Receptor Subtype Mediates Biochemical, Molecular, and Morphologic Features of Cultured Myocardial Cell Hypertrophy. J. Biol. Chem. 268 (21): 15374-15380.

73- Kolbek-Ruhmkorff, C., Horban, A., and Zimmer, H-G. 1993; Effect of Pressure and Volume Overload on Proto-Oncogene Expression in the Isolated Working Rat Heart. Cardiovasc. Res. 27: 1998-2004.

74- Komuro, I., Kaida, T., Shibazaki, Y., Kurabayashi, M., Katoh, Y., Hoh, E., Takaku, F., and Yazaki, Y. 1990; Stretching Cardiac Myocytes Stimulates Protooncogene Expression. J. Biol. Chem. 265 (7): 3595-3598.

75- Krebs, E. G. 1989; Role of the Cyclic AMP-Dependent Protein Kinase in Signal Transduction. J.A.M.A. 262: 1815-1818.

76- Kronenberg, M. W., McCain, R. W., Boucek. R. J., Grambow, D. W., Sagawa, K., and Friesinger, G. C. 1989; Effects of Methoxamine and Phenylephrine on left Ventricular Contractility in Rabbits. J. Am . Coll. Cardiol. 14: 1350-1358.

77- Laks, M. M., Morady, F., and Swan, H. J. C. 1973; Myocardial Hpertrophy Produced by Chronic Infusion of Subhypertensive Doses of Norepinephrine in the Dog. Chest 64: 75-78.

78- Lavendero, S., Caetagena, G., Guarda, E., Corbalan R., Godoy, I., Sapag-hagar,M., and Jalil, J. E. 1993; Changes in Cyclic AMP Dependent Protein Kinase and Active Stiffness in the Rat Volume Overload Model of Heart Hypertrophy. Cardiovasc. Res. 27: 1634-1638.

79- Leenen, F. H. H., and Myers, M. G. 1984; Pressor Mechanisms in Renovascular Hypertensive Rats. Handbook of Hypertens. 4: Experimental and Genetic Models of Hypertension.

80- Leenen, F. H. H., and Harmsen, E. 1991; Antihypertensive Drugs and Cardiac Trophic Mechanisms. J. Cardiovasc. Pharmacol. 17 (Suppl. 2): S50-S57.

81- Leenen, F. H. H., and Yuan, B. 1992; Dietary Sodium and the Antihypertensive Effect of Nifedipine in Spontaneously Hypertensive Rats. Am. J. Hypertens. 5: 515-519.

82- Lindpaintner, K., and Sen, S. 1985; Role of Sodium in Hypertensive Cardiac Hypertrophy. Circ. Res. 57: 610-617.

83- Lipp, J. A., and Rudolph, A. M. 1982; Sympathetic Nerve Development in the Rat and the Guinea-Pig Heart. Biol. Neonate, 21: 76-82.

84- Lomasney, J. W., Cottecchia, S., Lefkowitz, R. J., and caron M., G. 1991; Molecular Biology of α-Adrenergic Receptors: Implications for Receptor Classification and for Structure-Function Relatioships. Biochimica et Biophysica Acta, 1095: 127-139.

85- Long, C. S., Kariya, k., Krans, L., and Simpson, P. 1990; Trophic Factors for Cardiac Myocytes. J. Hypertens. 8 (Suppl.7): S219-S224.

86- Long, C. S., Kariya, K., Karns, L., and Simpson, P. 1991; Sympathetic Activity: Modulator of Myocardial Hypertrophy. J. Cardiovasc. Pharmacol. 17 (Suppl. 2): S20-S24.

87- Luft, F. C., Rankin, L. I., Henry, D. P., Bloch, R., Grim, C. E., Weyman, A. E., Murray, R. H. and Weinberger, M. H. 1979; Plasma and Urinary Norepinephrine Values at Extremes of Sodium Intake in Normal Man. Hypertens. 1: 261-266.

88- Mann, D. L., Kent, R. L., Cooper IV, G. 1989; Load Regulation of the Properties of Adult Feline Cardiocytes: Growth Induction by Cellular Deformation. Circ. Res. 46: 1079-1090.

89- Marban, E., and Koretsune, Y. 1990; Cell Calcium, Oncogenes, and Hypertrophy. Hypertens. 15: 652-658.

90- Maturi, M. F., Greene, R., Donahue, B., et al. 1986; Functional Consequences and Intracoronary Localization of Alpha-Adrenergic Stimulation of the Canine Coronary Circulation. J. Am. Coll. Cardiol. 8: 885-893.

91- Meggs, L. G., Ben-Ari, J., Gammon, D., Goodman, A. I. 1988; Myocardial Hypertrophy: The Effect of Sodium and the Role of Sympathetic Nervous System Activity. Am. J. Hypertens. 1: 11-15.

92- Meidell, R. S., Sen. A., Henderson, S. A., Slahetka, M. F., and Chien, K. R. 1986; α_1-Adrenergic Stimulation of Rat Myocardial Cells Increases Protein Synthesis. Am. J. Physiol. 251 (Heart Circ. Physiol. 20): H1076-H1084.

93- Mertens, M. J. F., Batink, H. D., Pfaffendorf, M., and van Zwieten, P. A. 1992; Impaired Inotropic Response to α_1- but not to ß-Adrenoceptor Stimulation in Isolated Hearts from Spontaneously Hypertensive Rats. J. Hypertens. 10: 143-148.

94- Mervaala, E. M. A., Himberg, J., Laakso, J., Tuomainen, P., and Karppanen, H. 1992; Beneficial Effects of a Potassium- and Magnesium-Enriched Salt

Alternative. Hypertens. 19: 535-540.

95- Michel, M. C., Knowlton, K. U., Gross, G., Chien, K. R., UC San Diego, La Jolla, CA, and University of Essen, Essen, Fed. Rep. Germany. 1990; α_1-Adrenergic Receptor Subtypes Mediate Distinct Functions in Adult and Neonatal Rat Heart. Circ. 82 (Suppl. III): 561.

96- Michel, M. C., Philipp, T., and Brodde, O. 1992; α- and ß-Adrenoceptors in Hypertension: Molecular Biology and Pharmacological Studies. Pharmacol. Toxicol. 70 (Suppl. II): s1-s10.

97- Michel, M. C., Hanft, G., and Grob, G. 1994; Functional Studies on α_1-Adrenoceptor Subtypes Mediating Inotropic Effects in Rat Right Ventricle. Br. J. Pharmacol. 111: 539-546.

98- Michel, M. C., Hanft, G., and Grob, G. 1994; Radioligand Binding Studies of α_1-Adrenoceptor Subtypes in Rat Heart. Br. J. Pharmacol. 111: 533-538.

99- Minneman, K. P. 1988; α_1-Adrenergic Receptor Subtypes, Inositol Phosphates, and Sources of Cell Ca^{2+}. Pharmacol. Rev. 40 (2): 87-100.

100- Minneman, K. P., Han, C., and Abel, P. W. 1988; Comparison of α_1-Adrenergic Receptor Subtypes Distinguished by Chlorethylclonidine and WB 4101. Mol. Pharmacol. 33: 509-514.

101- Morano, I., Lengsfeld, M., Ganten, U., Ganten, D., and Ruegg, J. C. 1988; Chronic Hypertension Changes Myosin Isoenzyme Pattern and Decreases Myosin Phosphorylation in the Rat Heart. J. Mol. Cell. Cardiol. 20: 875-886.

102- Morgan, H. E., and Baker, K. M. 1991; Cardiac Hypertrophy, Mechanical, Neutral, and Endocrine Dependence. Circ. 83 (1): 13-25.

103- Mozaffari, M. S., Jirakulsomchok, S., Oparil, S., and Wyss, J. M. 1990; Changes in Cerebrospinal Fluid Na^+ Concentration Do Not Underlie Hypertensive Responses to Dietary NaCl in Spontaneously Hypertensive rats. Brain Res. 506: 149-152.

104- Naslund, T., Silberstein, D. J., Merrell, W. J., Nadeau, J. H., and Wood, A. J. J. 1990; Low Sodium Intake Corrects Abnormality in ß-Receptor-Mediated Arterial Vasodilation in Patients with Hypertension: Correlation with ß-Receptor Function In Vitro. Clin. Pharmacol. Ther. 48 (1): 87-95.

105- Otani, H., Uria, T., Hara, M., Inoue, M., Omori, K., Cragoe, E. J., and Inagaki, C. 1990; Effects of inhibitiors of Protein Kinase C and Na^+/H^+ Exchange on α-Adrenoceptor-Mediated Inotropic Responses in the Rat Left Ventricular Papillary Muscle. Br. J. Pharmacol. 100: 207-210.

106- Overlack, A., Ruppert, M., Kolloch, R., Gobel, B., Kraft, K., Diehl, J., Schmitt, W., and Stumpe, K. O. 1993; Divergent Hemodynamic and Hormonal Responses to Varying Salt Intake in Normotensive Subjects. Hypertens. 22: 331-338.

107- Paul, M., and Ganten, D. 1992; The Molecular Basis of Cardiovascular Hypertrophy: The Role of Renin-Angiotensin System. J. Cardiovasc. Pharmacol. 19 (Suppl. 5): S51-S58.

108- Peeler, T. C., Baker, K. M., Esmurdoc, C. F., and Chernin, M. I. 1991; Angiotensin Converting Enzyme Inhibition in Dahl-Sensitive Rats. Mol. Cel.

Biochem. 104: 45-50.

109- Pfeffer, M. A., Pfeffer, J., Mirsky, I., and Heine, M. 1984; Cardiac Hypertrophy and Performance Dahl Hypertensive Rats on Graded Salt Diets. Hypertens. 6: 475-481.

110- Puceat, M., Clement, O., Lechene, P., Pelosin, J. M., Ventura-Clapier, R., and Vassort, G. 1990; Neurohormonal Control of Calcium Sensitivity of Myofilaments in Rat Single Heart Cells. Circ. Res. 67: 517-524.

111- Rabinowitz, B., Chuck, L., Kligerman, M., and Parmley W. W. 1975; Positive Inotropic Effects of methoxamine: Evidence for Alpha-Adrenergic Receptors in Ventricular Myocardium. Am J. Physiol. 229: 582-585.

112- Rankin, L. I., Luft, F. C., Henry, D. P., Gibbs, P. S., and Weinberger, M. H. 1981; Sodium Intake Alters the Effects of Norepinephrine on Blood Pressure. Hyprtens. 3: 650-656.

113- Reder, R. F., Danilo, P., Rosen, M. R. 1984; Developmental Changes in Alpha Adrenergic Effects on Canine Purkinjie Fibre Automaticity. Dev. Pharmacol. Ther. 7: 94-108.

114- Ruzicka, M., Yuan, B., Harmsen, E., and Leenen, F. H. H. 1993; The Renin-Angiotensin System and Volume Overload-Induced Cardiac Hypertrophy in Rats, Effect of Angiotensin Converting Enzyme Inhibitor Versus Angiotensin II Receptor Blocker. Circ. 87: 921-930.

115- Sadoshima, J., Izumo, S. 1993; Molecular Characterization of Angiotensin II-Induced Hypertrophy of Cardiac Myocytes and Hyperplasia of Cardiac Fibroblasts,

Critical Role of the AT_1 Receptor Subtype. Circ. Res. 73: 413-423.

116- Sadoshima, J., Xu, Y., and Slayter, H., S. 1993; Autocrine Release of Angiotensin II Mediates Stretch-Induced Hypertrophy of Cardiac Myocytes In Vitro. Cell. 75: 977-984.

117- Saito, K., Suetsugn, T., Oku, Y., Kuroda, A., and Tanaka, H. 1994; α_1-Adrenoceptors in the Conduction System of Rat Hearts. Br. J. Pharmacol. 111: 465-468.

118- Schluter, K. D., and Piper, H. M. 1992; Trophic Effects of Catecholamines and Parathyroid Hormone on Adult Ventricular Cardiomyocytes. Am. J. Physiol. 263 (Heart Circ. Physiol. 32): H1739-H1746.

119- Schmieder, R. E., Messerli, F. H., Garavagalia, G. E., and Nunez, B. D. 1988; Dietary Salt Intake (A Determinant of Cardiac Involvement in Essential Hypertension). Circ. 78: 951-956.

120- Schmieder, R. E., Messerli, F. H., Ruddel, H., Garavagalia, G. G., Grube, E., Nunez, B., and Schulte, W. 1988; Sodium Intake Modulates Left Ventricular Hypertrophy in Essential Hypertension. J. Hypertens. 6 (Suppl. 4): S148-S150.

121- Schmitz, W., Kohl, C., Neumann, J., Scholz, H., Scholz, J. 1989; On the Mechanism of Positive Inotropic Effects of Alpha-Adrenoceptor Agonists. Basic Res. Cardiol. 84 (Suppl. I): 23-33.

122- Schneider, M. D., and Parker, T. G. 1990; Cardiac Myocytes as Targets for the Action of Peptide Growth Factors. Circ. 81 (5): 1443-1454.

123- Schunkert, H., Dzau, V. J., Tang, S. S., Hirsch, A. T., Apstein, C. S., and Lorell, B. 1990; Increased Rat Cardiac Angiotensin Converting Enzyme Activity and mRNA Expression in Pressure Overload Left Ventricular Hypertrophy, Effect on Coronary Resistance, Contractility, and Relaxation. J. Clin. Invest. 86:1913-1920.

124- Schwartz, D. D., and Malik, K. U. 1991; Mechanism of Neurotransmitter Release Elicited by the Preferential α_1-Adrenergic Receptor Agonist Phenylephrine in Superfused Superior Cervical Ganglion Cells in Culture. J. Neurochem. 57: 831-838.

125- Sedaa, K. O., Bjur, R. A., Shinozuka, K., and Westfall, D. P. 1989; Nerve and Drug-Induced Release of Adenine Nucleosides and Nucleotides from Rabbit Aorta. J. Pharmacol. Exp. Ther. 252: 1060-1067.

126- Sei, C. A., Irons, C. E., Sprenkle, A. B., McDonough, P. M., Brown, J. H., and Glembotski, C. C. 1991; The α-Adrenergic Stimulation of Atrial Natriuretic Factor Expression in Cardiac Myocytes Requires Calcium Influx, Protein Kinase C, and Calmodlin-Regulated Pathways. J. Biol. Chem. 266 (24): 15910-15916.

127- Sen, S., and Young, D. R. 1986; Role of Sodium in Modulation of Myocardial Hypertrophy in Renal Hypertensive Rats. Hypertens. 8: 918-924.

128- Simpson, P. 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.

129- Simpson, P. C. 1989; Proto-Oncogenes and Cardiac Hypertrophy. Annu. Rev. Physiol. 51: 189-202.

130- Simpson, P. C., Cuenco. R. G., Paningbatan, M. O., and murphy, M. D. 1990; An α_1-Adrenergic Receptor Subtype Sensitive to WB-4101 Transduce Cardiac Myocyte Growth. Circ. 82 (Suppl. III): 561.

131- Skomedal, T., Schiander, I. G., and Osnes, J. 1988; Both Alpha and Beta Adrenoceptor-Mediated Components Contribute to Final Inotropic Response to Norepinephrine in Rat Heart. J. Pharmacol. Exp. Ther. 247 (3): 1204-1210.

132- Smith, J. B., Fineberg, N. S., Wade, M. B., and Weinberger, M. H. 1989; Acute Sodium Loading Alerts Sodium Pump in Caucasian Hypertensive Subjects. Hypertens. 13: 15-21.

133- Soltoff, S. and Cantley, L. C. 1989; Mitogens and Ion Fluxes. Annu. Rev. Physiol. 50: 207-223.

134- Steinfath, M., Chen, Y., Lavicky, J., Magnussen, O., Nose, M., Rosswag, S., Schmitz, W., and Scholz, H. 1992; Cardiac α_1-Adrnoceptor Densities in Different Mammalian Species. Br. J. Pharmacol. 107: 185-188.

135- Stewart, J. M., Patel, M. B., Wang, J., Ochoa, M., Gewitz, M., Loud, A. V., Anversa, P., and Hintze, T. H. 1992; Chronic Elevation of Norepinephrine in Conscious Dogs Produces Hypertrophy with No Loss of LV Reserve. Am. J. Physiol. 262 (Heart Circ. Physiol. 31): H331-H339.

136- Sullivan, J. M., Ratts, T. E., Taylor, J. C., Kraus, D. H., Barton, B. R., Patrick, D. R., and Reed, S. W. 1980; Hemodynamic Effects of Dietary Sodium in Man.

Hypertens. 2: 506-514.

137- Terzic, A., Puceat, M., Vassort, G., and Vogel, S. M. 1993; Cardiac α_1-Adrenoceptors: An Overview. Pharmacol. Rev. 45 (2): 147-169.

138- Toal, C. B., and Leenen, F. H. H. 1983; Dietary Sodium Restriction and Development of Hypertension in Spontaneously Hypertensive Rats. Am. J. Physiol. 245 (Heart Circ. Physiol. 14): H1081-H1084.

139- Tsoporis, J., Yuan, B., and Leenen, F. H. H. 1989; Arterial Vasodilators, Cardiac Volume Overload, and Cardiac Hypertrophy in Normotensive Rats. Am. J. Physiol. 265 (Heart Circ. Physiol. 25): H876-H880.

140- Tsuchida, A., Liu, Y., Liu, G., Cohen, M., and Downey, J. M. 1994; α_1-Adrenergic Agonists Precondition Rabbit Ischemic Myocardium Independent of Adenosine by Direct Activation of Protein Kinase C. Circ. Res. 75: 576-585.

141- Tsujimoto, G., Tsujimoto, A., Suzuki, E., and Hashimoto, K. 1989; Glycogen Phosphorylase Activation by Two Different α_1-Adrenergic Receptor Subtypes: Methoxamine Selectivity Stimulates a Putative α_1-Adrenergic Receptor Subtype (α_{1a}) that Couples with Ca^{2+} Influx. Mol. Pharmacol. 36: 166-176.

142- Vago, T., Bevilacqua, M., Dagani, R., Meroni, R., Frigeni, G., Santolib, C., and Norbiato, G. 1984; Comparsion of Rat and Human Left Ventricular ß-Adrenergic Receptors: Subtype Heterogeneity Delineated by Direct Radioligand Binding. Biochemical and Biophysical Research Communication, 121 (1): 346-354.

143- Vargas, H. M., Cunningham, D., Zhou, L., Hartman, H. B., and Gorman, A. J. 1993; Cardiovascular Effects of Chloroethylclonidine, an Irreversible α_{1b}-

Adrenoceptor Antagonist, in the Unanesthetized Rat: A Pharmacological Analysis In Vivo and In Vitro. J. Pharmacol. Exp. Ther. 266 (2): 864-871.

144- Vleeming, W., van Rooij, H. H., Wemer, J., and Porsius, A. J. 1991; Cardiovascular Responses to the Stereoisomers of Dobutamine in Isolated Rat Hearts 48 Hours After Acute Myocardial Infarction. J. Cardiovasc. Pharmacol. 17: 634-640.

145- Wang, X., Wettwer, E., Gross, G., and Ravens, U. 1991; Reduction of Cardiac Outward Currents by Alpha-1 Adrenoceptor Stimulation: A Subtype-Specific Effect. J. Pharmacol. Exp. Ther. 259 (2): 783-788.

146- Waspe, L. E., Ordahl, C. P., and Simpson, P. C. 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.

147- Watson, P. A., Haneda, T., and Morgan, H. E. 1989; Effect of higher Aortic Pressure on Ribosome Formation and cAMP Content in Rat Heart. Am. J. Physiol. 256 (Cell Physio. 25): C1257-C1261.

148- Wiener, H. L., and Thalody, G. P. 1993; Differential Attenuation of the Responses to Adenosine and Methoxamine in Isolated Rabbit Aorta. J. Pharmacol. Exp. Ther. 267 (2): 828-837.

149- Wilczynski, E. A., Yuan, B., and Leenen, F. H. H. 1992; Dietary Sodium Restriction and the Developement of two-kidney, one-clip Hypertension in Young versus Adult Rats. Can. J. Physiol. Pharmacol. 70: 452-457.

150- Williamson, A. P., Kennedy, R. H., Seifen, E., Lindemann, J. P., and Stimers, J. P. 1993; α_{1b}-Adrenoceptor-Mediated Stimulation of Na-K Pump Current in Adult Rat Ventricular Myocytes. Am. J. Physiol. (Heart Circ. Physiol. 33): H1315-H1318.

151- Wilson, K. M., and Minneman, K. P. 1990; Different Pathways of [^{3}H]Inositol Phosphate Formation Mediated by α_{1a}-and α_{1b}-Adrenergic Receptors. J. Biol. Chem. 265 (29): 17601-17606.

152- Woodcock, E. A., Arnolda, L. and McGarth, B. P. 1988; Ventricular Beta-Adrenoceptors in Adriamycin-Induced Cardiomyopathy in the Rabbit. J. Mol. Cardiol. 20: 771-777.

153- Xenophontos, X. P., Watson, P. A., Chua, B. H. L., Haneda, T., and Morgan, H. E. 1989; Increased Cyclic AMP Content Accelarates Protein Synthesis in Rat Heart. Circ. Res. 65: 647-656.

154- Yarden, Y., and Ulrich, A. 1988; Growth Factor Receptor Tyrosine Kinases. Annu. Rev. Biochem. 57: 443-478.

155- Yuan, B., and Leenen, F. H. H. 1991; Dietary Sodium Intake and Left Ventricular Hypertrophy in Normotensive Rats. Am. J. Physiol. 261 (Heart Circ Physiol. 30): H1397-H1401.

156- Zak, R. 1973; Cell Proliferation During Cardiac Growth. Am. J. Cardiol. 31: 211-219.

157- Zierhut, W., and Zimmer, H. G. 1989; Significance of Myocardial α- and ß-Adrenoceptors in Catecholamine-Induced Cardiac Hypertrophy. Circ. Res. 65:

1417-1425.