INTERACTION BETWEEN HYDROGEN SULPHIDE (H_2S) AND NITRIC OXIDE (NO) IN LEFT VENTRICULAR HYPERTROPHY AND THEIR EFFECT ON RESPONSIVENESS OF ALPHA 1-ADRENERGIC RECEPTORS SUBTYPES IN THE RAT KIDNEY

by

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To

My mother Kaniz Fatima, my wife Tabinda Fatima and my sister sister Nusrat Perveen and all members of my happy family

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LIST OF ABBREVIATIONS

LVH	Left ventricular hypertrophy
CHF	Congestive heart failure
CVS	Cardiovascular system
AV	Atrio-ventricle valves
MAP	Mean arterial pressure
CO	Cardiac output
RAAS	Renin angiotensin aldosterone system
SNS	Sympathetic nervous sytem
PNS	Parasympathetic nervous sytem
Ach	Acetylecholine
NA	Noradrenaline
HR	Heart rate
α_1-adrenergic receptor	Alpha one adrenergic receptor
β_1-adrenergic receptor	Beta one adrenergic receptors
TPR	Total peripheral resistance
GDP	Guanosine di phosphate
GTP	Guanosine tri phosphate
IP3	Inositol 1, 4, 5- triphosphate
DAG	Diacylglycerol
AD	Aldosterone
MAPK	Mitogen activated protein kinase
HT	Hypertension
LDL	Low density lipoprotein
DM	Diabetes mellitus
ACTH	Adrenocorticotropic hormone
DHEA	Dihydroepiandroandrenosterone
ROS	Reactive oxygen species
ACEi	Angiotensin converting enzyme inhibitor
NADPH oxidase	Nicotinamide adenine dinucleotide phosphate-oxidase
H_2O_2	Hudrogen peroxide
SOD	Superoxide dismutase
TNF	Tumor necrosis factor
SHR	Spontaneously hypertensive rats
I/C	Isoprenaline/caffeine
S/C	Sub-cutaneous
RSNA	Renal sympathetic nerve activity
SA node	Sino-arterial node
AV node	Arterio-ventricle node
ECG	Electrocardiogram
PCT	Proximal convoluted tubules
DCT	Distal convoluted tubules
MeU	Methylurapidil
CEC	Chloroethylclonidine
BMY	(8-(2-[4-(2-methoxyphenyl)-1-piperazinyl) ethyl]-8-azaspiro (4·5) decane-7,9-dione dihydrochloride

H_2S	Hydrogen sulphide
NO	Nitric oxide
CO	Carbon monoxide
MST	Mercaptopyruvate sulphur transferase
3-MP	3 Mercaptopyruvate
CAT	Cystein amino transferase
CSE	Cystathion gamma lyase
CBS	Cystathione beta synthase
KCL	Potassium chloride
Mm	Micromole
Mm	Milimole
MMP	Matrix metalloproteinase
TIMP	Inhibitor of matrix metalloproteinase
NOS	Nitric oxide synthase
BH_4	Tetrahydrobiotin
L-NMMA	NG-monomethyl-L-arginine
EDRF	Endothelium derived relaxing factor
L-NAME	L-N^G-Nitroarginine methyl ester
Enos	Endothelial nitric oxide synthase
TAC	Transverse aortic constriction
MI	Myocardial infarction
GSH	Glutathione reductase
T-AOC	Total antioxidant capacity
MDA	Malanodialdehyde
STZ	Streptozotocin
µg	Micro gram
Ng	Nano gram
NIBP	Non invasive blood pressure
mmHg	Millimeter mercury
mg /dl	Milligram per desiliter
mL/min/kg	Millilitre per minute per kilogram
mg/kg	Milligram per kilogram
i.p.	Intraperitoneal
mL	Millilitre
%	Percentage
PWV	Pulse Wave velocity
m/s	Meter per second
µM	Micro moles
nmol/mL	Nano moles per millilitre
µL	Microliter
Ucr.	Urinary creatinine
Pcr.	Plasma creatinine
Bw	Body weight
MAP	Mean arterial blood pressure
SBP	Systolic blood pressure
HR	Heart rate
$U_{Na}V$	Absolute sodium excretion
U_kV	Absolute potassium excretion
FE_{Na}	Fractional sodium excretion

UFR	Urine flow rate
BPU	Blood perfusion unit
RCBP	Renal cortical blood perfusion
U/mL	Units per millilitre
PCR	Polymerase chain reaction
qPCR	Real-time PCR
RT	Reverse transcription
RT-PCR	Reverse transcription polymerase chain reaction
RNA	Ribonucleic acid
mRNA	Messenger Ribonucleic acid
DNA	Deoxyribonucleic acid
cDNA	Complementary Deoxyribonucleic acid

INTERAKSI YANG KETARA ANTARA HIDROGEN SULFIDA DAN NITRIK OKSIDA TERHADAP HIPERTROPI VENTRIKEL KIRI DAN TINDAK BALAS RESEPTOR ADRENERGIC SUBJENIS ALFA-1 DALAM GINJAL TIKUS

ABSTRAK

Penyelidikan ini telah dijalankan untuk menyiasat kesan rangsangan hipertrofi ventrikel kiri (LVH) ke atas reseptor adrenergik subjenis α_1 terhadap stimulus adrenergik di dalam tikus. Peranan sistem hidrogen sulphida (H_2S) dan nitrik oksida (NO) dan interaksinya dalam perkembangan LVH telah dikaji dengan memeriksa kesan pengubahsuaian ekspresi cistation γ liase (CSE mRNA) dan enzim endotelial nitrogen oksida sintase (eNOS mRNA) di dalam jantung semasa LVH. Parameter kardiovaskular seperti geometri kardiak, tekanan oksidatif, kekejangan arteri dan rangsangan vaskular kepada stimulus vasoaktif telah dikaji. Di samping itu, kajian ini telah memeriksa fungsi perkumuhan ginjal, haemodinamik dan perubahan histologi selepas NaHS, iaitu penderma H_2S, L-arginine, iaitu penderma NO dan kombinasi NaHS dan L-arginine yang diberi secara eksogenus. Tikus Wistar-Kyoto (WKY) telah dibahagikan kepada dua kumpulan utama iaitu kawalan dan LVH. LVH telah diaruh dengan menggunakan isoprenalin (5mg/kg, melalui suntikan secara subkutaneus setiap 7 jam selama 2 minggu) dan kafein (62mg/L di dalam air minuman selama 2 minggu). Kumpulan ini telah dipecah bahagian lagi kepada 8 kumpulan yang berdasarkan rawatan. NaHS (56μM/kg disuntik secara intraperitoneal selama 5 minggu) atau L-arginine (1.25g/L diberi selama 5 minggu di dalam air minuman) kumpulan kawalan yang dirawat atau kumpulan-kumpulan LVH. Reseptor adrenergik subjenis alfa-1 telah dikaji melalui pemeriksaan tindak balas kepada noradrenalin (NA), fenilefrin (PE) dan metoxamin (ME) di dalam

kehadiran latar belakang infusi intraginjal oleh reseptor penghalang adrenergik subjenis alfa-1 yang selektif (5-metilurapidil (5-MeU), kloroetilclonidin (CEC) dan BMY 7378). Data masa-sebenar tindakan rantaian polymerase kuantitatif (telah dinormalisasikan kepada β-actin dan relatif kepada WKY) telah dikira melalui cara $2^{-\Delta\Delta CT}$. Data mewakili min ± S.E.M telah dianalisis menggunakan ANOVA satu-hala atau dua-hala yang bersesuaian dan diikuti dengan ujian pos hoc dengan singnifikasi $P<0.05$. Tikus LVH yang dirawat dengan H_2S, NO dan kombinasi H_2S dan NO telah menunjukkan peningkatan CSE, eNOS dan eNOS mRNA masing-masing yang signifikan ($P<0.05$) di dalam myocardium. Di samping itu, terdapat pengurangan yang signifikan (semua adalah $P<0.05$) di dalam jisim jantung, parameter tekanan oksidatif di dalam plasma dan kekejangan arteri di dalam kumpulan tikus LVH yang dirawat dengan H_2S dan kumpulan tikus LVH yang dirawat dengan NO. Tindak balas reseptor adrenergik subjenis alfa-1 kepada stimulus adrenergik telah menunjukkan peningkatan yang signifikan (semua adalah $P<0.05$) di dalam LVH-H_2S, LVH-NO atau LVH-H_2S+NO. Kesimpulannya, parameter normal kardiak dan parameter fungsi ginjal bergantung kepada system H_2S dan NO yang utuh. Di dalam kumpulan tikus LVH, pengurangan ekpresi eNOS dan CSE mRNA didapati dalam jantung dan ginjal. Walaubagaimanapun, rawatan dengan H_2S dan NO telah membaikpulihkan ekpresi eNOS dan CSE dan telah meningkatkan parameter fungsi ginjal dan kardiovaskular. Sesungguhnya, dapatan ini menunjukkan kepentingan eNOS dan CSE di dalam patofisiologi model penyakit jantung ini.

INTERACTION BETWEEN HYDROGEN SULPHIDE (H_2S) AND NITRIC OXIDE (NO) IN LEFT VENTRICULAR HYPERTROPHY AND THEIR EFFECT ON RESPONSIVENESS OF ALPHA 1-ADRENERGIC RECEPTORS SUBTYPES IN THE RAT KIDNEY

ABSTRACT

The present study investigated the effect of left ventricular hypertrophy (LVH) on the responsiveness of α_1-adrenergic receptor subtypes to adrenergic stimuli in the rat. The role of hydrogen sulphide (H_2S) and nitric oxide (NO) systems and their interaction in the progression of LVH was studied by examining the effect of altered expression of cystathione γ lyase (CSE mRNA) and endothelial nitric oxide synthase (eNOS mRNA) in the heart during LVH. Cardiovascular parameters such as cardiac geometry, oxidative stress, arterial stiffness and vascular responsiveness to vasoactive stimuli were studied. In addition, this study examined renal excretory functions, haemodynamics and histopathological changes after exogenous administration of NaHS, an H_2S donor, L-arginine, an NO donor and a combination of NaHS and L-arginine. Wistar-Kyoto (WKY) rats were divided into two major groups of Control and LVH. These groups were then subdivided into 8 groups based on treatment. NaHS (56μM/kg I.P. for 5 weeks) or L-arginine (1.25g/L for 5 weeks in drinking water) treated control or LVH groups. LVH was induced using isoprenaline (5mg/kg, S.C. every 72 hours for 2 weeks) and caffeine (62mg/L in drinking water for 2 weeks). The α_1-adrenergic receptors subtypes was studied by examining the responsiveness to noradrenaline (NA), phenylephrine (PE) and methoxamine (ME) in the presence of a background intrarenal infusion of selective α_1-adrenergic receptors blockers (5-methylurapidil (5-MeU), chloroethylclonidine (CEC) and BMY 7378). Real-time quantitative PCR data (normalized to β-actin and relative to WKY) were calculated by $2^{-\Delta\Delta CT}$ method. Data, mean±SEM were

subjected to one or two-way ANOVA when appropriate followed by a *post hoc* test with significance at $P<0.05$. Treatment of LVH rats with H_2S, NO and combination of H_2S+NO enhanced significantly ($P<0.05$) the expression of CSE, eNOS and eNOS mRNAs in the myocardium respectively. In addition, there was a significant decrease (all $P<0.05$) in heart mass, oxidative stress parameters in the plasma and arterial stiffness in H_2S and NO groups of LVH rats. The responsiveness of α_1-adrenergic receptors to adrenergic stimuli was significantly enhanced (all $P<0.05$) in LVH-H_2S, LVH-NO or LVH-H_2S+NO. In conclusion, the normal cardiac and renal functional parameters are dependent on intact H_2S and NO systems. In the LVH rats, there is a down regulation of eNOS and CSE mRNA expressions in the heart and kidney. However, treatment with H_2S and NO restored the expression of eNOS and CSE and enhanced the renal and cardiovascular functional parameters. These findings indicate the importance of eNOS and CSE in the pathophysiology of this model of heart disease.

CHAPTER 1

INTRODUCTION

Left ventricular hypertrophy (LVH) is an inflammatory and compensatory mechanism of heart as a result of elevated afterload. Physiological adaptations in structure lead to pathologically malfunction of heart which ultimately worsens the cardiovascular morbidity and mortality. In compensatory mechanism, heart has to pump more blood against elevated afterload so in this effort left ventricle walls have to stretch more than the normal force of contraction leading to dilation of left ventricle walls thickness. Starting from adaptation to increased work load LVH contributes to increased cardiovascular events by affecting ventricular dysfunction, coronary circulation and arrythmogenesis (Clement et al., 1993).

The prevalence of LVH is age dependent and it is found to be increasing from 6 % under the age of 30 years in Framingham subjects to 43% in those ≥ 70 years old (LEVY et al., 1988). Hypertension also contributes to development of LVH (Breslin et al., 1966; Frohlich et al., 1971; Kannel et al., 1969; Savage et al., 1979) which further worsen the cardiovascular system that is evident of hypertensive target organ damage (Dunn et al., 1977; Frohlich et al., 1992; Frohlich, Tarazi, & Dustan, 1971). Severity of hypertension leads to prevalence of LVH ranging from less than 20 % in mild hypertension to ≥ 50 % in severe hypertension (Hammond et al., 1986; Savage et al., 1979). Overlooked diagnosis or inappropriate treatment

outcomes will make LVH as harbinger of cardiovascular events. Conversely, reduction in LVH may lead to reduced cardiovascular morbidity (Verdecchia et al., 1998).

LVH usually leads to heart hypertrophy leading to congestive heart failure (CHF) associated with increased risk of cardiovascular morbidity and mortality (de Simone et al., 2008; Levy et al., 1990; Vakili et al., 2001). Therefore, diagnosing the cause of LVH is important for targeted therapeutic outcome. Induction of LVH results in many geometrical changes in the heart and pathological changes in cardiovascular system. Lower incidences of cardiovascular events have been reported with the reduction of LVH (Dahlöf et al., 2002; Okin et al., 2006).

The understanding of cardiovascular system is important to know about development, complications and prognosis of LVH.

1.1 Cardiovascular system

The cardiovascular system (CVS) is responsible for the circulation of blood from heart to different parts of body along with the nutritional supply to different organs. The CVS also contributes to theremoval of waste materials from blood by filtration through kidneys and diffusion via lungs. The heart, vessels, red blood cells, white blood cells and platelets collectively constitute the CVS and responsible for the release of several hormones.

1.2 Functional anatomy of the heart

The heart is the organ responsible to pump blood to different organs through the blood vessels. It is enclosed in a thin membrane called the pericardium and located in thoracic cavity just above the diaphragm. Heart muscles form a functional syncitium.

The heart consists of four chambers, the upper two are called atria while thelower two chambers are called ventricles. Heart usually receives deoxygenated blood from different parts of body through vena cavas in right atrium and oxygenated blood into left atrium. Ventricles are usually larger in size than atria because ventricles are responsible to receive blood from atria and pump it to elsewhere. In this effort both ventricles undergo physiological hypertrophy. As such atria are specialized in receiving blood while both ventricles pump the blood to different parts of body. Whole heart muscle is called myocardium and narrow lower portion is called apex while upper broader part is called the base of heart.

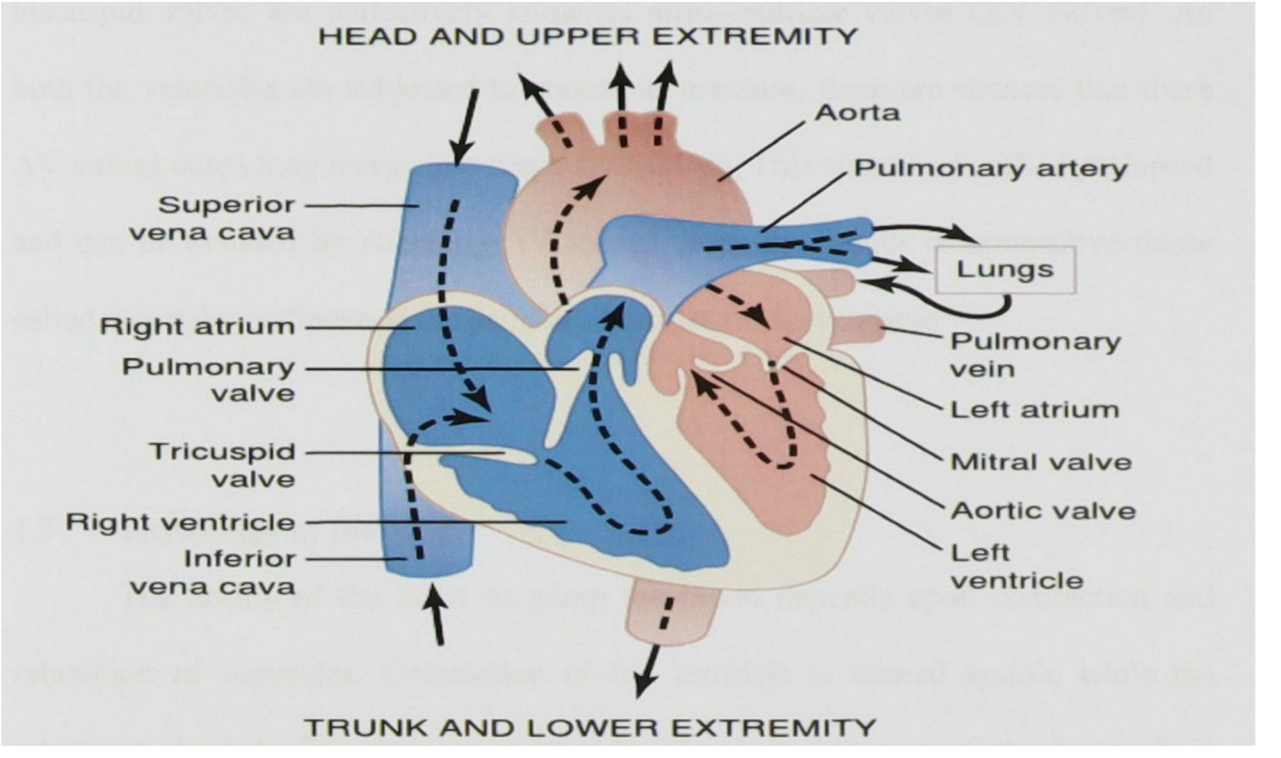


Figure 1.1: Functional anatomy of heart (Adopted from Guyton 2006)

The blood is received from superior and inferior vena cava into the right atrium which pumps the blood into right ventricle. The right atrium and right ventricle are connected with a tricuspid valve as shown in Figure 1.1. The right ventricle pushes deoxygenated blood into the lungs by means of pulmonary artery. This deoxygenated blood is oxygenated in the lungs and received into the left atrium of the heart through pulmonary vein. In this respect, pulmonary artery is the onlyartery containing deoxygenated blood while pulmonary vein is the only vein containing oxygenated blood. The left atrium and left ventricles are connected by biscupid mitral valve. Oxygenated blood is pushed into the left ventricle and from here it is pushed to different organs through aorta. The opening of aorta also has semilunar valve as in the pulmonary vein. The left ventricle has to pump blood with great force and eventually undergoes functional hypertrophy and this is the reason for greater chamber size and mass as compared to right ventricle. The backflow of blood into their respective atrial chamber is prevented by mitral valve. Tricuspid and

biscuspid valves are collectively knownas atrio-ventricle valves (AV valves). As both the ventricles are subjected to enormous pressure, there are chances that these AV valves cusps may merge into respective atrium. This situation is called prolapsed and can be avoided by attaching AV valves cusps by strands of connective tissue called chordate tendineae to the papillary muscles of the ventricle

1.3 Physiology of the heart

The ability of the heart to pump the blood depends upon contraction and relaxation of ventricles. Contraction of the ventricle is termed systole while the relaxation diastole. The pressure exerted by blood on unit area of blood vessels is termed as blood pressure (BP). The blood pressure consists of the systolic (SBP) and diastolic blood (DBP) pressures. Normal values of blood pressure is around 120-140 mmHg for SBP and 70-90 mmHg for DBP. The average pressure or force required to pump the blood into the blood vessels is knownas mean arterial blood pressure (MAP). The value of MAP is measured by one third of SBP plus two third of DBP and value is generally between 90-110 mmHg.

Blood pressure can be calculated by multiplying heart rate (HR) and total peripheral resistance (TPR). The normal HR is 70-80 beats per minutes (BPM). When this rate is increased (above 100 bpm) it is called as tachycardia and when heart rate is decreased (below 60 bpm) is called as bradycardia. The chronotropic action of the heart is controlled by the β_1-adrenoreceptors.

The beginning of one heart beat to the beginning of other is knownas cardiac cycle. Each cardiac cycle is initiated by spontaneous generation of an action potential at the sinus node (SA node). The amount of blood pumped by heart in one minute is called as cardiac output (CO) and its average value is 5.6 L/min. in healthy individual in males while this value is around 4.9 L/min. in females.

1.4 Molecular mechanism for the development of left ventricular hypertrophy

The increased workload on the heart due to increased demand of body for blood and nutrition is most common reason for the onset of LVH. Increased activity of the heart is linked with increased physiological needs such as when doing physical exercise (Russell et al., 2000). Thus, as a result of increased activity there are changes in the structure of heart as a result of new demand. The LVH comprises of structure changes due to increased dimensions of cardiomyocyte, the proliferation of interstitial tissues and rarefaction of coronary circulation (Wollert & Drexler, 2002). When cardiomyocyte are stimulated by any hypertrophic agent or stimulus, it is translated inside the body as second (cytosolic) and third (nucleus) messengers responsible for their actions inside the cells thus regulating the process of transcription. This transcription will cause expression of specific genes responsible for LVH. This is shown in Figure 1.2.

The growth of myocardium cells in LVH may occur either by the addition of sarcomeres in parallel position (pressure overload hypertrophy) or by series addition

(volume overload hypertrophy). This allows the myocardium either to increase in length or increase in diameter leading to eccentric or concentric hypertrophy respectively (Kempf & Wollert, 2004). Hypertrophy due to volume overload may be adaptive (physiological) or may be maladaptive (pathological) (Kempf & Wollert, 2004). The physiological hypertrophy can be observed during adolescence stage, pregnancy and exercise due to transient volume overload while persistent volume overload can lead to pathological hypertrophy as shown in Figure. 1.3.

These anatomical and molecular changes not only occur in the heart but also in the vasculature and intercellular matrix (Gradman & Alfayoumi, 2006). Following pressure load due to aortic stenosis, valvular disease or hypertension the heart is exposed to increased hemodynamic overload (Lorell & Carabello, 2000). Thickness of the myocardium is increased due to persistent volume load. Thus in pressure load hypertrophy (concentric hypertrophy) the thickness of myocardium to chamber dimension ratio is increased (Lorell & Carabello, 2000).

LVH is not only the hypertrophy of the muscle but some molecular and structural changes also occur. Along with muscle hypertrophy, the cardiac vasculature undergo as remodelling (Gradman & Alfayoumi, 2006). The extracellular matrix of the heart is disturbed and elastin to collagen ratio is altered. Major changes occur in interstitium where increased amount of collagen is observed in the heart (Gao et al., 2005). It is of interest that hypertrophy of the cardiomyocyte without fibrosis or vascular changes do not appear to have adverse prognosis (Gradman & Alfayoumi, 2006).

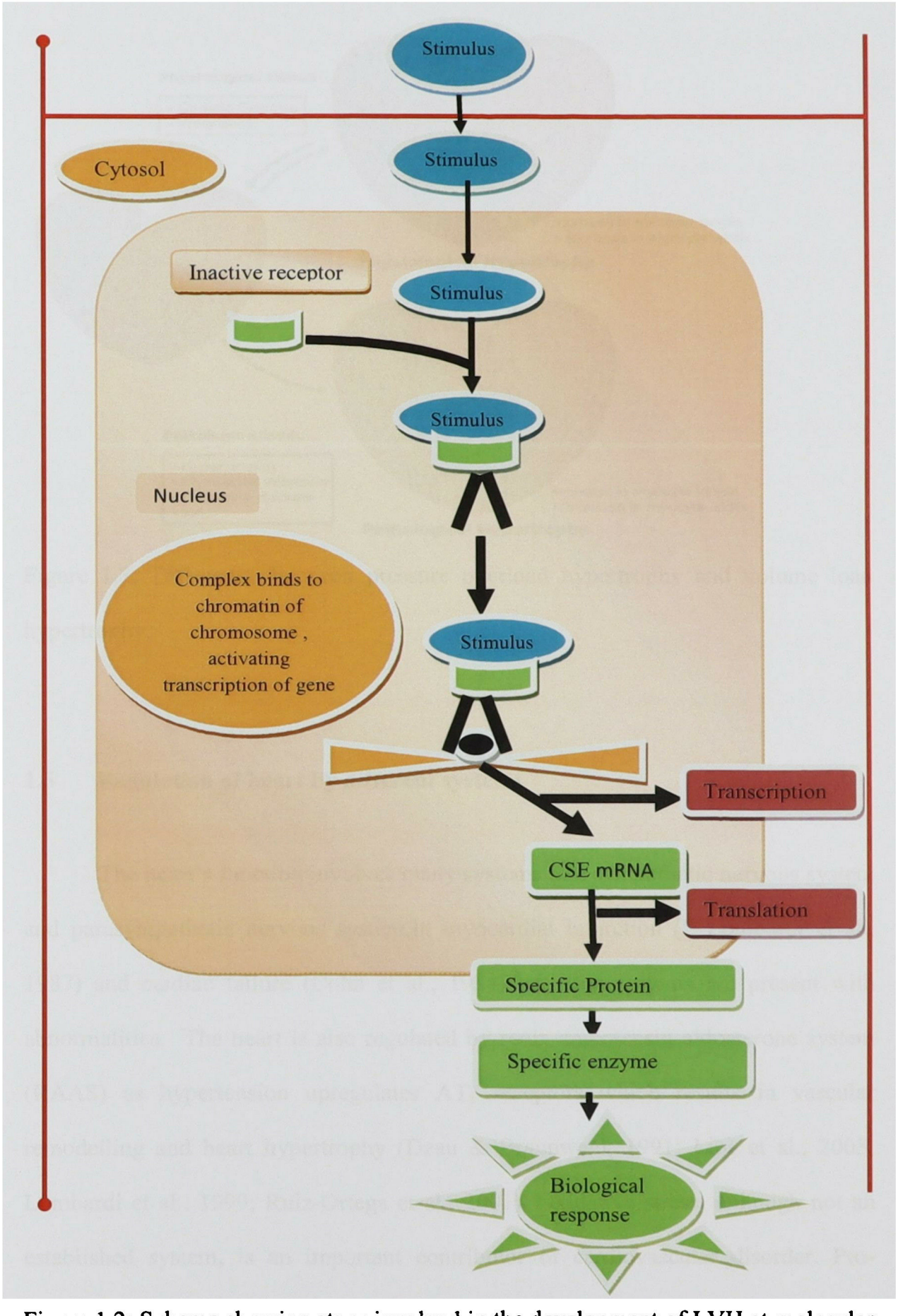


Figure 1.2: Scheme showing steps involved in the development of LVH at molecular level

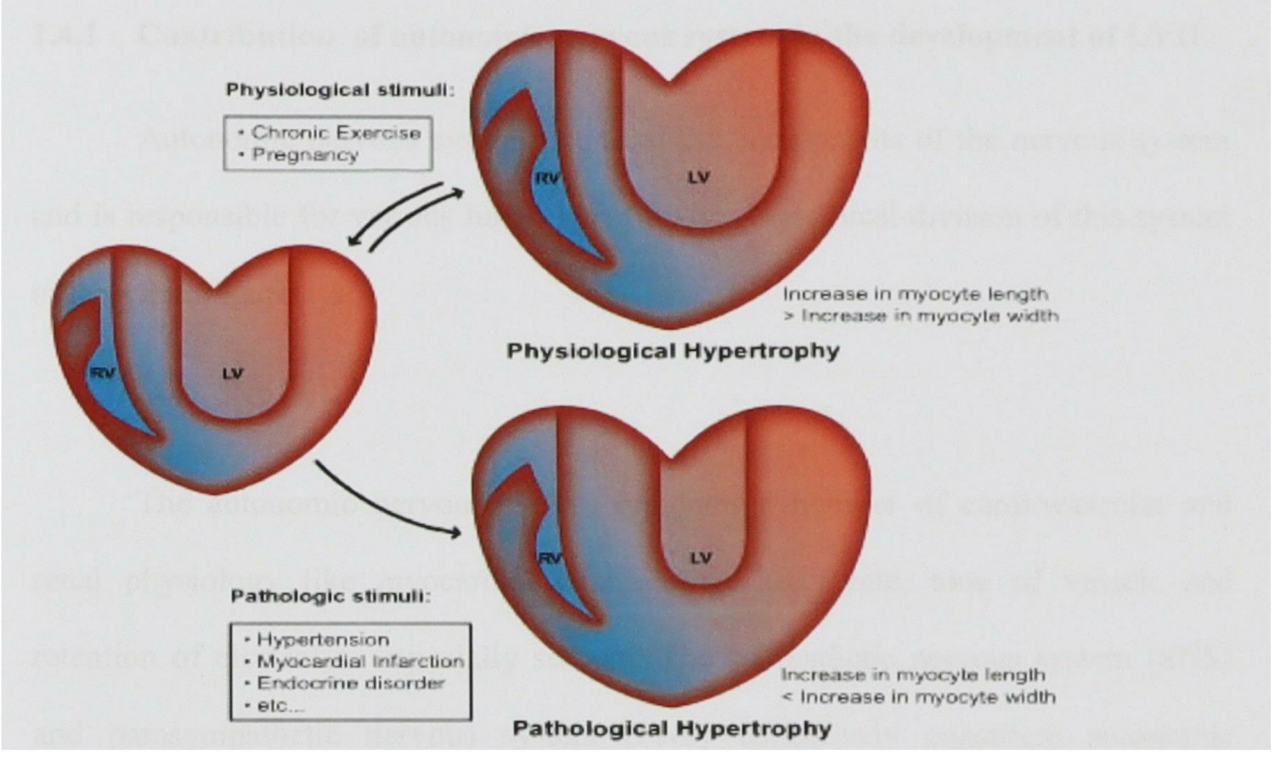


Figure 1.3: Difference between pressure overload hypertrophy and volume load hypertrophy.

1.5 Regulation of heart by different systems

The heart's function involves many systems like sympathetic nervous system and parasympathetic nervous system.In myocardial infarction (F Lombardi et al., 1987) and cardiac failure (Cohn et al., 1984) both the systems are present with abnormalities. The heart is also regulated by renin-angiotensin aldosterone system (RAAS) as hypertension upregulates AT_1 receptors which results in vascular remodelling and heart hypertrophy (Dzau & Braunwald, 1991; Lips et al., 2003; Lombardi et al., 1999; Ruiz-Ortega et al., 2001). Oxidative stress, although not an established system, is an important contributor of cardiovascular disorder. Pro-oxidants and antioxidants play a vital role in pathogenesis and treatment of cardiovascular ailments.

1.4.1 Contribution of autonomic nervous system in the development of LVH

Autonomic nervous system is one of the components of the nervous system and is responsible for various functions of body. Anatomical division of this system is shown in Figure 1.4.

The autonomic nervous system regulates a number of cardiovascular and renal physiology like myocardial contractility, heart rate, tone of vessels and retention of electrolyte especially sodium. The sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) collectively constitute autonomic nervous system which is responsible for the regulation of normal cardiovascular system. Normally the parasympathetic nervous system is dominant over the SNS with the regulation of vascular tone and cardiac output. It has been observed that abnormalities in sympathetic and parasympathetic nervous systems exist in cardiovascular disease states like heart failure (HF) (Casolo et al., 1989; Cohn et al., 1984; Hasking et al., 1986; Kaye et al., 1995) and myocardial infarction (MI) (Lombardi et al., 1987). Studies have demonstrated that in myocardial infarction and heart failure the degree of abnormality in the autonomic nervous system dysfunction is strong and can be an indicator of prognosis (Nolan et al., 1998; Rovere et al., 1998).

1.5.1.(a) Role of Sympathetic and parasympathetic nervous system in cardiovascular system and left ventricular hypertrophy

There are two neurotransmitters of the autonomic nervous system, acetyl choline (Ach) and noradrenaline (NA). The Ach is released from parasympathetic post ganglionic neurons while NA is released from post ganglionic neurons of the sympathetic nervous system. Ach produces its pharmacological action by binding to receptors called cholinergic receptors. NA produces its pharmacological actions by binding to receptors called adrenergic receptors. The autonomics nervous system regulates different actions of the body by acting on these cholinergic and adrenergic receptors receptors. However, with the development of cardiovascular complications, the SNS predominates over PNS. This heightened SNS activity leads to several pathologies like vasoconstriction, sodium reabsorption and ventricular hypertrophy thus leading to heart failure. Adrenergic receptors are divided into two main categories (1) α-adrenoreceptors (2) β-adrenoreceptors. These receptors are further classified into sub-categories. These receptors exhibit various functions and are shown in Figure 1.4, whereas the location of these adrenoreceptors and their physiological action on different organs are shown in Figure 1.5.

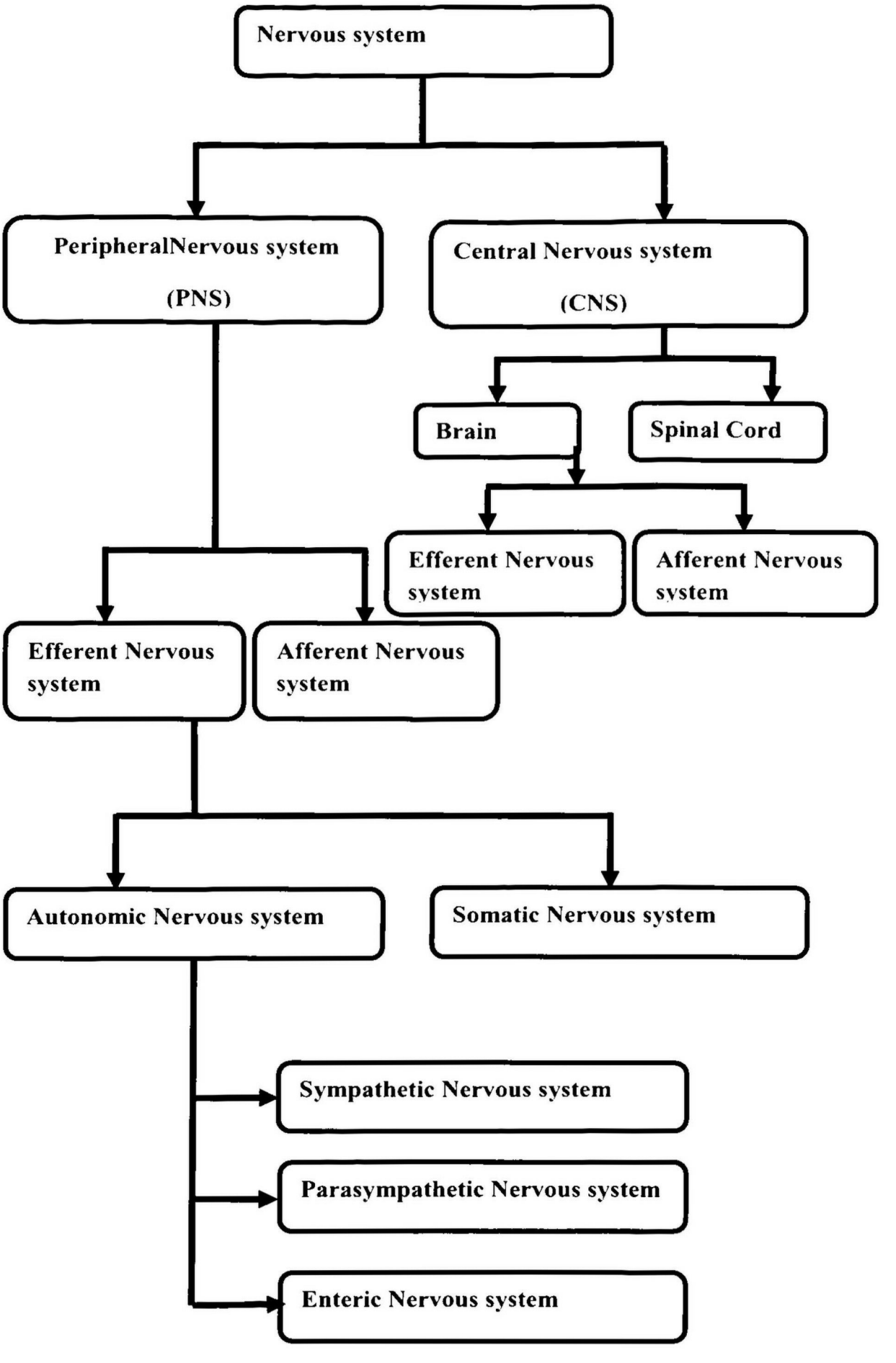


Figure 1.4: Anatomical organization of the nervous system

The sympathetic nervous system plays a key role in the pathogenesis of LVH and its activation is implicated in the occurance of LVH (Julius, 1998; Mancia et al., 1999). Noradrenaline (NA), a neurotransmitter of the SNS, and is involved in the hypertrophy by stimulating the growth of myocyte protein. It has been proventhat SNS and RAS contribute to the development of LVH and are involved in regression of LVH by antihypertensive drugs (Pfeffer et al., 1982). In cardiac tissue, the regional concentration of NA contributes to the development of LVH in early stages (Dang et al., 1999). Some studies suggested that SNS is important in the early stages of development of LVH and hypertension in SHR (Adams et al., 1989; Bevan, 1984; Folkow, 1982; Folkow et al., 1972). The sympathetic hyperactivity results in the imbalance of the ANS in LVH and heart failure (HF) is reflected by the beta-blockade which reduces the heart rate (HR) that is marked as twice than the control (Coumel et al., 1991). Another study documented that LVH with hypertension is associated with increased sympathetic activity that is confined to the heart, suggesting cardiac NA is responsible for the development of LVH (Schlaich et al., 2003).

There is an increasing body of evidence that suggests non hemodynamic factorsto stimulate the LVH (Kelm et al., 1996; Patel et al., 1991). However, these findings have not been seen in humans. Animal experimental data have shown that NA is linked to the mass of LVH and thus, called a myocardial hypertrophic neurohormone (Patel et al., 1991; Simpson, 1983). An increasing number of studies account the SNS for the onset of LVH. NA concentration in regional cardiac tissue as well as systemic circulation is considered to increase the mass of LV by stimulating cell myocyte protein. Noradrenaline is a neurotransmitter of the SNS and

excitation of sympathetic nervous system causesthe release of catecholamines. These catecholamines have effects on the heart and peripheral tissues. On the heart, locally they enhances the cardiac stimulation by acting on β_1 receptors which increases the heart rate along with regional cardiac tissue proliferation. An increase in the systemic concentration elevates the total peripheral resistance by constricting the arteries by its actionon α-adrenergic receptors. The different functions of α and β receptors and their classification is shown in Figure 1.5. The involvement and functions of SNS and PNS in different organ is shown in Figure 1.6.

The activity of the heart is controlled by β_1 adrenoreceptors which are located on the heart. These adrenoreceptors are responsible for chronotropic, ionotropic and dromotropic activities as shown in Figure 1.4. The establishment of LVH is due to an overactivity of heart, which results in over performance of the left ventricle. This enhanced activity is due to the excited β_1 adrenoreceptor which is under direct control of SNS. As such in LVH, over stimulation or excitation is expected to trigger LVH and offset the SNS and PNS activities. Noradrenaline acts on β_1, α_1 and α_2 receptors. Thus, NA not only increases the heart rate but also the total peripheral resistance. It is thought that the heart contractility is increased by NA acting on β_1 receptors which ultimately makes left ventricle to pump more blood into the peripheral parts of the body and increase the thickness of the left ventricle. This action of NA in the onset of LVH along with angiotensin II is supported by the literature (Huang et al., 2012; Kelm et al., 1996).

Conversly, total peripheral resistance increases the afterload and makes the left ventricle to apply more pressure to force blood out of the heart. Hence, increased contractility and increased after load contributes to the onset of LVH. Furthermore, another function of NA is myocyte proliferation. Hence, by acting on the SNS, NA contributes to the onset of LVH and systemic elevation of NA contributes to damage of other key organs like kidneys. This notion can be supported by the evidence that plasma noradrenaline level predicts the survival and incidence of cardiovascular events in end stage renal disease (Zoccali et al., 2002).

It has been reported in the data that an increase in LV mass is related to renal nerve sympathetic activity in LVH model of isoprenaline and caffeine (Burns et al., 2007). It can be predicted that any drug having sympathetic inhibition activity can be an effective therapeutic option in LVH. In human, renal denervation technique by means of sympathoinhibition result in amelioration of heart mass and LV function (Mathias C Brandt et al., 2012). Although catecholamines are considered the culprit for cardiovascular abnormality, reported data shown that central activation of SNS is associated with human LV mass irrespective of the fact that this increase is categorized as LVH (Burns et al., 2007).

1.5.1.(b) Physiological functions of adrenoreceptors

α-Adrenoreceptors have been classified into α_1-adrenoreceptors and α_2-adrenoreceptors.

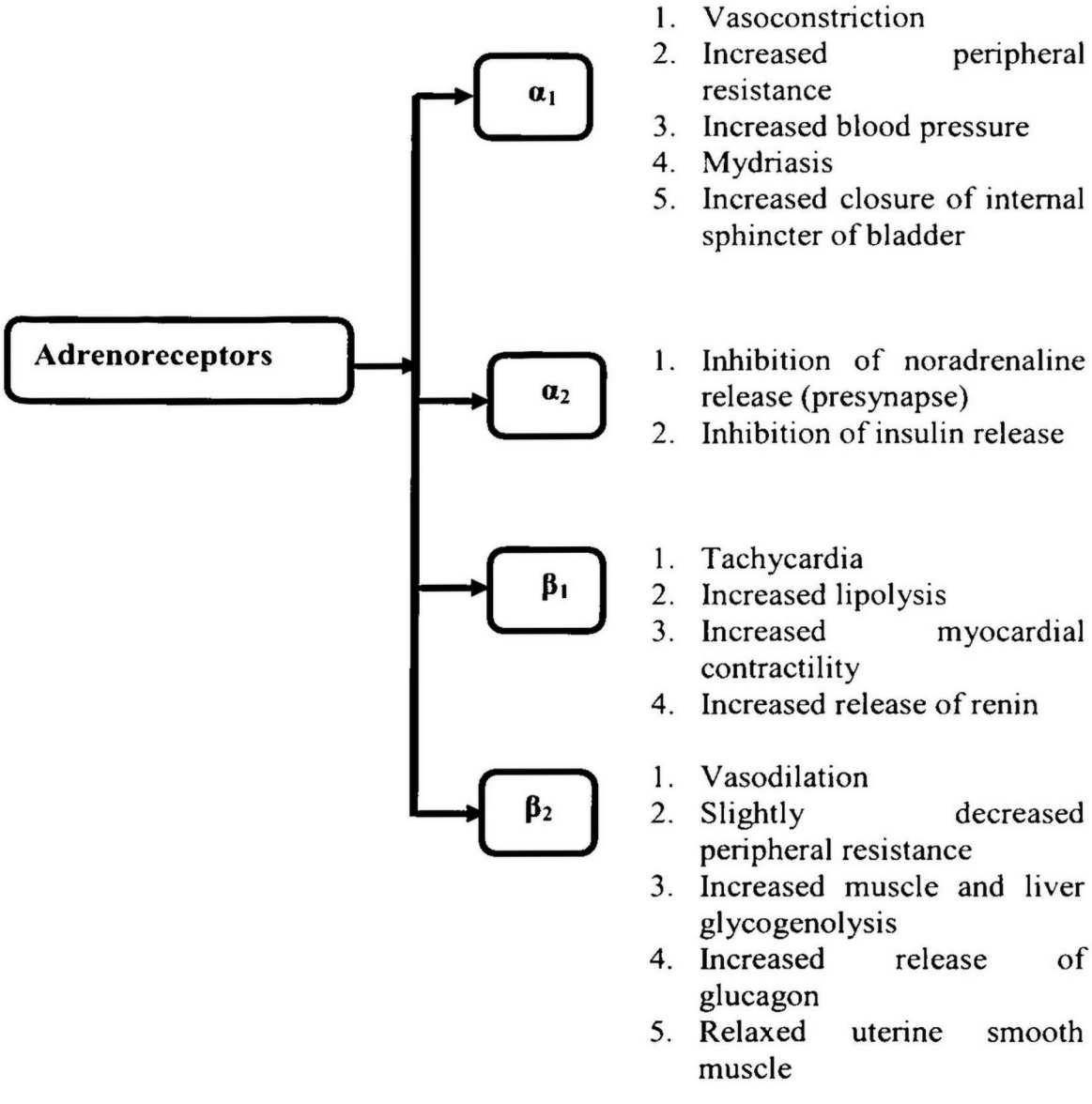


Figure 1.5: Classification and various functions mediated by adrenoreceptors

	Functions	Dominant Receptor	SNS Adrenergic responses	PNS Cholinergic responses
Heart	Rate of contraction	β_1	High	Low
	Force of contraction	β_1	Increase	None
	Conduction velocity	β_1	Faster	Slower
Eye	Pupil size	α_1	Mydriasis	Miosis
Bronchial smooth muscle		β_2	Relaxation	Contraction
Veins				
GIT	Tone, motility and secretory activity	α_2, β_2	Decrease	Increase
Skeletal muscle		α_1, β_2	Contraction	No enervation
Urinary bladder	Detrusor muscle	B	Relaxation	Contraction
Liver		α_1, β_2	Increase adrenergic activity	None
Renin secretion		β_1	Increase	None
Insulin secretion		α_2	Decrease	Increase

Figure 1.6: Locations of adrenoreceptors in various parts of body and their physiological functions (drawn by using MS word)

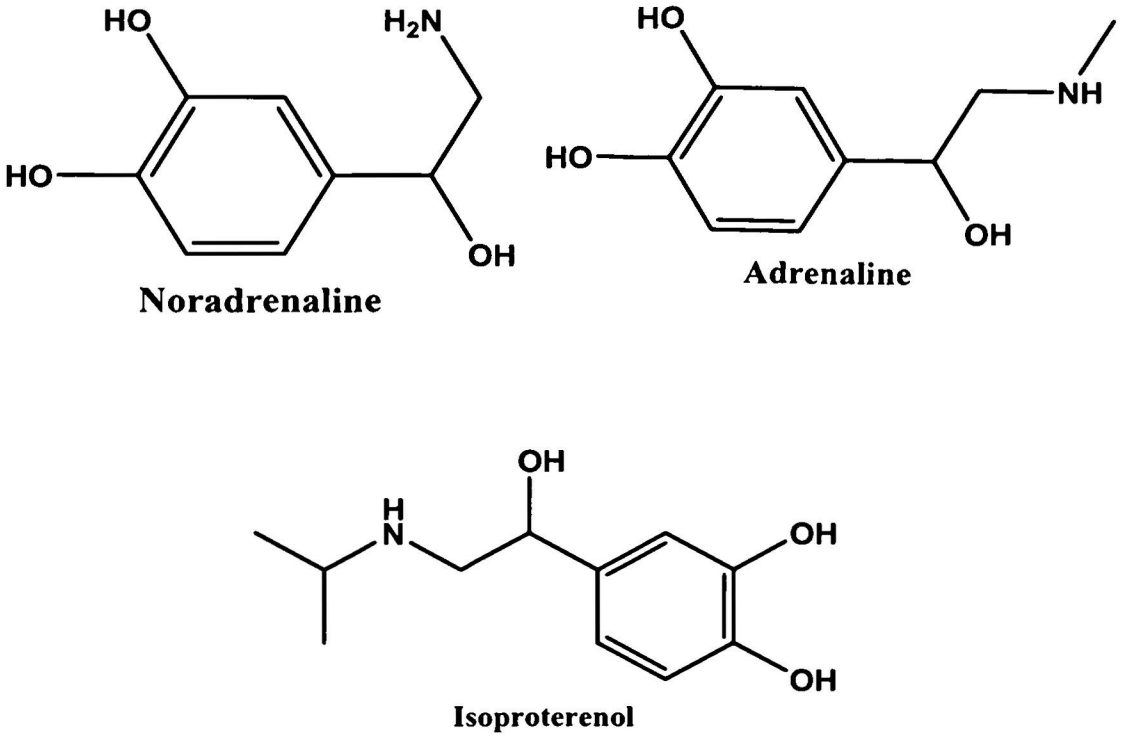


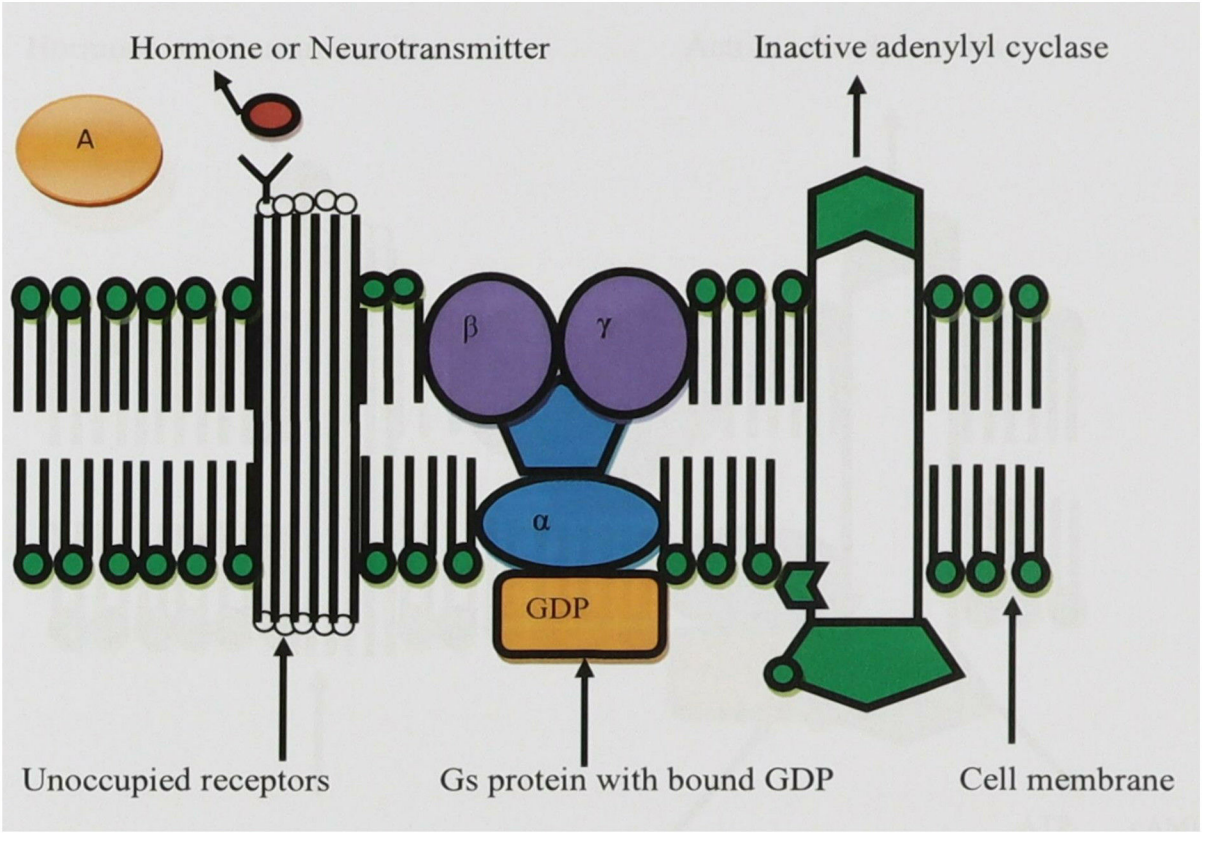
Figure 1.7: Structure of few noradrenaline, adrenaline and isoproterenol responsible for hyperactivity of sympathetic nervous system.

It is established that heart hypertrophy or LVH is due to over activity of the heart due to increased work load. This work load could be the consequence of increased peripheral resistance or due to increased excitability of the heart. In the former situation α_1 receptors are responsible for increased vasoconstriction in peripheral parts of the body. However, in the latter case, β_1 adrenergic receptors are responsible for chronotropic action. The therapeutic option may either decrease the total peripheral resistance (TPR) or decrease the chronotropic action of the heart. It is reported that blocking either α_1 or $\beta_1$receptors resulted in partial reduction of trophic responses while trophic responses were abolished by blocking both receptorss (Zierhut & Zimmer, 1989). Hence, inhibition of hyperactivities of the heart will not only reduce TPR and HR but also attenuate SNS which is implicated in LVH. The

SNS activation is due to increased release of norepinephrine and hyperactivation of β receptors. Thus continuous exposure to NA leads to ventricular remodelling (Adams, 2004). It is also worthwhile mentioning that adrenergic receptors are G-protein coupled receptors and exhibit their responses by acting on G protein.

1.5.1.(c) G-protein coupled receptors and second messenger pathway system for adrenergic receptor

Each subtype of adrenergic receptors has specific affinity for G-protein type either Gq or Gi. α_1 adrenoceptors show preference for Gq, α_2 adrenoceptors link with Gi while β_2 adrenoceptors also couple with Gi (Kirstein & Insel, 2004). G proteins having subunits, an α subunit that bind to guanosine triphosphate (GTP) and βγ subunits. The binding of an appropriate ligand to the receptors on the extracellular surface activates G proteins. This activation leads to the replacement of GDP by GTP on α subunit so α-GTP and βγ subunits act on the other cellular effectors called second messenger as shown in Figure 1.7. A common pathway for the activation of Gs protein is activation of adenylyl cyclase by α-GTP thatresults in the production of cyclic adenosine mono phosphate (cAMP). This cAMP is second messenger and involved in protein phosphorylation. Other than cAMP, G protein also activates phospholipase C responsible for the generation of inositol-1,4,5- triphosphate (IP3) and diacylglycerol (DAG). These effectors regulate the calcium concentration in the cell. This family of receptor transduce signals from light, odors and neurotransmitter like norepinephrine, dopamine, serotonin and acetylecholine.



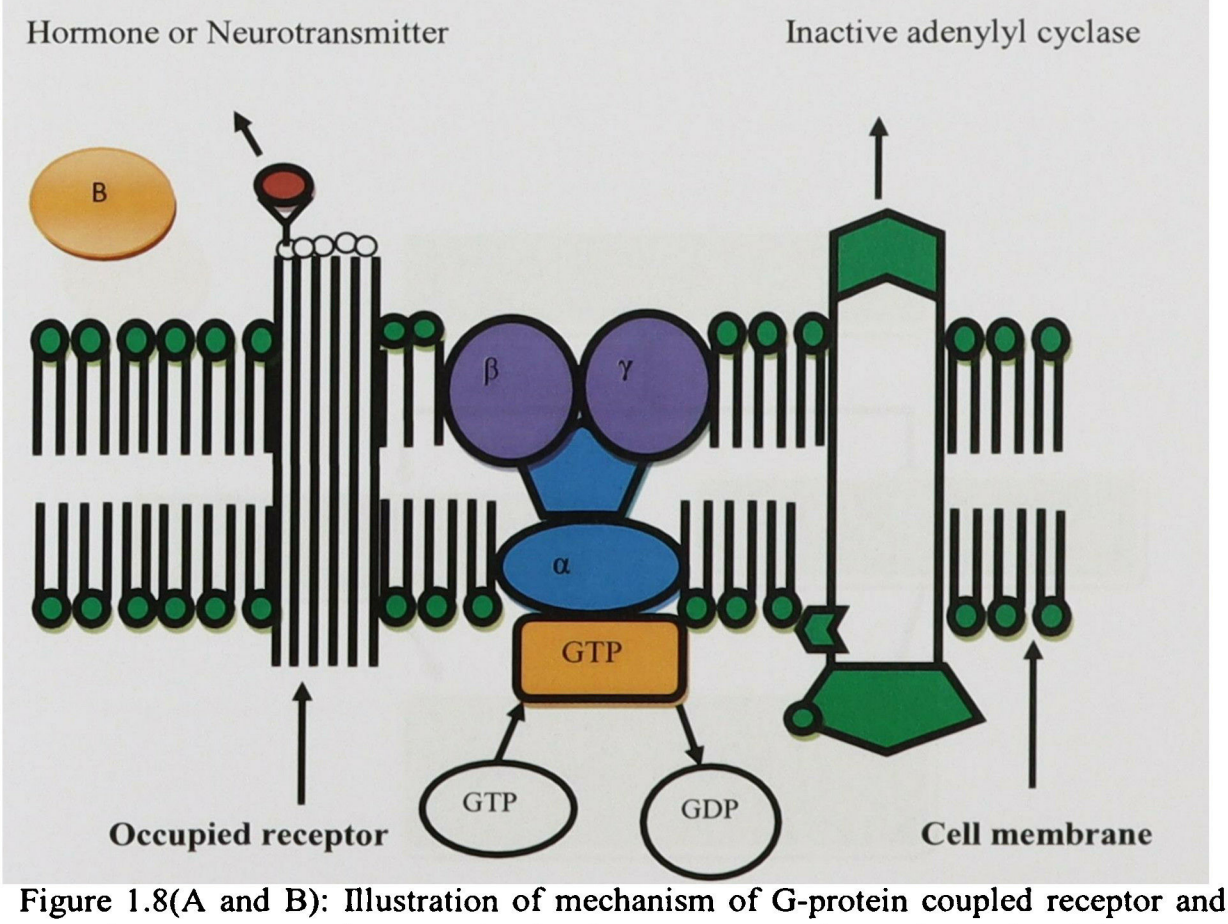
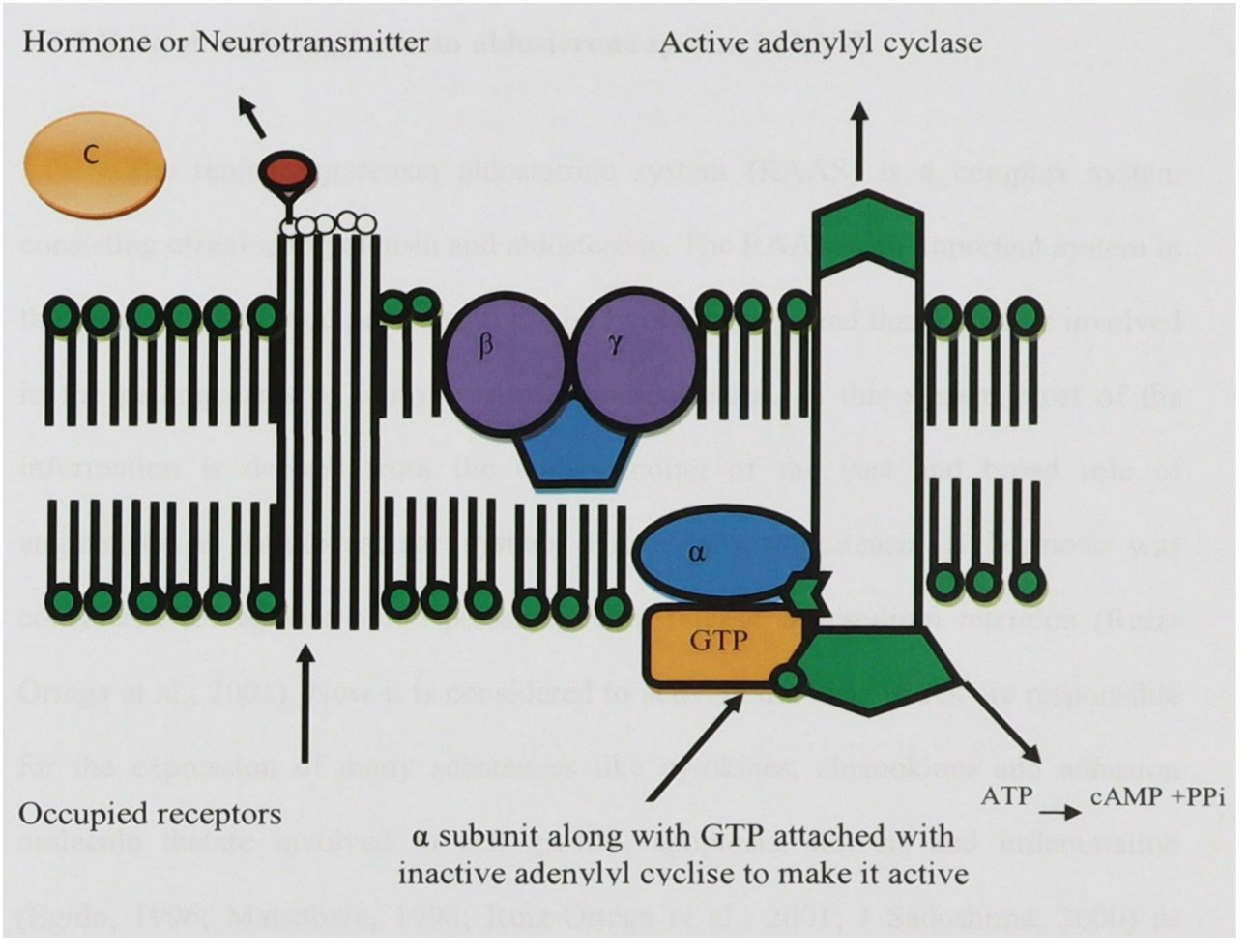


Figure 1.8(A and B): Illustration of mechanism of G-protein coupled receptor and second messenger pathway activation by neurotransmitter when adenylyl cyslase inactive.



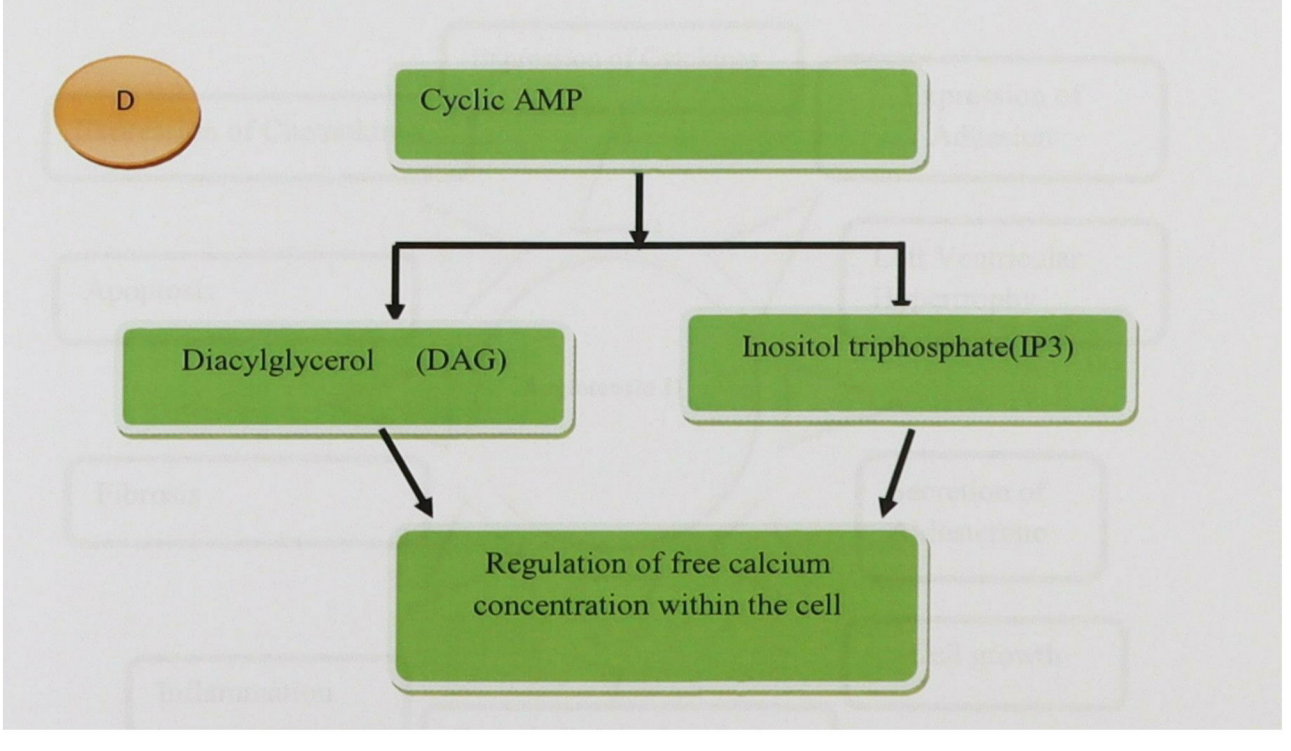


Figure 1.8 (C and D) Illustration of mechanism of G-protein coupled receptor activation (C) by adenylyl cyclase and second messenger pathway activation (D).

1.5.2 Role of renin angiotensin aldosterone system in LVH

The renin angiotensin aldosterone system (RAAS) is a complex system consisting ofrenin, angiotensin and aldosterone. The RAAS is an important system in the regulation of blood pressure. Huge body of data reported that RAAS is involved in the pathogenesis of many cardiovascular diseases. In this system most of the information is derived from the understanding of the vast and broad role of angiotensin in cardiovascular system. Historically angiotensin II hormone was considered to regulate blood pressure, renin release and sodium retention (Ruiz-Ortega et al., 2001). Now it is considered to activate the cells which are responsible for the expression of many substances like cytokines, chemokines and adhesion molecule thatare involved in cell growth, apoptosis, fibrosis and inflammation (Egido, 1996; Matsubara, 1998; Ruiz-Ortega et al., 2001; J Sadoshima, 2000) as shown in Figure 1.9.

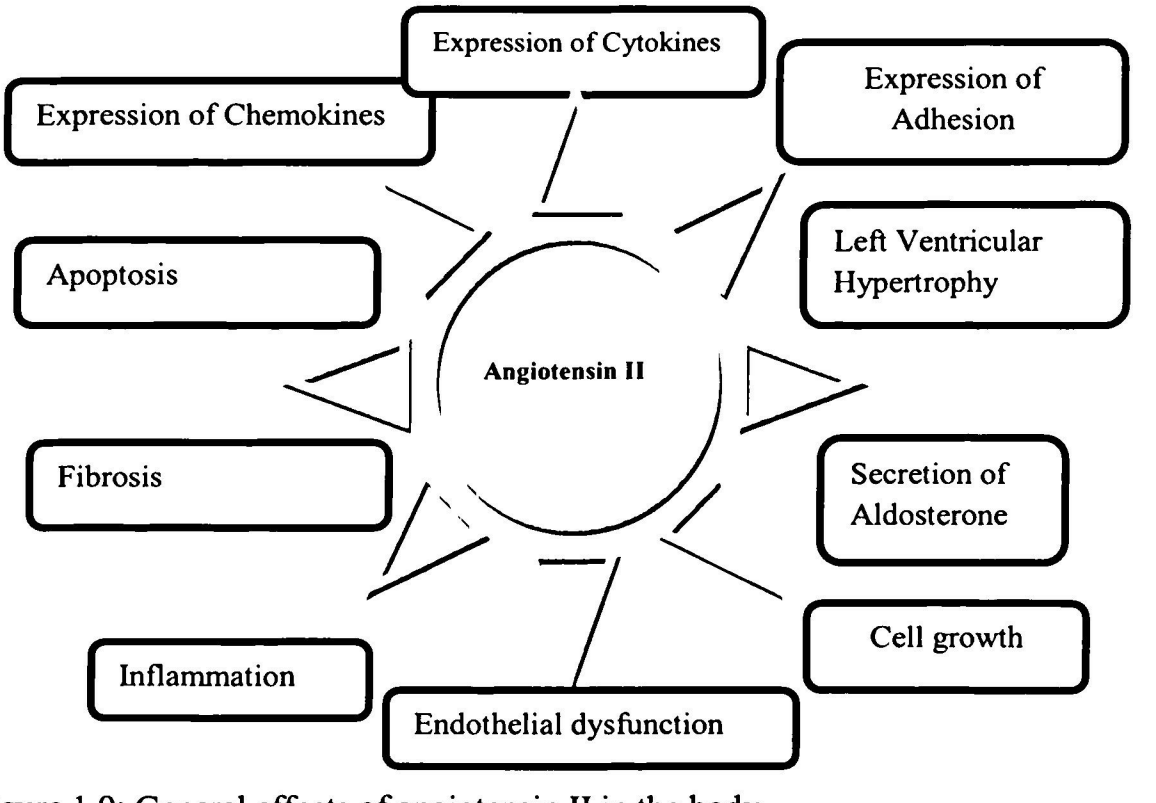


Figure 1.9: General effects of angiotensin II in the body

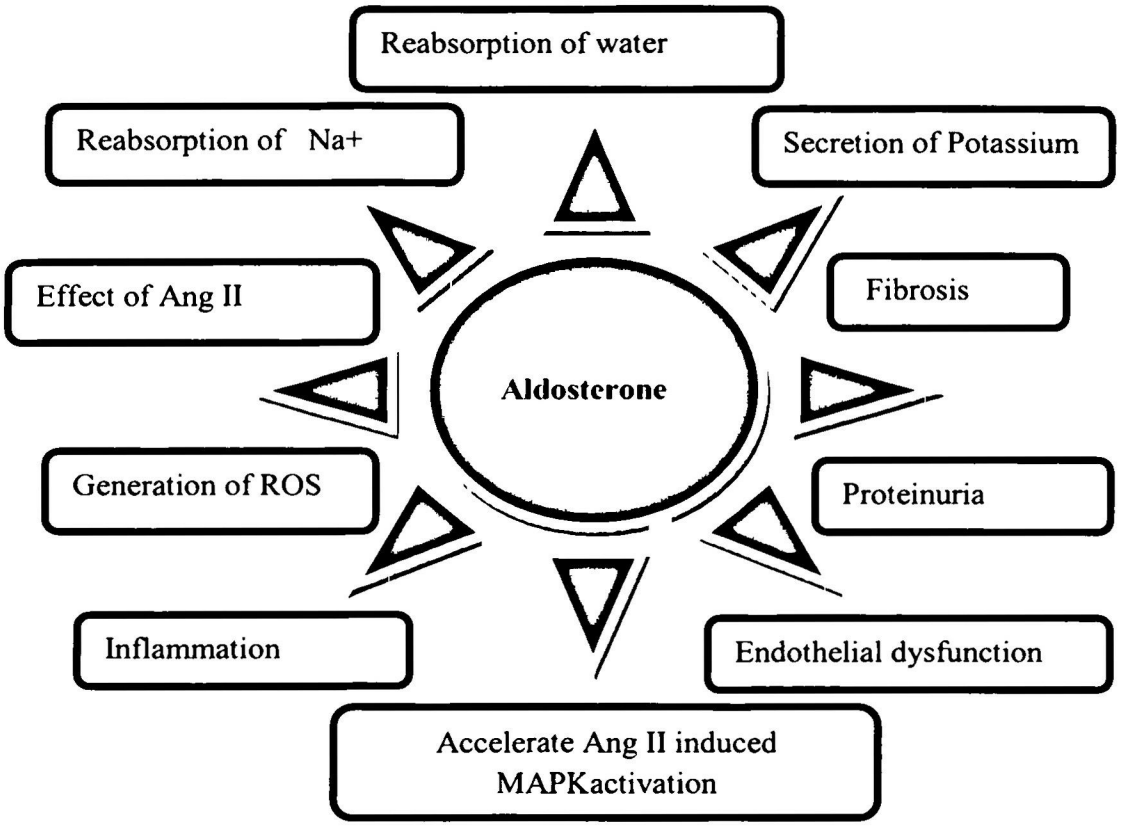


Figure 1.10: Effects of aldosterone in the body

The activation of this pathway start from renin and is completed with the secretion of aldosterone (AD). The AD has substantial importance in RAAS as a controlling mechanism of renal hemodynamics by altering renal reabsorptions and excretions. Various functions of AD are shown in figure 1.10. Different steps involved in this system are shown in figure 1.11.

The RAAS is a multicomponent system with a broad role in different biological systems. The cardiovascular system is mainly controlled by SNS and

RAAS. Of the component of RAAS, angiotensin II and aldosterone are more important as shown in figure 1.10 and are involved in the regulation of CVS.

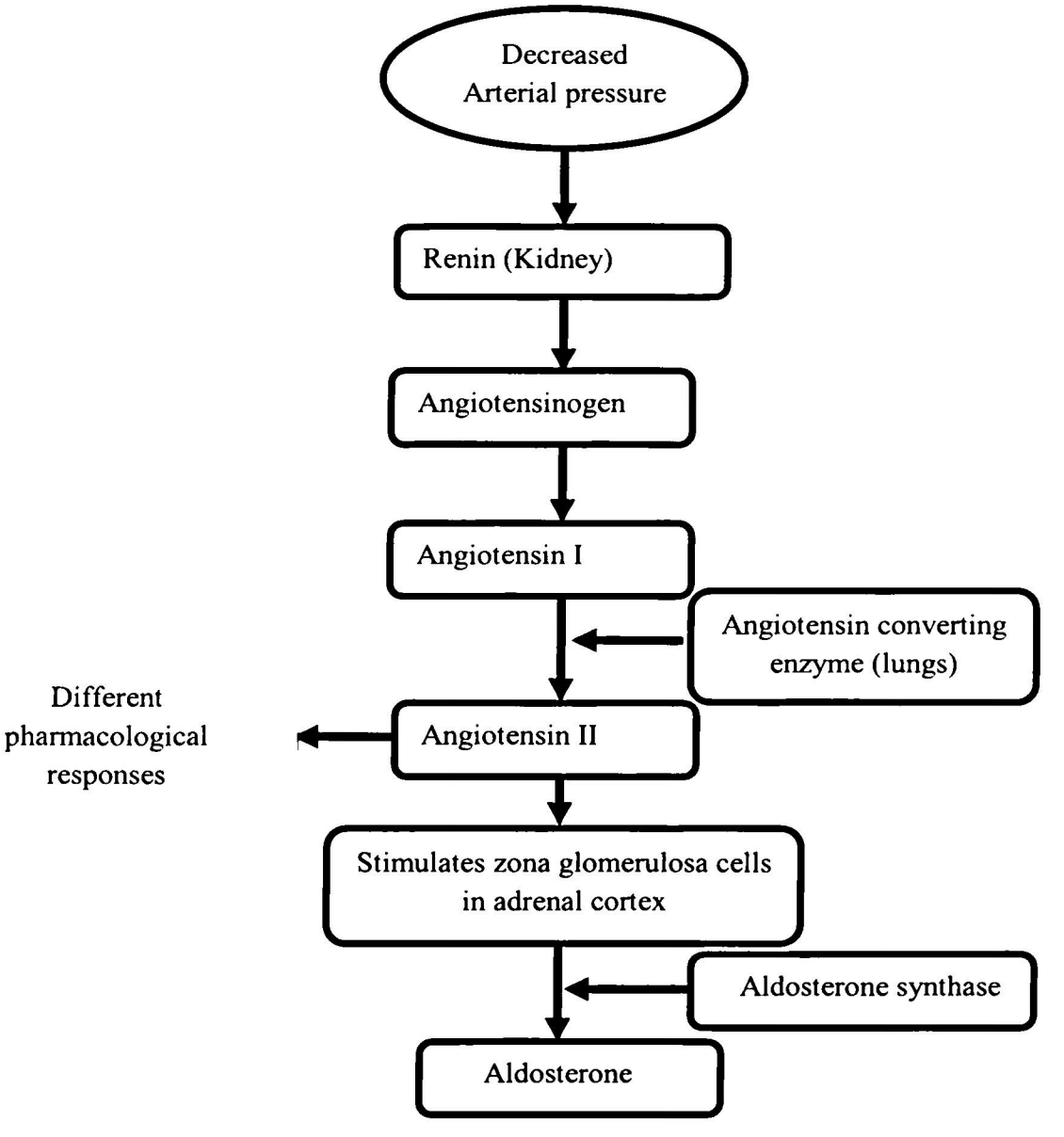


Figure 1.11: Schematic pathway of Renin-Angiotensin Aldosterone System (RAAS)

The RAAS is considered to have an inverse relationship with LV systolic functions in primary and secondary hypertension (Devereux et al., 1987).