INTERACTION OF SYMPATHETIC NERVOUS SYSTEM AND RENIN ANGIOTENSIN SYSTEM IN SYSTEMIC CIRCULATION OF CARDIAC HYPERTROPHIC RAT MODEL WITH Gα PROTEIN EXPRESSION INTERFACE

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by

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NurJannah binti Mohamad Hussain
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%	percent
^{o}C	degree celsius
α	alpha
β	beta
βARK-1	β-adrenoceptors kinase-1
γ	gamma
δ	delta
ε	epsilon
ζ	zeta
µg	microgram
µg/ml	microgram per mililiter
µl	microliter
µm	micrometer
µl/min	microliter per minute
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
AMP	adenosine monophosphate
Ang II	angiotensin II
Ang	angiotensin
ANOVA	analysis of variance
ALLHAT	Antihypertensive and Lipid-Lowering Teatment to Prevent Heart Attack Trial
Ao	aorta
AT_1	angiotensin II receptor type 1
AT_2	angiotensin II receptor type 2
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
AV	atriaventricular
BLAST	Basic Local Alignment Search Tool
BP	blood pressure
Ca^{2+}	calcium ion

cAMP	cyclic adenosine monophosphate
cDNA	complimentary deoxyribonucleic acid
cGMP	cyclic guanosine monophosphate
CH	cardiac hypertrophy
CHATE	cardiac hypertrophic rats treated with atenolol
CHDOX	cardiac hypertrophic rats treated with doxazosin
CHLOS	cardiac hypertrophic rats treated with losartan
CO	cardiac output
COMET	Carvedilol or Metoprolol European Trial
COX-1	cyclooxygenase
DAG	diacylglycerol
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
DOCA	deoxycorticosterone acetate
DPX	dextropropoxyphene
e.g.	for example
ECG	electrocardiogram
et al	and others
ET	endothelin
g	gram
GDP	guanine diphosphate
GPCRs	G protein coupled receptors
GRKs	GPCR kinases
GTP	guanine triphosphate
GTPase	guanine triphosphatase
Gα	alpha subunit of G protein
h	hour
HR	heart rate
I_{CaL}	L-type calcium current
i.e.	that is
i.p.	intraperitoneally
i.v.	intravenously
IP_3	inositol triphosphate
K^+	potassium ion

kb	kilobasepair
kDa	kilodalton
kg	kilogram
kpa	kilopascal
LA	left atrium
LIFE	Losartan Intervention for Endpoint Reduction in Hypertension
LV	left ventricle
M	muscarinic receptor
MAP	mean arterial pressure
MAPKs	mitogen activated protein kinases
ME	methoxamine
mg	milligram
mg/kg	milligram per kilogram
mg/ml	miligram per mililiter
$MgCl_2$	magnesium chloride
ml	mililiter
ml/cycle	mililiter per cycle
ml/min	mililiter per minute
mM	milimol
M-MLV	Moloney Murine Leukemia Virus
mRNA	messenger ribonucleic acid
n	number of animals
NA	noradrenaline
NAD	nicotinamide adenine dinucleotide
NCBI	National Center for Biotechnology Information
ng	nanogram
nm	nanometer
nNOS	neuronal NO syntase
NO	nitric oxide
NOS	nitric oxide syntase
O_2	oxygen
PA	pulmonary artery
PCR	polymerase chain reaction
PE	phenylephrine

pi	pound-force per square inch
PIP_2	phosphatidylinositol biphosphate
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PR	peripheral resistance
RA	right atrium
RAS	renin angiotensin system
RGS	regulation of G protein signalling
RNA	ribonucleic acid
RNase	ribonuclease
RT-PCR	reverse transcriptase-polymerase chain reaction/ real time polymerase chain reaction
RV	right ventricle
SA	sinoatrial
SHR	spontaneous hypertensive rat
SNP	single nucleotide polymorphism
SNS	sympathetic nervous system
SV	stroke volume
TPR	total peripheral resistance
U/µl	unit per microliter
UV	ultraviolet
V	volt
w/v	weight per volume
v/v	volume per volume
WKY	Wistar Kyoto
WKYATE	Wistar Kyoto rats treated with atenolol
WKYDOX	Wistar Kyoto rats treated with doxazosin
WKYLOS	Wistar Kyoto rats treated with losartan

INTERAKSI SISTEM SARAF SIMPATETIK DAN SISTEM RENIN ANGIOTENSIN PADA PEREDARAN DARAH SISTEMIK DI MODEL TIKUS HIPERTROFIK JANTUNG BERSAMA EKSPRESI Gα PROTEIN

ABSTRAK

Penyelidikan ini mengkaji kaitan di antara sistem saraf simpatetik (SNS) dan sistem renin angiotensin (RAS) di dalam hipertrofi jantung. Kajian hemodinamik dilakukan untuk menilai respon vasokonstriksi adrenoseptor (iaitu adrenoseptor α_1 dan β_1) dan reseptor angiotensin II jenis 1 (AT_1) untuk memahami peranan fungsi SNS dan RAS dengan menggunakan model tikus hipertrofi jantung. Selanjutnya, penyelidikan ekspresi mRNA protein Gα melalui real-time PCR (RT-PCR) juga dilakukan untuk menilai beberapa isotype daripada 3 kumpulan protein Gα yang berbeza iaitu protein $G\alpha_s$, $G\alpha_i$ dan $G\alpha_{q/11}$ pada jantung tikus. Tikus WKY digunakan dan induksi hipertrofi jantung dilaksanakan dengan 2 dos isoproterenol (5 mg/kg) secara subkutenius dan dengan 40 mg/kg kafein di dalam larutan 1% sebanyak dua kali sehari secara oral selama 7 hari. Selain itu, blocker iaitu atenolol, doxazosin dan losartan juga diberikan, untuk menyiasat ciri antagonis ubat-ubat ini dalam menyekat reseptor tersebut, untuk melihat reaksi reseptor dan mentafsirkan ekspresi protein Gα. Peningkatan yang signifikan pada indeks jantung kepada tubuh dan garis dasar MAP pada tikus hipertrofi jantung berbanding dengan tikus WKY menunjukkan pengaktifan simpatetik. Selain itu, penilaian histologi pada jantung mengesahkan pembentukan hipertrofi jantung di dalam model tikus. Pengurangan fungsi adrenoseptor α_1 pada tikus hipertrofi jantung, mencadangkan rangsangan berlawanan untuk merendahkan SNS yang semakin meningkat yang disebabkan oleh peningkatan fungsi adrenoseptors β_1. Peningkatan fungsi adrenoseptors β_1 ini disokong oleh pemerhatian terhadap amplifikasi ekspresi mRNA protein $G\alpha s_{45}$ di dalam jantung hipertrofi. Tampaknya terdapat kaitan di antara kedua-dua

adrenoseptor di dalam pengaturan SNS dimana adrenoseptors α_1 boleh menyekat stimulasi adrenoseptors β_1 namun begitu, adrenoseptors β_1 tidak mempunyai sebarang kesan terhadap stimulasi adrenoseptors α_1. Fenomena ini juga diamati dalam hubungan di antara SNS dan RAS dimana reseptor AT_1 berperanan mempengaruhi pengaktifan adrenoseptors α_1 tetapi interaksi ini tidak berfungsi sebaliknya. Penyelidikan ini juga menunjukkan interaksi lain yang menghubungkan RAS dan SNS iaitu interaksi langsung di antara reseptor AT_1 dengan adrenoseptor β_1. Fungsi reseptor AT_1 dan interaksi dengan adrenoseptor α_1 dan β_1 di dapati dimodulasi oleh kedua-dua protein $G\alpha_q$ dan $G\alpha_{11}$ di dalam keadaan fisiologi normal mahupun jantung hipertrofi. Namun yang demikian, interaksi di antara adrenoseptor dengan protein $G\alpha_{q/11}$ di dalam jantung berbeza di mana protein $G\alpha_q$ berkait rapat dengan stimulasi adrenoseptors β_1 sedangkan protein $G\alpha_{11}$ lebih berkaitan dengan stimulasi adrenoseptors α_1. Perbezaan di antara subtype protein $G\alpha s_{45}$ dan $G\alpha s_{52}$ juga dicatat untuk pengesahkan penglibatan adrenoseptors β_1 di dalam keadaan patofisiologi dan fisiologi. Sebagai kesimpulan, data-data ini mempunyai maklumat yang berharga mengenai hubungan di antara SNS dan RAS di dalam keadaan hipertrofi jantung pada tahap reseptor dan juga pada tahap protein Gα di mana beberapa kes menunjukkan yang protein Gα yang berbeza subtype boleh bertindak berlainan walaupun termasuk di dalam kumpulan yang sama.

INTERACTION OF SYMPATHETIC NERVOUS SYSTEM AND RENIN ANGIOTENSIN SYSTEM IN SYSTEMIC CIRCULATION OF CARDIAC HYPERTROPHIC RAT MODEL WITH Gα PROTEIN EXPRESSION INTERFACE

ABSTRACT

The present study investigates the interrelation between sympathetic nervous system (SNS) and renin angiotensin system (RAS) in cardiac hypertrophy. Haemodynamic studies were performed to evaluate the vasoconstriction responses of adrenoceptors (i.e. α_1- and β_1- adrenoceptors) and angiotensin II type 1 receptors (AT_1) to understand the functional role of SNS and RAS respectively using the cardiac hypertrophic rat model. Furthermore, the mRNA expression studies of Gα proteins via real time-PCR (RT-PCR) were also conducted to assess 2 isotypes from 3 different groups of Gα proteins which are $G\alpha_s$, $G\alpha_i$ and $G\alpha_{q/11}$ proteins in the rat's heart. WKY rats were used and cardiac hypertrophy was induced subcutaneously with 2 doses of isoproterenol (5 mg/kg) and 40 mg/kg of caffeine twice daily by gavage as 1% solution for 7 days. In addition, blockers namely atenolol, doxazosin and losartan were administered to investigate the antagonistic features of the respective drugs in blocking the intended receptors, in order to contemplate the receptors reaction and interpret the expression of Gα protein. Significant increments in the mean heart to body index and MAP baseline values were observed in cardiac hypertrophic rats compared to the WKY rats which depicted sympathetic activation. Moreover, histological evaluation of the heart sections confirmed cardiac hypertrophy in the rat model. Reduced functionality of α_1- adrenoceptors in cardiac hypertrophic rats was suggested to counteract persistent SNS stimulation caused by the increased functionality of β_1-adrenoceptors which was made apparent by the $G\alpha_{s45}$ mRNA expression amplification in the hypertrophic heart. It appears that

crosstalk exists between both adrenoceptors in SNS regulation whereby α_1-adrenoceptors could interrupt β_1-adrenoceptors stimulation, however, β_1-adrenoceptors does not exert any effect on α_1-adrenoceptors. This phenomenon was also observed in a connection between SNS and RAS whereby AT_1 receptors functional role may influence α_1- adrenoceptors activation but the interaction does not work the other way round. Another support of the correlation linking SNS and RAS was also shown in the direct interaction of AT_1 receptors and β_1-adrenoceptors. The functionality of AT_1 receptors and its interaction with α_1- and β_1- adrenoceptors were observed to be modulated by both $G\alpha_q$ and $G\alpha_{11}$ protein mediated pathway in cardiac hypertrophic and physiological states. Nevertheless, the interaction between adrenoceptors with $G\alpha_{q/11}$ protein in normal heart differs as $G\alpha_q$ protein may have interrelated effect with β_1-adrenoceptors signalling while $G\alpha_{11}$ protein may acquire control over α_1-adrenoceptors activation. Discrepancies amongst the $G\alpha_{s45}$ and $G\alpha_{s52}$ protein subtype were also noted in confirming β_1-adrenoceptors participation under pathophysiological and physiological condition. In conclusion, the data provide valuable information regarding the crosstalks between SNS and RAS in cardiac hypertrophic condition at the level of receptors and Gα protein interface where in some cases the different subtypes of Gα protein may act differently despite belonging to the same group.

CHAPTER 1

INTRODUCTION

1.1 Introductory to Cardiovascular Physiology

1.1.1 The Number One Killer

Heart failure is an alarming non communicable disease associated with a dynamic myocardial dysfunction and with poor clinical outcome (El-Demerdash *et al*., 2005). This idiopathic disease is a common clinical problem resulting in significant morbidity and mortality around the world. It has become the number one killer across the globe. Statistics from The World Health Organization (http://www.who.int/en/) estimated that 17 million people died from cardiovascular diseases every year, a staggering 30% of all global deaths.

For the past decade, cardiovascular diseases have claimed 25% death in Malaysia and it is a fatal epidemic that tops the chart of all causes of death in this country and also other developing countries. Through statistical collection, it was shown that acute coronory disease has no race boundaries and the prevalence is close to active smoking habit (Chin *et al*., 2008).

Heart disease causes numerous complications and implications not only to the patients, but it also extends to the community and the nation as a whole. For the past decades, a considerable attention has been put into the health system of assessment and management of heart diseases (Thomas, 2007). Furthermore, multitudes of experimental and clinical studies have been done to comprehend the physiology and pathtophysiology of the heart. Each attempt has brought closer in understanding and appreciating the physiological aspect of cardiovascular system towards improving the mortality and morbidity of heart diseases.

1.1.2 Heart Anatomy

The heart is a muscular pump responsible for pumping blood through the blood vessels by repeated, rhythmic contractions, which provide the necessary force to circulate the blood to all the tissues in the body (Applegate, 2000). The *kardia,* similar to the word cardiac came from ancient Greek which refers to the heart (Opie, 2004). Basically, the heart contains four chambers namely of the right atrium, right ventricle, left atrium and left ventricle. The heart wall is made up of three layers of tissues, i.e. an outer layer epicardium, a middle myocadium and the internal endocardium layer (van Wijk *et al*., 2009).

The heart provides tissues with continuous supply of oxygen and nutrients while removing metabolic waste products from them. Its function is modulated into circulatory system that can be broken down to the systemic circulation, pulmonary circulation and coronary circulation (Applegate, 2000)(Figure 1.1).

The circulations that institute the pathway of the blood through the heart is altogether a continuous flow and we need to apprehend that both of the atrias and ventricles contract at the same time. However, for the ease of understanding, attempts to explain the right heart blood route first, then the left may be applied (Applegate, 2000).

The right atrium receives blood relatively low in oxygen and high in carbon dioxide venous return via the superior vena cava and inferior vena cava. The right atrium also receives blood from the coronory sinus of the coronary circulation, the circulation that provides the heart muscle itself with sufficient supply of oxygen and nutrients. The deoxygenated blood then flow through the tricuspid valve to fill the right ventricle during diastole phase.

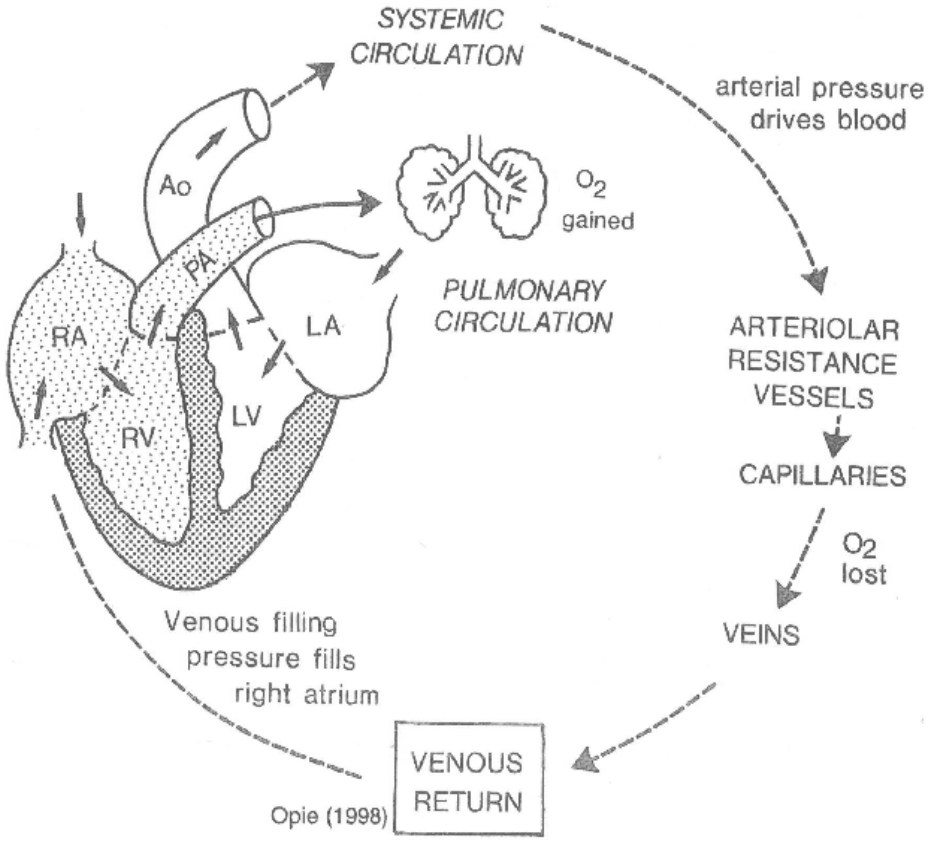

Figure 1.1 Systemic and pulmonary circulation of the heart. The circulation of the blood from right atrium to right ventricle; from the right ventricle through passage of the lung into the left atrium, then to the left ventricle and aorta; to the vascular beds by way of the arteries past the tissues into the veins; eventually back to the base of the heart. RA, right atrium; RV, right ventricle; PA, pulmonary artery; LA, left atrium; LV, left ventricle; Ao, aorta; O_2, oxygen. The faded dotted areas represent deoxygenated blood (Adapted from Opie, 2004).

Subsequently the right ventricle contracts and propels the venous return through the pulmonary semilunar valve and to the pulmonary vascular tree where it gets oxygenated in the capillaries of the lungs. This part of explanation belongs to the pulmonary circulation by which the systolic pressure generated by the right ventricle is much less then the left, as it only requires pushing the blood to the lungs. In addition, the right side of the heart is made out of thinner walled muscular chambers, corresponding to the lesser force it needs compared to the left side of the heart (Burkhoff *et al*., 1984).

Applying to the same analogy as the pulmonary circulation, the systemic circulation begins by the oxygenated blood from the lungs entering the left atrium and during the diastole period, the bicuspid valve opens and allows the blood to the left ventricle. Then, throughout the systole phase, the thick wall of left ventricle contracts building a reasonable huge force that abruptly closes the bicuspid valve and forces the opening of aortic valve, sending the blood to the whole body (Applegate, 2000).

1.1.3 Conduction System

The heart beat and contraction of the four chambers are coordinated and initiated by a wave of electricity that arises voluntarily by specialized cells which are the main players of the heart conduction system. The sinoatrial (SA) nodes, situated at the posterior wall of the right atrium, commonly initiates the electrical impulses for contraction. The impulses spread rapidly through the left and right atria and reach to the atriaventricular (AV) node, which is located beneath the right atrium and close to the interatrial septum. There is a brief delay of impulse travelling in this node, giving time for the atrium to finish their contraction before the ventricle starts contracting. Then, the impulses accelerate to the bundle of His and consequently to the right and left bundles branches which extend to form Purkinje fibers that distribute the impulses throughout the ventricles to produce ventricle contraction (Applegate, 2000; Opie, 2004; Lily, 2007).

1.1.4 Arterial Blood Pressure and Haemodynamic Factors

During the left ventricle contraction, blood is propeled into the aorta which divides into many arteries and later smaller arteries known as arterioles before

reaching the capillaries. The arterial walls are thicker to withstand the pressure, compared to the subsequent vessels heading to the right atrium, known as the veins (Opie, 2004).

Both the arteries and the veins possess the same three layers of intima, media and adventitia (Applegate, 2000). Not only is the composition of each layer between the arteries and veins different, there are also many crucial variances between the bigger vessels and the smaller ones (e.g. aorta and arteriole). This is to accommodate the sudden changes of pressure pattern throughout the circulation in different vessels, from high arterial pressure to low venous pressure (Parati *et al*., 1989).

The aorta stretches during the high pressure of systole and recoil during the diastole in order to attune to the oscillating pressure from the left ventricle (Lily, 2007). However, the high pressure is actually more prominent in the later arteries and arterioles where it provides resistance as the arterial lumen decreases. The arteries play an important role in regulating the blood flow and its velocity hence, controlling the arterial blood pressure or simply known as blood pressure (Opie, 2004).

Blood pressure changes during exercise; responding to emotional status; and having a diurnal variation. The blood pressure from the brachial artery can easily be measured using the sphygmomanometer (Applegate, 2000). It is crucial to monitor the blood pressure because it is an accredit to the hemodynamic factors which impose to cardiac output (CO) and peripheral resistance (PR) that also affect other attributes such as stroke volume (SV) and heart rate (HR). On that account, blood pressure could mirror the well-being of the cardiovascular system. Notably, the progressive and linear increase of blood pressure can predispose a person to hypertension which more often than not, leads to alarming cardiovascular complications (Lily, 2007).

The relations between these hemodynamic factors are depicted from the formulae below.

$$BP = CO \times TPR$$

$$CO = HR \times SV$$

Blood pressure (BP) is influenced by ways of cardiac output (CO) and total peripheral resistance (TPR). In other equation, CO is proportional to the effect of heart rate (HR) and stroke volume (SV). CO (ml/min) is the blood volume ejected from the ventricle per minute, whereas SV (ml/cycle) is the blood volume out from the ventricle per contraction (Applegate, 2000).

SV is dependent on several factors namely preload (i.e. end-diastolic volume), venous return, cardiac contractility and cardiac strength (Applegate, 2000; Lily, 2007). All these extrinsic factors contribute to the Starling law of the heart. Starling law explains that the cardiac muscle fibers stretches as the venous filling pressure increases and the extension of the heart increases its force of contraction which in return augments the SV (Rang *et al*., 2003). The Starling law also proposes about the longitudinal fibrils of cardiac muscles which refers to the cross bridge interaction and also give a view on the intrinsic factors of the heart itself which refer to the aberrant molecular active compound of heart failure that imparts to the modern view of calcium cycle abnormalities in heart failure (Dauterman *et al*., 1995).

All these hemodynamic factors that apply to the heart, blood vessels and the circulation of the cardiovascular system are constantly under the regulation of several signals that change to accommodate the homeostasis of the body. Signals from the autonomic nervous system, renin angiotensin system, baroreflex central control, and also from the vascular bed itself (e.g. nitric oxide signaling, endothelin) are very pivotal in modulating throughout the physiologic adjustment (e.g. during exercise or

rest) and most importantly during the challenges of pathologic alteration in cardiovascular system (Applegate, 2000; Opie, 2004; Lily, 2007).

1.2 Autonomic Nervous System

1.2.1 The Basic of Autonomic Nervous System

The autonomic nervous system comprises of two distinguish systems which is the sympathetic (i.e. adrenergic) nervous system and parasympathetic (i.e cholinergic or vagal) nervous system (Alexander *et al*., 1994). Some would also like to include the enteric nervous system as one of the systems which consists of underlying neurons in the intramural plexuses of the gastrointestinal system (Rang *et al*., 2003).

Both the sympathetic and parasympathetic systems have their own fair share of duty where they work hand in hand and either can be more or less active depending on the particular requirement of the physiological state. The former predominates in states of excitation, for instance during emotional stress and exercise, whereas the latter predominates during satiation and repose. Each system conveys all their output from the central nervous system via two series of neurons (i.e. preganglionic and postganglionic) to the terminal nerve fibers which position proximate to the cells of the organ to be controlled (Rang *et al*., 2003). The synaptic bulb of the nerve ending releases neurotransmitters which travel through the synaptic junction that serves as messengers to evoke the actions of their corresponding neurons and eventually act upon the receptors on the specific cell membrane sites (Missler *et al*., 2003).

The neurotransmitters that serve the sympathetic nervous system are catecholamines, namely adrenaline and noradrenaline, while acetylcholine is the

neurotransmitter that attends to parasympathetic nervous system. Basically the adrenaline and noradrenaline act on adrenoreceptors which comprise of α adrenoceptors (α_1 and α_2) and β adrenoceptors (β_1, β_2 and β_3). On the other hand acetylcholine acts on muscarinic (M_1, M_2, M_3, M_4 and M_5) and nicotinic receptors. All these receptors are also known as G protein coupled receptors (GPCRs) (Rang *et al*., 2003).

In addition, there is also a regulatory neuronal activity called neuromodulation which influences the release of noradrenaline from the terminal neurons. Depending on the location, the neuromodulation process can be pre- or post-synaptic. It is known that mutual presynaptic inhibition can occur by which the release of noradrenaline from sympathethic nerve terminal can inhibit the release of acetylcholine, and acetylcholine can also act on noradrenaline the same way. This interaction is called heterotropic interaction, where one neurotransmitter affects the release of another. It can also be a homotropic interaction where the effect of one neurotransmitter is from the same neuron. This is particularly true for noradrenaline as it is known to inhibit its own release by acting on α_2-adrenoceptors (Rang *et al*., 2003; Opie, 2004; Brink *et al*., 2004).

1.2.2 Sympathetic Nervous System

The avenue of vasculature system is greatly authorized by the changes in the release of catecholamines from both the sympathetic nerves terminals and the adrenal medulla (Guimaraes and Moura, 2001).

During excitatory state (e.g. dynamic exercise), the nerve ending of sympathetic nervous system (SNS) increases the release of noradrenaline to the sinus and atrioventricular node which causes sinus tachycardia and increases the rate of

conduction. This increases the heart rate and therefore brings positive chronotropic effects (Opie, 2004; Rang *et al*., 2003) (Figure 1.2). The release of noradrenaline also increases in the left ventricle and concurrently the adrenal medulla liberates adrenaline to all parts of the heart. Noradrenaline and adrenaline activate β_1-adrenoceptors in the heart, increasing intracellular cyclic 3', 5'-adenosine monophosphate (cAMP) formation and hence intensifying inward Ca^{2+} current. As a result, the force of the heart contractility increases and gives rise to positive inotropic effect (Rang *et al*., 2003).

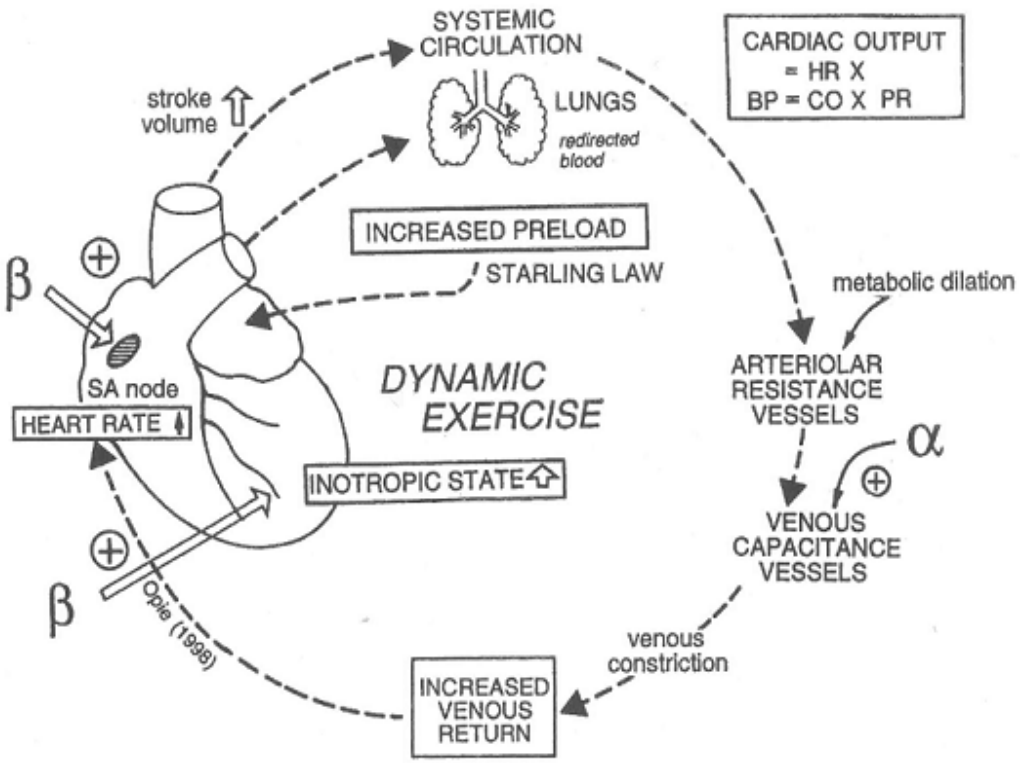

Figure 1.2: Effects of SNS on heart circulatory system during dynamic exercise. The profound effect is the increase of cardiac output which brings about augmentation of preload (Starling mechanism) and inotropic activity. β-adrenergic stimulation (β) increases heart rate (HR) and inotropic state. α-adrenergic stimulation (α) contracts venous capacitance vessels to increase venous return (Adapted from Opie, 2004).

Although the effects of noradrenaline and adrenaline on the heart are compatible, their effects on the vasculature bed are conflicting. Adrenaline activates the β_2-adrenoceptor on the arterioles which causes vasodilatation and thus increasing the limb's blood flow. Due to the vasodilatation, the systolic BP augments even more; however, tending to decrease the diastolic BP. As a result, the increase of mean BP may not be as much. On the other hand, the noradrenaline is prone to act on arteriolar α_1-adrenoceptors causing arterioles contraction and causing resistance to the heart work. In contrast to adrenaline, noradrenaline causes a rise in both the systolic and diastolic BP. In this condition where there is an excessive increase in BP, the baroreflexes will take over. The question is what are the net adrenergic effects on the vascular bed? The answer to this question remains obscure and complex. It was suggested that during muscle exertion activities, the adrenaline opposes the noradrenaline-mediated vasoconstrictive effects and the adrenaline-mediated vasodilatory effects of β_2-adrenoceptors prevail. However, it remains inconclusive as the general sympathetic stimulation on the arterioles varies from one individual to another. However, it is well documented that those who are hypertensive or prone to be hypertensive are likely to show vasoconstrictive effects (Opie, 2004).

1.2.3 Parasympathetic Nervous System

In parasympathetic activity, the muscarinic receptors respond to their messenger, acetylcholine and basically having an opposing effect to those of catecholamines. Principally, parasympathetic activation promotes cardiac slowing and inhibition of heart conductivity. The latter actualize seeing that the nodal tissue

seems to be the main control of parasympathetic activity over the heart (Schauerte *et al*., 2001).

This maybe due to the high occupancies of M_2 receptors in this tissue, which contribute to the negative inotropic effect (Rang *et al*., 2003). The vagal effect on the heart muscle in decreasing the force of heart contractility is mostly of the atria rather than the ventricle, despite the similar densities of M_2 receptors in these chambers. The responsiveness of ventricles towards acetylcholine may be less and there are probably some differences in downstream postreceptors actions (Opie, 2004).

The M_2 receptor activation reduces cAMP formation, which narrows down the β-adrenoceptors activation. Inhibiting sympathetic stimulation was also believed to be the main role of muscarinic receptors modulation, known as accentuated antagonism (Harvey and Belevych, 2003). Furthermore, nitric oxide (NO) may also assist in cholinergic stimulation by generating the second messenger, cyclic 3',5'-guanosine monophosphate (cGMP) that inhibits calcium channel opening and/or promoting the release of acetylcholine from vagal nerve ending (Massion *et al*., 2003).

The parasympathetic effect on the vascular bed is pretty straight forward where it stimulates overall vasodilatation. However, the main influence in vasodilatation effects on the arterioles is the local messengers, namely adenosine and nitric oxide. The former has a direct effect on the adenosine receptors on the vascular smooth muscle cells and a negative neuromodulatory effects by inhibiting the release of noradrenaline. The latter is a highly interactive free radical where it is synthesize in the vascular endothelium and the neuronal nitric oxide synthase (nNOS) is synthesized in the nitroxidergic nerves. In addition, it is also produce locally in the sinoatrial node to promote bradycardia effect (Opie, 2004).

1.2.4 Renin Angiotensin System

The long term control of arterial blood pressure depends on the regulation of blood volume. This is where the renin angiotensin system (RAS) comes in responding to fluid and electrolyte (i.e. sodium) counterbalance. RAS imposes to encounter low renal perfusion, decreased in blood volume, a fall in BP or decreased in tubular reabsoption of sodium (Opie, 2004). During such events, renin is released from the kidneys which cleaves circulating angiotensinogen to angiotensin I in the liver. Then, the latter is rapidly split by angiotensin-converting enzyme (ACE) to form angiotensin II (Ang II), a powerful vasoconstrictor (Lily, 2007). Ang II is the chief executor peptide of RAS above other cognate peptides such as Ang 1-7, Ang III and Ang IV, which as well attracted much research interest on their effect towards cardiovascular function (Jones *et al*., 2008).

Ang II acts on angiotensin II type 1 receptors (AT_1) of arterial smooth muscle to cause vasoconstriction of the arterioles and therefore increasing TPR, which in turn regulates the systemic BP. In response to maintaining the intravascular volume, Ang II stimulates the release of aldosterone from the adrenal cortex that promotes sodium reabsorption in the kidney. Furthermore, Ang II also helps to release antidiuretic hormone that causes water retention, thus augmenting the intravascular volume, as well as acting on hypothalamus to enhance thirst and encourage water intake. In the heart, the Ang II evokes contractility and plays a chief role in the ventricular hypertrophy (Lily, 2007).

Despite its compelling significance on the vascular/cardiac contractility and fluid/electrolyte balance, Ang II is also known to serve the role of autocrine and paracrine agent. Local synthesis of Ang II can manipulate cellular growth and regional haemodynamics of tissue *in situ* (Jones, 2008).

Additionally, RAS is also recognized to interact with other modulators such as those related to SNS where Ang II facilitates the release of noradrenaline. Likewise, bradykinin system too enhances noradrenaline released. However, having a different agenda that contradicts the RAS, the bradykinin production is counteracted by angiotensin converting enzyme (Cox *et al*., 2000). Moreover, RAS interacts with baroreflexes as well, attempting to offset the baroreflexes mediating bradycardia (Fabiani *et al*., 2001).

Given all the benefits and crucial roles of the RAS in regulating BP, its effects in chronic disease state such as hypertension and heart failure could be decrementing (Opie, 2004). Studies through fetal, neonatal and adult cardiomyocytes have revealed many novel findings that implicate RAS as the culprit behind heart failure development (Schlüter *et al*., 2008).

1.2.5 Baroreflexes

Baroreflexes together with autonomic responses offer an acute regulation on the BP variation by detecting an increase or decrease in arterial pressure. The baroreceptors which are situated at the carotid sinus and aortic arch are a pressure sensitive cells that liberate their signals via glossopharyngeal and vagus nerves respectively. These signals are then carried to the vasomotor control in the brainstem where the signals evoke an effect on the sympathetic and parasympathetic nervous system. In a situation where an acute hypertension is detected, the baroreceptors stretch sending neuronal impulses to the vasomotor control which consequently inhibits sympathetic activities and in turn stimulate the parasympathetic activities. As a result, the TPR declines and reduction of heart rate and heart contractility become apparent, thus returning the BP to its basal level (Lily, 2007).

However, baroreflexes do not respond well to long term regulation of BP and therefore do not assist to counteract chronic hypertension. The reason for this is that the baroreceptors constantly reset themselves. After a day or two of exposure to higher than baseline pressures, the baroreceptor firing rate returns to its control value (Lily, 2007).

1.3 G-Protein Coupled Receptors (GPCRs)

1.3.1 Signal Transduction

The vast GPCRs family represents many receptors from the autonomic nervous system. Adrenoceptors, muscurinic receptors, acethylcholine receptors, dopamine receptors and opiate receptors are all under the same family of GPCRs. GPCRs and their downstream effectors are often the subject of multitude research concerning cardiovascular system as it is vital to understand the physiological as well as the pharmacological perspective of these receptors (Wieland and Mittmann, 2003).

Through cloning, the molecular structures of GPCRs and their orphan receptors have been identified. All of them share a consistent architecture which is why there are collectively known as the seven transmembrane spanning (heptahelical) receptors with an extracellular N-terminal region of varying length and an intracellular C-terminal region (Rang *et al*., 2003). The latter region provides control interactions with other proteins that affect signal transduction GPCRs. The signal transduction of GPCRs begins from its activation by their corresponding neurotransmitter, and then coupled to their analogous G proteins before proceeding to the intracellular signal systems (Brink *et al*., 2004).

There are three major types of intracellular signal systems that serve as the targets of G proteins. The first system, involve the activation of a membrane-bound enzyme known as adenylate cyclase. When activated, this membrane-bound enzyme produces the second messenger, cAMP from adenosine 5'-triphospate (ATP). Adenylate cyclase is the only exclusive enzyme system that is capable to yield cAMP. The series of cascade depends on the respective G protein that the GPCRs are coupled to. G protein is the determinant of whether the catalytic activity of adenylate cyclase is to be increased or decreased where it is equivalent to the production of cAMP within the cell. Consequently, cAMP activates protein kinases and commences the many regulation of cellular function (Rang *et al*., 2003 and Wheeler-Jones, 2005).

There are many agents that influence the formation of cAMP. The enzyme called phosphodiesterases inactivates cAMP by hydrolysis to adenosine 5'-monophosphate (5'-AMP). Others such as β-adrenergic agonist, glucagon and forskolin stimulate cAMP production in the heart and consequently increase heart rate and contractility. However, the increase of intracellular cAMP level in the heart may show different concentration in different cellular compartment in the heart. This cAMP compartmentation incident explains that even though, for example the forskolin or β-adrenergic agonist produce their effects by increasing the cAMP level, it does not mean that they will produce a uniform positive effect on the myocyte contractility. This phenomenon might act as a protective mechanism for the heart muscle from a harmful Ca^{2+} overload, amid to extensive term of cAMP activation (Georget et al., 2003).

The second signal system is the phosphoinositide system. In this system, the activated receptors (e.g. α_1-adrenoceptors, muscurinic receptors and angiotensin II

receptors) will couple to the appropriate G protein, where later triggers phospholipase C. Five phospholipase C (PLC) families have been identified as β (beta), γ (gamma), δ (delta), ζ (zeta), and ε (epsilon). PLC_β, PLC_γ and PLC_ε are well expressed in the cardiomyocyte and are suggested to interconnect with many cardiac signalling networks (Wang *et al*., 2005).

The activation of phospholipase C results in the breakdown of its substrate identified as phosphatidylinositol 4,5-biphosphate (PIP_2) to emanate two second messengers, diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). Ultimately, the turnover of the system is restored as DAG is phosphorylated to form phosphatidic acid while the IP_3 is dephosphorylated and recoupled with phosphatidic acid to form PIP_2 once more (Rang *et al*., 2003).

IP_3 is a hydrophilic mediator that stimulates the release of Ca^{2+} from the sarcoplasmic reticulum. DAG is highly lipophilic and exerts its effects through activation of protein kinase C (PKC), where it also results in the increase of Ca^{2+} intracellular level. Together the IP_3 and DAG being duo mediators elicit vascular contraction by raising cytosol calcium in the vascular smooth muscle (Kamp and Hell, 2000 and Opie, 2004).

With regard to cardiac function, the activation of phospholipase C may give either positive or negative inotropic effects (Opie, 2004). In heart failure, up regulation of phospholipase C might be an aid to compensate the β-adrenoceptor desensitization and down regulation, by promoting heart contractility. This situation can also exemplify the fact that communication exists between the adenylate cyclase system and phospholipase C system (Fan *et al*., 1998).

However, the negative inotropic effect of phospholipase C, is not well documented and needs to be deduced further (Wang *et al*., 2005). Nonetheless, a

study proposed that there is a possibility that phospholipase C activation through α-adrenoceptors' transmission may depict Ca^{2+} dependence and resulting in negative inotropic effects (Capogrossi *et al*., 1991).

The final G protein excitatory target is the ion channel, which denotes that G protein can directly interact with ion channels, either in an activating or inhibiting manner, without going through any second messengers (e.g. cAMP and phospholipase C). One good example that describes a direct interaction of G protein with ion channels would be the M_2 receptors that regulate the activation of K^+ channels, enhancing the K^+ permeability, hence, creating hyperpolarization in the cells. However, several studies have suggested that there is no exclusive pathway in which the ion channels are activated, proposing that both direct and indirect G protein-mediated receptors have their own justly way of modulating the ion channels. (Breitwieser, 1991).

1.3.2 GPCRs Homodimerization and Heterodimerization

The studies of GPCRs have evolved to another aspect. Rather than defining every single GPCRs as a single quantity, many researches are now looking at how these GPCRs physically interact with one another. A growing body of evidence indicates that GPCRs can form homodimers and heterodimers (Barnes, 2006). Homodimerization distinguishes from heterodimerization, where the former implies two identical GPCRs forming a complex, whereas the latter imply two different types of GPCRs forming a complex (Brink *et al*., 2004). This event of GPCRs has evolved several aspects and many agree that dimerization may be necessary for efficient agonist binding and signalling in physiological and pathophysiological state (Brink *et al*., 2004; Xiao *et al*., 2006). It has been investigated in culture cells that

adrenoceptors form dimers to facilitate receptor–ligand interactions in optimizing the signalling efficiency towards better cardiac contractility regulation and cardiac growth (Xiao *et al*., 2006).

Moreover, receptors dimers may generate new drug binding sites and this can tremendously affect pharmacological approach of drug intervention. It has been shown that adenosine and dopaminergic receptors are able to form dimers, suggesting that future drug target for Parkinson's disease may be at adenosine receptors rather than at dopaminergic receptors (Brink *et al*., 2004). Nevertheless, further research is obligatory to fully appreciate this fascinating fine-tuning of GPCRs (Xiao *et al*., 2006).

1.3.3 GPCR Sequestration

GPCRs are the first line recognition of extracellular stimuli (e.g. drugs and neurotransmitter) before any intracellular events could take place. However, through continuous or repeated activation, GPCRs could endure configurational changes that serve as a negative feedback to prevent excessive stimulation of a particular GPCR. (Lemaire and Rockman, 2004). There are many terms used to describe this phenomena, namely desensitization, downregulation and internalization. Although many researchers use these terms interchangeably, in fact each term defines and expresses different mechanism of the same end result, which is to inhibit further stimulation of any given GPCR. Thus GPCRs sequestration is presumed to be an act of compensatory feedback adapting to aberrant stimuli (Brink *et al*., 2004).

When abide to desensitization, GPCRs are engulfed inwards into the membrane folding to form vesicles. These GPCR containing vesicles are freed into the cellular cytoplasm by a transaction called internalization resembles endocytosis.

Once internalized, the GPCR can either be metabolized by lysosomes (down-regulation) or dephosphorylated (desensitization) and recycled to the cell membrane to replenish function (resensitization) (Brink *et al*., 2004).

The features of desensitisation is referring to phosphorylation by serine and theonine kinases such as protein kinase A (PKA), protein kinase C (PKC) and specific membrane-bound GPCR kinases (GRKs). It can also occur as a heterologous desensitisation which is known as non-agonist specific desensitization, where the phosphorylation of one type of receptors is due to the activation of kinases by other receptors which share the same agonist (Lemaire and Rockman, 2004; Rang *et al*., 2003; Freedman et al., 1995).

Eminently, an agonist-specific desensitisation can also occur in a process known as homologous desensitisation which accounts for most GPCRs. In this case, phosphorylation is by GRKs and the phosphorylated receptors bind to inhibitory proteins, the β-arrestin isoforms that can result in blocked interaction with their respective G proteins. Basically the effect of homologous desensitisation is more intense and long lasting compared to the heterologous desensitisation which only offers the bases of seconds to minutes sequestration (Lemaire and Rockman, 2004).

1.3.4 G proteins

1.3.4.1 The Function of G protein and Subclasses

Upon activation of GPCR, the responsible ligand either stabilizes or induces conformational changes in the receptor that activate one or many heterotrimeric G protein(s) on the inner membrane surface of the cell. G proteins are a family of guanine triphosphate (GTP) - binding proteins, assigned as the middle man to

orchestrate the interaction between the GPCRs with the regulation of a variety signal transduction systems (Evi K *et al*., 2005).

The heterotrimeric structure of G protein is an assembly of subunits characterized as α, β and γ. The α-subunit contains the helical domain that apprehends the GTP binding site known to be unique to each G protein. This helical domain uphold the specificity of receptor and distinct effector of every GPCR (Lefkowitz, 2004 and Itoh *et al*., 1988). During inactive state, the G protein exists as a trimeric structure whereby the βγ-dimers stays clinch to α-subunit, with guanine diphosphate (GDP) occupying the site on the α-subunit (Rang *et al*., 2003 and Cabrera-Vera *et al*., 2003).

The stoichiometry of G protein begins when a certain transmitter or agonist binds to the corresponding GPCR and triggers the configurational changes of intracellular domains of the receptor. These modifications require the receptor to associate with a number of G proteins. Activation of the GPCR promotes molecule exchange of GDP to GTP at the active site of α-subunit. This putative GDP/GTP exchange, in turn causes dissociation of the heterotrimeric complex, and releases the GTP-bound α-subunit and the βγ-dimer to interact with downstream intracellular or membrane effectors (Cabrera-Vera *et al*., 2003)(Figure 1.3).