CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

Humans have used plant materials as herbal medicines since prehistoric times. In some countries such as China documentary evidence shows that herbal medicines have been used for at least 7000 years, while in Europe there is also a rich history in the use of herbal plants (Wilkinson, 1998). In Thailand, Thai traditional medicine draws from Chinese and Indian traditions. In 1985, the Thai Ministry of Public Health made herbal medicines and their use in the Public Health Care program legal. The National Institute of Thai Traditional Medicine, Ministry of Public Health was established in 1994 to undertake research on Thai traditional medicine and herbs, to promote their use in the health services (Pornsiripongse, 2003).

Interest in herbal medicines began to decline in the 1700's when the reduction approach to science began to dominate. Plants were extracted and pure compounds were isolated, produced and synthesized. This eventually led to the development of the pharmaceutical industry where the synthetic approach to drug design still dominates today. Recently consumers have become more interested in natural products and this has created opportunities for the development of complementary medicine. There is now a great potential for the development of herbal medicine especially in the emerging nutraceutical industry. Many herbal medicines are also often considered to be foods and are used in preventative and curative treatments throughout the world (Wilkinson, 1998). Although there is a large amount of anecdotal evidence to support the use of herbal medicines, the scientific studies to support these claims are in most cases in their infancy. Therefore there is interest in the scientific community to conduct research to purify the active constituents from the herbal medicine and prove that the constituents are effective clinically and to clarify their potential use as drugs.

Cardiovascular disease, and its complications, is the main cause of death for both males and females among all racial and ethnic groups in almost countries of the world including the technologically advanced countries and developing countries. In USA, more than half the total deaths occur due to cardiovascular complications (Witztum, 1996). In Europe and the European

Union, cardiovascular disease also causes nearly half of all deaths, which are 49% and 42%, respectively. Each year it causes over 4.35 million deaths in Europe and over 1.9 million deaths in the European Union (Petersen, 2005). Cardiovascular disease has now become a major public health problem in less-developed countries. It is usually in the top five causes of death (Akinkugbe, 1990; Dodu, 1984). About two-thirds of the estimated 14.3 million annual cardiovascular disease deaths occur in the developing world (Bonita and Beaglchole, 1994).

Because cardiovascular disease has now become the main cause of mortality in the history of mankind, most breakthroughs that have discovered medical uses for natural products have been in the area of cardiovascular research. Presently, about 25% of prescribed medications are derived from naturally occurring compounds. Almost 90% of all current classes of therapeutic agents were developed from naturally occurring chemical templates. Cardiovascular agents account for approximately 15% of all prescriptions filled each year, and a large number of these are based on naturally occurring substances (Cutler S.J. and Culter H.G., 2005). The drugs: digitalis, reserpine, ajmaline, quinidine, ergotamine, atropine and lovastatin, are a few examples of important therapeutic agents isolated from plants (Gilani, 1998).

However, modern medicine is now beginning to accept the use of standardized plant extracts. *Ginko bioloba*, *Hawthorn*, *Gensing* and *Garlic* are a few examples of botanicals that have undergone extensive clinical trials and found to be highly useful in a variety of cardiovascular disorders, such as cereberal and cardiac insufficiency, hyperlipidaemias and hypertension (Gilani, 1998). Recently, Dr. Harunobu Amagase presented details at WorldNutra, 2003 showing that several recent clinical research studies had found that people at risk of heart disease may improve their cardiovascular health by taking an Aged Garlic Extract. This extract can reduce or inhibit multiple risk factors for heart diseases, especially slowing down the plaque formation in the heart's arteries that may lower homocysteine levels in the blood, a major risk factor for heart attacks (Tattelman, 2005).

Therefore, it is of interest to find new cardiovascular drugs from Thai traditional plants by extracting them and isolating and purifying cardiovascular active substances using current scientific methods.

1.2 Cardiovascular system

The cardiovascular system consists of three anatomical components: the heart, the vascular system and the autonomic nervous system (Hoffman et al., 1996). The heart consists of two separate pumps: the left heart and the right heart. The vascular system consists of the pulmonary and systemic circulation. These four components are arranged in series, which means that blood must flow through them in sequence (Rhoades and Pflanzer, 2003). The functions of the heart and the vascular system are mainly controlled by the autonomic nervous system, that consists of sympathetic and parasympathetic nervous systems, acting via adrenergic receptors and muscarinic acetylcholine receptors (Opie, 2004). These three components: the heart, the vascular system and the autonomic nervous system, interact in a complex manner to control blood flow to organs throughout the body by transporting and distributing essential substances to the tissues and removing the by-products of metabolism.

1.2.1 The Heart

The heart is a pump composed of various parts: two atria and two ventricles, which are divided laterally and vertically by a series of one-way valves separating the chambers and directing flow. The left and right atria act as receptacles for blood returning from pulmonary and systemic circulations, via pulmonary veins and the superior and inferior vena cava respectively (Leung, 2004).

Heart function, in terms of contractile force (inotropy) and beating frequency (heart rate) relies on a three-tiered control system: (1) an immediate and fast feedback response of the cardiac tissue to the actual mechanical load, (2) regulation of cardiac performance by the autonomous nervous system involving humoral primary messengers affecting the intracellular signaling systems, and (3) long-term adaptation to altered physiological and pathological conditions produced by changes in gene expression. However, the first two modes of regulation partially overlap and primarily depend on the sympathetic nervous system (Zaugg and Schaub, 2004).

Contraction of the heart

Most of the heart, approximately 75% of the total volume of the myocardium (Brilla et al., 1991), consists of contractile muscle cells known as cardiomyocytes. The rest consists of the pacemaker and conducting tissues, which are concerned with the generation and propagation of the heart's electrical activity, as well as blood vessels and the extracellular space (Opie, 2004).

The cardiomyocytes consist of three systems: (1) a sarcolemmal excitation system that participates in the spread of the action potential and functions as a switch initiating intracellular events, (2) an intracellular excitation-contraction coupling system that converts the electric excitation signal produced by the 1st system to a chemical signal, and then it activates (3) the contractile system that is a molecular motor based on the formation of chemical bridges between actin and myosin (LeWinter and Osol, 2004).

The action potential

The action potential is necessary to make the myocardial contraction due to the transmission of a membrane-based depolarizing current that is propagated through the heart by way of the specialized conduction system tissue (LeWinter and Osol, 2004). Each cardiomyocyte that connects to other cells by gap junctions can spread the action potentials from one cell to another. Thus, the initial excitation of one myocardial cell results in excitation of all cells. The initial depolarization normally arises in a small group of conducting-system cells, called the sinoatrial (SA) node (Irisawa et al., 1993). The SA node is located in the right atrium near the entrance of the superior vena cava. It behaves as a pacemaker region by generating the cardiac electrical impulse that spreads throughout the heart in a sequential manner.

As shown in Figure 1, the SA node initiates an electrical impulse that flows through the right ①nd left atria ,②aking them contract. When the electrical impulse reaches the atrioventricular (AV) node ③, it is delayed slightly. The impulse then travels down the bundle of His ④ that divides into the right bundle branch for the right ventricle and the left bundle branch ⑤

for the left ventricle . The impulse then spreads through the ventricles making the contraction.
This normal electrical activation causes a coordinated sequence of contraction and relaxation of
the cardiac chambers that results in ejection of blood by the ventricle into the aorta and
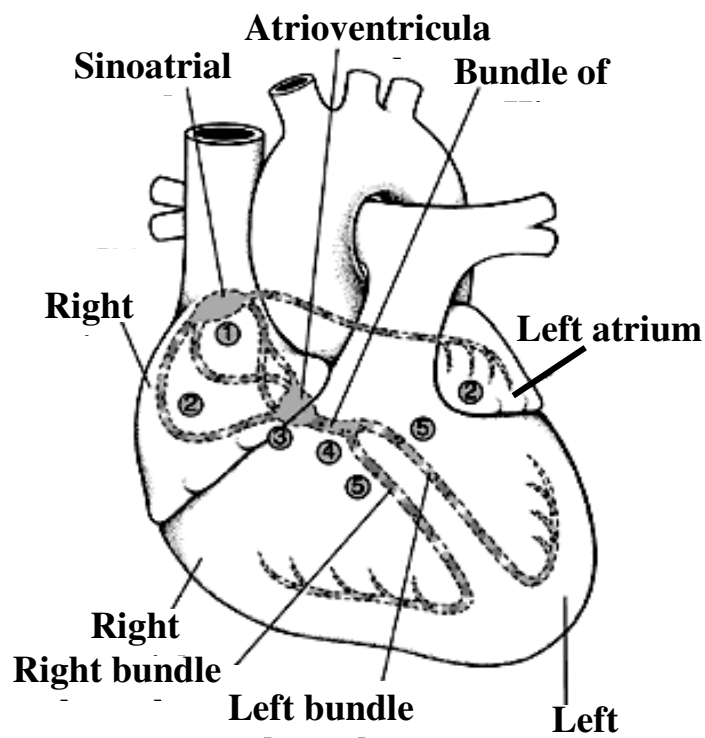pulmonary artery followed by relaxation and filling (Rhoades and Pflanzer, 2003).

Atrioventricula
Sinoatrial
Bundle of
Right
Left atrium
Right
Right bundle
Left bundle
Left

Figure 1 Normal electrical pathways of the heart (modified from Rhoades and Pflanzer,

2003)

In the polarized state of the cardiomyocyte, the K^+ concentration is much higher
inside than outside the cell, whereas Na^+ is much higher outside than inside the cells. These
gradients are maintained by the activity of the Na^+-K^+ pump using energy in the form of
adenosine triphosphate (ATP). A high internal K^+ concentration in turn promotes outward trans-

sarcolemmal diffusion of K^+ to create a negative charge within the sarcolemma and hence the polarized state. This polarity is lost and reversed during the ionic movements that accompany the wave of electrical excitation, a process called depolarization (Opie, 2004).

During depolarization, the opening of ion-selective channels alters the charges across the sarcolemma. This highly regulated series of changes in electrical charge is called the action potential. As shown in Figure 2, Na^+ channels open first to bring in positively charged Na^+ at an extremely rapid rate that causes the equally rapid upstroke phase of the action potential (Balser, 2001). The entry of these positive charges causes the sarcolemma to lose its resting negative charge or polarity, becoming depolarized (phase 0). When most of the rapid entry of Na^+ has ceased, the calcium channel openings that are relatively selective for the entry of Ca^{2+} occurs somewhat more slowly (phase 1). The Ca^{2+} entry accounts for at least part of the plateau phase (phase 2) of the action potential. Potassium channels open toward the end of the plateau phase of the action potential to carry positive charges outward across the sacrolemma (phase 3) that terminates the action potential (phase 4) (Opie, 2004).
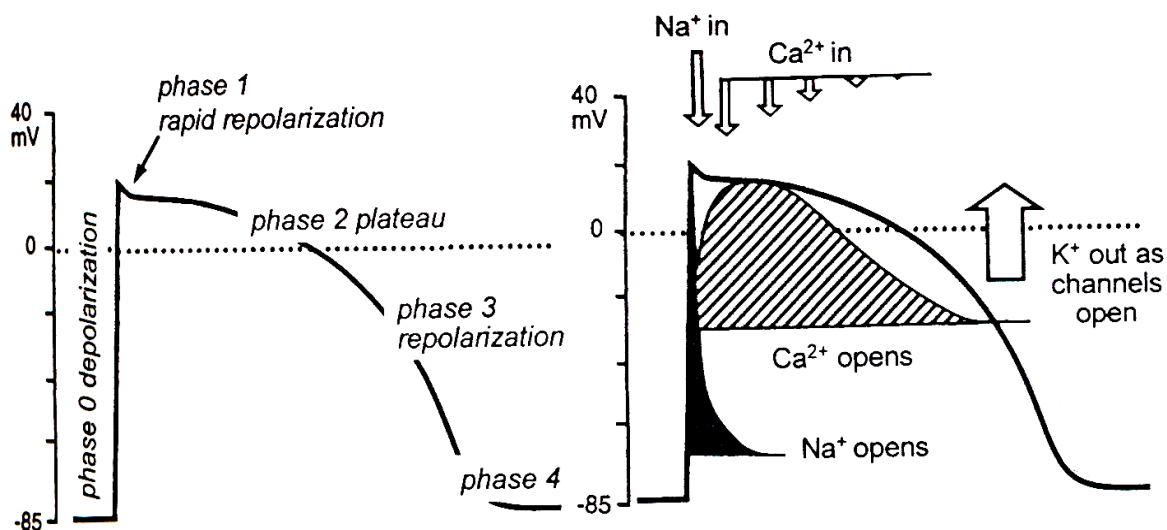

Figure 2 Action potential phases and currents. The four phases of the cardiac action
potential (left) and the underlying currents (right) (Opie, 2004).

Cardiac excitation-contraction coupling

Cardiac excitation-contraction coupling is the process that occurs from electrical excitation of the cardiomyocyte to contraction of the heart that makes the ventricle propel blood out (Bers, 2002). Excitation of cardiac muscle starts when the cardiac action potential spreads rapidly along the myocardial sarcolemma from cell to cell via gap junctions and spreads into the interior of the cells via the T-tubules.

Figure 3 shows that during the cardiac action potential, Ca^{2+} enters the cell through depolarization-activated L-type Ca^{2+} channels as an inward Ca^{2+} current ①. The Ca^{2+} entry binds to RyR (ryanodine receptor) to trigger Ca^{2+} release from the sarcoplasmic reticulum (SR) through ligand-gated Ca^{2+} release channels ②. This process is called Ca^{2+}-induced Ca^{2+} release. The combination of Ca^{2+} influx and release raises the free intracellular Ca^{2+} concentration ③. The Ca^{2+} binds to the myofilament protein troponin C (TnC) that then switches on actin-myosin crossbridge cycle ④, causing contraction of the cardiomyocyte (Bers, 2000).

This contraction and cell activity in general are terminated by removing Ca^{2+} from its regulatory sites on TnC and other proteins in order to decrease the intracellular Ca^{2+} (Zaugg and Schaub, 2004). The intracellular Ca^{2+} is transported out of the cytoplasm by four pathways (Figure 3): the SR Ca^{2+}-ATPase Ⓐ, a sarcolemmal Na^+ / Ca^{2+} exchange (antiporter) Ⓑ, a sarcolemmal Ca^{2+}-ATPase Ⓒ, and mitochondrial Ca^{2+} uniport Ⓓ (Bers, 2002). However, it was found that in the rat and mouse ventricles, the SR Ca^{2+}-ATPase can remove 92% of the activating Ca^{2+} (Hove-Madsen and Bers, 1993; Li et al., 1998), whereas that in the ferret, dog, cat, guinea-pig and human ventricles are 70% (Bers, 2002).
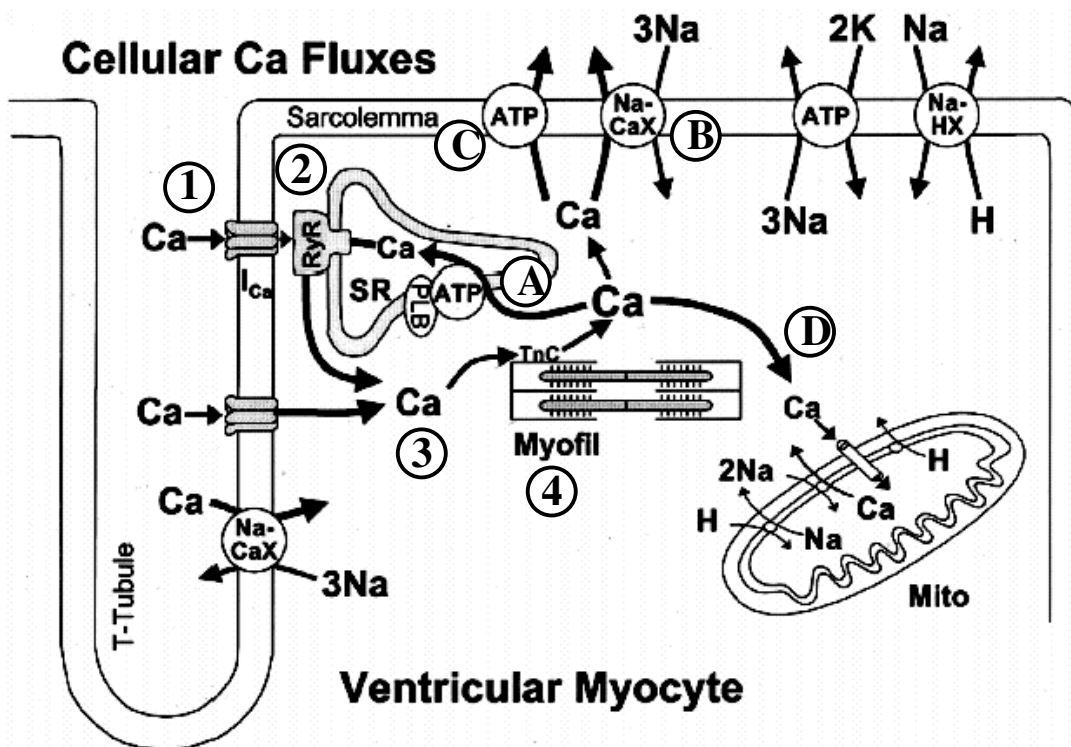
Figure 3 Cardiac excitation-contraction coupling and Ca^{2+} transport in a ventricular

myocyte. NCX=Na^+/Ca^{2+} exchange; RyR=ryanodine receptor; ATP=ATPase;

PLB=phospholamban; SR=sarcoplasmic reticulum; TnC=troponin C;

Myofil=myofilament (modified from Bers, 2000).

Heart rate

Heart rate is the numbers of times the heart contracts per minute (beats/min).

The cardiac cycle is the amount of time needed to complete systole and diastole; in which systole

is the period of ventricular contraction and blood expulsion, while diastole is the period of

ventricular relaxation and filling. The heart rate is determined by the discharge rate of the SA

node, which is a component of the specialized conduction system and has the property of

spontaneous electrical depolarization (LeWinter and Osol, 2004). Due to the rapid spontaneous

depolarization rate of the SA node, it therefore normally controls the heart rate. However, when the activity of the SA node is depressed or the conduction is blocked, another portion of the conducting system can take over as pacemaker and set the new heart rate (Vander et al., 1994).

The heart rate of a resting adult human is 50-100 beats/min, while in small mammals it is faster and in large mammals slower (Levick, 2000). The heart rate is mainly controlled by the autonomic nervous system; sympathetic and parasympathetic nervous system and neuroendocrine systems that modulate beat-to-beat and longer-term variations in heart rate (LeWinter and Osol, 2004). A change in activity of the sympathetic nerves increases the heart rate (positive chronotropic effect), whereas an activity of the parasympathetic (vagus) nerves causes a decreased heart rate (negative chronotropic effect) (Opie, 2004). The positive chronotropic effect results from an increase in the slope of the pacemaker potential by the sympathetic stimulation to cause the SA node cells to reach their threshold more rapidly (Vander et al., 1994), whereas the parasympathetic stimulation causes the negative chronotropic effect by increasing the number of K^+ channel openings that induces the hyperpolarization of the plasma membrane of the SA node cells (DiFrancesco, 1989). This effect results in the pacemaker potential starting from lower values and then the heart rate is decreased.

1.2.2 The vascular system

The vascular system is both a conduit for the flowing blood (blood vessel) and a dynamic system that controls the distribution of blood in the body. Normal vascular function requires the continual adjustment of both total and peripheral resistance to provide the distribution of blood flow (Levick, 2000). Blood vessels are functionally classified into (1) elastic arteries: the pulmonary artery, aorta and major branches, (2) muscular arteries: the medium to small arteries, (3) resistance vessels: the smallest terminal arteries with the diameter of 100-500 $\square$m, and arterioles with the diameter less than 100 $\square$m, (4) exchange vessels: the capillaries with the diameter of 4-7 $\square$m, and (5) capacitance vessels: venules with the diameter of 50-200 $\square$m and veins (Levick, 2000).

The vessels chiefly involved in regulating the rate of blood flow throughout the body are called the resistance vessels or arterioles. The walls of these vessels are composed in

large part of smooth muscle fibers that regulate the diameter of the vessels. Thus, the presence of the smooth muscle in resistance vessels allows the vessel lumen diameter to vary (Berne and Levy, 1998). When this smooth muscle contracts strongly, the endothelial lining folds inward and completely obliterates the vessel lumen. When the smooth muscle is completely relaxed, the vessel lumen is maximally dilated.

Contraction and relaxation of blood vessels

The contractile activity of vascular smooth muscle cells is generally elicited by neural or humoral stimuli that increases the Ca^{2+} concentrations in the myoplasm through (1) voltage-gated calcium channels (electromechanical coupling), (2) receptor-mediated calcium channels (pharmacomechanical coupling) in the sarcolemma, and (3) the release of Ca^{2+} from the sarcoplasmic reticulum. However, the pharmacomechanical coupling is a predominant mechanism for eliciting contraction of vascular smooth muscle.

Figure 4 shows excitation-contraction coupling in vascular smooth muscle. The chemical stimuli activate receptors in the membrane of vascular smooth muscle that in ① turn activate phospholipase C (PLC) in a reaction coupled to guanine nucleotide binding proteins, G-proteins ②. The PLC hydrolyzes phosphatidyl inositol bisphosphate (PIP$_2$) in the membrane to yield diacylglycerol (DAG) and inositol triphosphate (IP$_3$) ③ that latter stimulates the release of ④ Ca^{2+} from the sarcoplasmic reticulum (SR). This Ca^{2+} binds to calmodulin (Cal), which in turn binds to a myosin light chain kinase (MLCK). It activates the Ca^{2+}-calmodulin-myosin kinase complex to phosphorylate the light chains of myosin. Then, the phosphorylated myosin ATPase is also activated by actin, and the result ⑤ cross-bridge cycling initiates contraction (Berne and Levy, 1998).

Control of the contraction-relaxation cycle in vascular smooth muscle is mainly regulated by the cytosolic Ca^{2+} through synthesis of the second messengers, cAMP and cGMP, as well as by acting on the protein kinases: MLCK, protein kinase C, cAMP dependent protein kinase, and cGMP dependent protein kinase (Silver, 1985). These activities are also induced by the activation of post-junctional receptors by neurotransmitters released from sympathetic nerve terminals (Kitamura et al., 1991).

In addition, the sensitivity of the contractile regulatory apparatus to Ca^{2+} is also increased by agonists, which appear to involve G-proteins (Morgan, 1987; Silver, 1985), whereas the relaxation of vascular smooth muscle occurs when MLCK is inactivated by dephosphorylation (Aksoy et al., 1982), and the cytosolic Ca^{2+} is lowered by the SR uptake, the Ca^{2+} pump and the Na^+-Ca^{2+} exchanger (Figure 4).
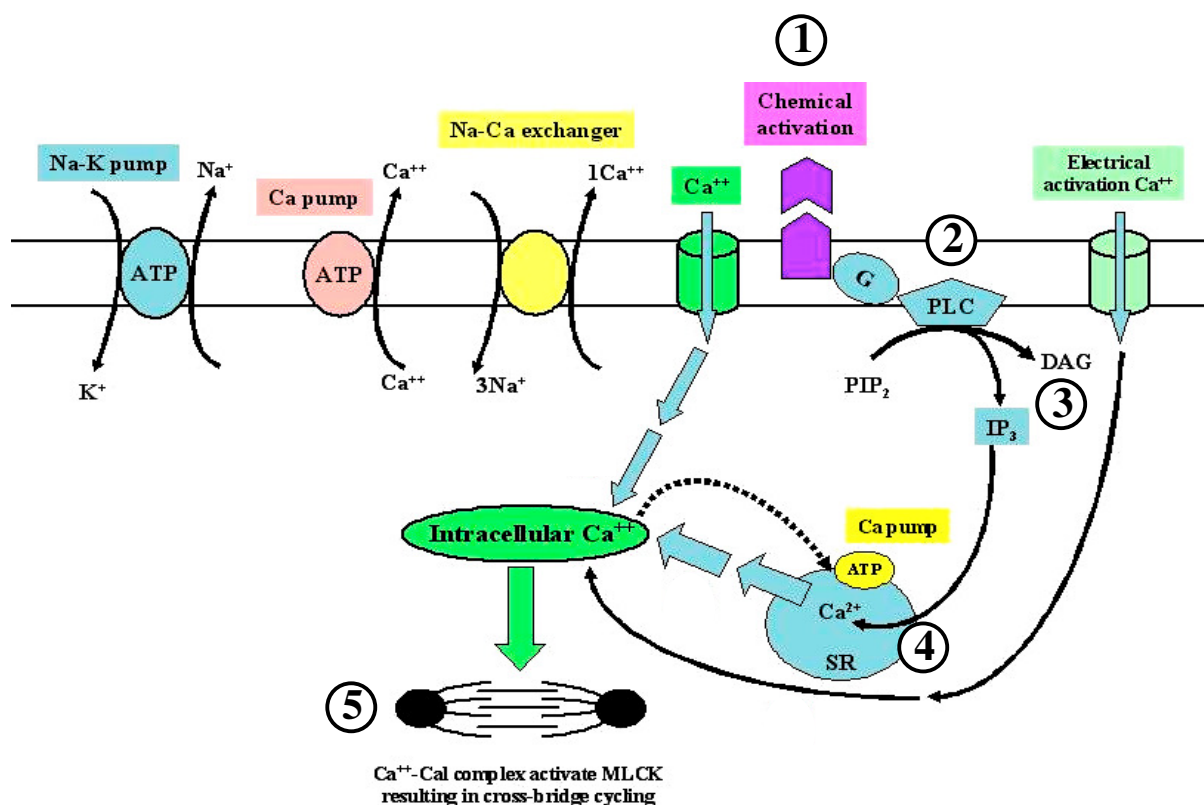

Figure 4 Excitation-contraction coupling in vascular smooth muscle (modified from

Berne and Levy, 1998).

Blood pressure

Blood pressure is the pressure exerted by the blood on the walls of the blood vessels. It refers to systemic arterial blood pressure which is the pressure in the large arteries, the aorta and other systemic arteries, delivering blood to body parts. Blood pressure values are universally stated in millimeters of mercury (mmHg) (Martini et al., 2006). The maximum

pressure reached during peak ventricular ejection is called systolic pressure (SP) and the minimum pressure occurring just before ventricular ejection begins is called diastolic pressure (DP). The arterial pressure is continuously changing throughout the cardiac cycle. Therefore, the average pressure or the mean arterial blood pressure (MAP) is used. The MAP is the pressure driving blood into the tissues average over the entire cardiac cycle. It is approximately equal to the DP plus one-third of the pulse pressure (SP-DP), MAP = DP + 1/3(SP-DP). This MAP is not specific to any artery, because the aorta and other large arteries have such large diameters that they offer only negligible resistance to flow, and the mean pressure is therefore similar everywhere in the large arteries (Vander et al., 1994).

The control of blood pressure is mediated through complex, overlapping mechanisms that interact to produce appropriate responses in a wide variety of circumstances. The pressure pattern in the circulation varies from high arterial to low venous values. It changes quite abruptly at the level of arterioles, for these are small arteries approximately 300 $\square$m in lumen diameter with relatively thick muscular walls. The arterioles are the site of the major resistance component of the systemic vascular resistance because they constitute the major resistance against which the ventricular pump, and collectively constitute the peripheral or systemic vascular resistance. If the arterioles dilate, the vascular resistance falls and more blood enters the capillaries (Opie, 2004).

Acute regulation of the peripheral vascular resistance over minutes and hours is achieved largely by (1) autonomic control and baroreflexes that provide rapid responses of the circulation to minute-by-minute blood pressure variations, and (2) regulation of the peripheral arteriolar tone by both local metabolic factors, such as nitric oxide and adenosine, and by variable adrenergic or cholinergic signals.

Autonomic control and baroreflexes

The vasomotor center at the base of the brain coordinates autonomic control of the circulation by receiving incoming signals from the baroreflexes and then transmitting efferent impulses through vasoconstrictor or vasodilator fibers to the arterioles. Baroreceptors (high-pressure receptors), situated on the arterial side of the circulation in the carotid sinus and aortic

arch, play a key role in the short-term regulation of blood pressure. They form the first line of defense against acute hypertension or hypotension by adjusting both the vagal tone and sympathetic outflow against the level of receptor input.

In response to acute hypertension, the baroreflexes engender increased neuronal traffic to the vasomotor center with consequent inhibition of sympathetic outflow and increase in vagal tone causing decreases in the heart rate, contractility, and cardiac output, as well as inducing a decrease in peripheral vascular resistance. Thus, the acute increase in blood pressure induces self-correcting changes.

In response to acute hypotension or drug-induced vasodilatation, there is a decrease in the distending pressure in the baroreceptors resulting in a decreased frequency of afferent stimuli to the vasomotor center. This event consequently increases in sympathetic outflow and inhibits vagal tone. The □-mediated reflex response will increase the heart rate and contractility, whereas the □-mediated increase in peripheral vascular resistance will also help to elevate blood pressure (Opie, 2004).

Endothelial cells of blood vessels

The endothelium is a single cell layer lining the blood vessels that has a significant effect on vascular function and blood flow in ways that are still being explored. Capillaries consist only of endothelium, whereas all other vessels have, in addition, layers of connective tissue and smooth muscle (Levick, 2000). Endothelial cells have a large number of active functions especially the important function associated with the cardiovascular system. Vascular endothelium plays an important role in the regulation of arterial tone via the production of the powerful vasodilators: prostacyclin (Moncada et al, 1976) and endothelium-derived relaxing factors (EDRF) the best known being nitric oxide (Furchgott and Zawadzki, 1980), as well as the metabolic vasoactive substances: catecholamine, angiotensin, bradykinin and prostaglandins (Vane, 1969).

Nitric oxide

Nitric oxide (NO) is a lipophilic, freely diffusible, soluble gas. It accounts for the biological activities of EDRF. The NO is synthesized in endothelial cells of the blood vessels from the amino acid L-arginine that was transported into the cell via a cation amino acid transporter system (system y+) in the cell membrane (Wiesinger, 2001). L-arginine is changed to NO by the enzyme, endothelial nitric oxide synthase (eNOS) using tetrahydrobiopterin (BH_4) as a co-factor and L-citrulline being produced as a co-product (Boulanger et al., 1998). Three main factors affecting overall NOS activity are pharmacological agonists, shear stress due to blood flow, and NOS formation (Levick, 2000). The analogue of arginine such as N-monomethyl-L-arginine (L-NMMA) can also interrupt NO production (Palmer and Moncada, 1989).

The pharmacological agonists induce NO secretion by a dual process (Figure 5). Agonists, such as acetylcholine, bradykinin and substance P, activate the receptor-operated cation channels resulting in an influx of extracellular Ca^{2+} ions to the cells. ① The agonist-G-protein complex ② activates a membrane-bound enzyme phospholipase C (R-G-PLC complex) that catalyzes the production of a cytoplasmic messenger, inositol triphosphate (IP_3) causing the activation of Ca^{2+} release from SR. Both processes induce a transient increase in intracellular Ca^{2+}, which then binds to calmodulin to form the calcium-calmodulin complex ③ ④ . This complex ⑤ activates eNOS to produce NO. Then, NO diffuses out of the endothelial cell into adjacent smooth muscle cells, where it binds to the heme group of the ⑥ enzyme guanylate cyclase . The activation of guanylate cyclase produces cyclic guaosine monophosphate (cGMP) from guanosine triphosphate ⑦ (GTP) . The cGMP activates a protein kinase G (PKG) by a number of phosphorylating actions to reduce the cytosolic Ca^{2+} concentration in the vascular myocyte leading to vasodilatation (Levick, 2000).
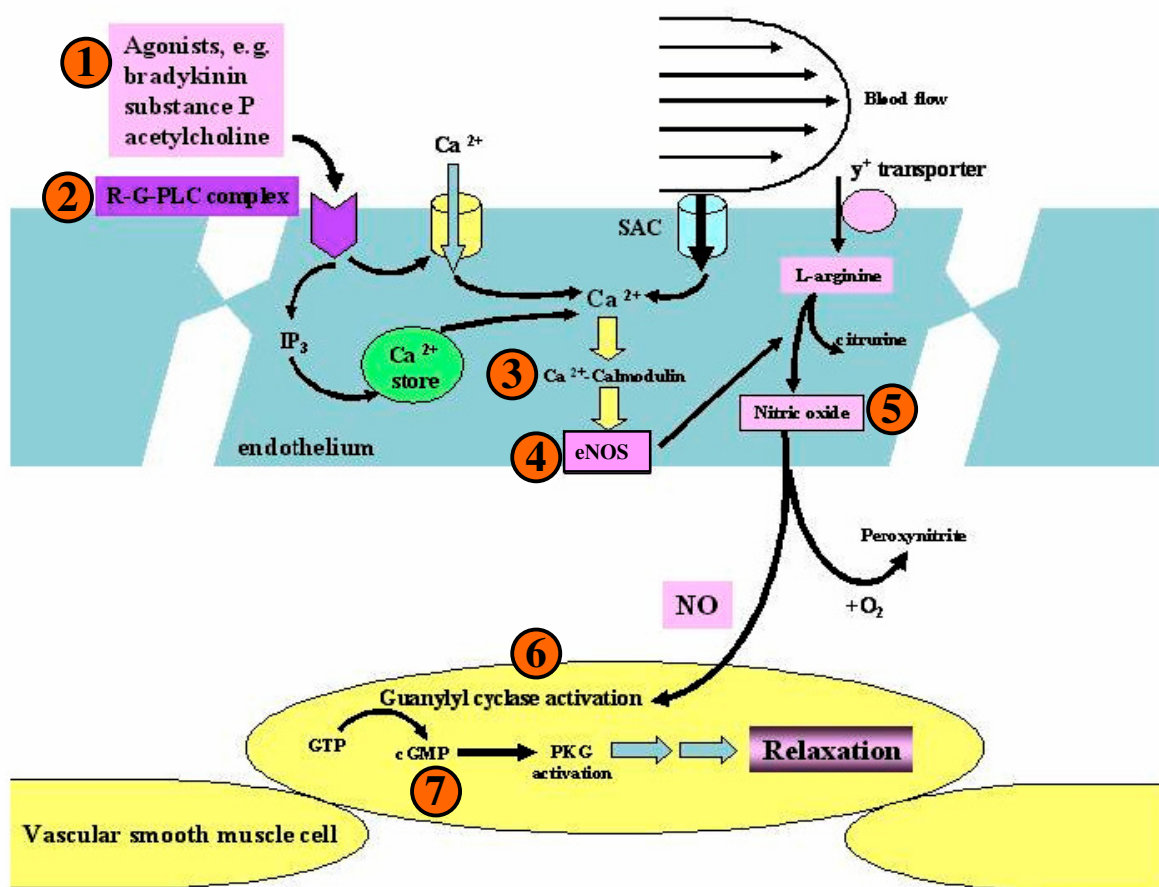
Figure 5 Local controls of blood vessels by nitric oxide inducing vasodilatation (modified from Levick, 2000).

In some vessels and species, NO causes a hyperpolarization of vascular smooth muscle that is a well known mechanism for inducing vascular relaxation. The hyperpolarization of myocyte occurs when K^+ channels open, and it appears that NO at high concentrations can directly activate K_{Ca} channels (calcium-activated K^+ channels) in the myocyte membrane (Cohen et al., 1995). Under physiological conditions, a membrane hyperpolarization leads the myocyte to inhibit a depolarization-induced Ca^{2+} influx and reduces the Ca^{2+} mobilization from the intracellular stores (Calderone and Martinotti, 2000) that results in a decrease of intracellular Ca^{2+} leading to vasodilatation.

In addition, it is well established that NO has a leading role as an inhibitory neurotransmitter of peripheral non-adrenergic, non-cholinergic (NANC) nerves. The nerve whose transmitter function depends on the release of NO is called "nitroxidergic" (Toda and Okamura, 1991) or "nitrergic" (Rand, 1992). Peripheral nitrergic nerves have a widespread distribution, and are particularly important in that they produce relaxation of smooth muscle in the gastrointestinal, respiratory, vascular and urogenital systems (Esplugues, 2002). Neuronal nitric oxide synthase (nNOS), the enzyme producing NO in nitrergic neurons in the central nervous system, is found in the perivascular nerves of various blood vessels and appears to constitute an alternative regional control mechanism for blood flow, independent of eNOS (Bredt et al., 1990; Huang et al., 1999). There is evidence implicating central NO in the regulation of blood pressure (Togashi et al., 1992) and heart rate (Sakuma et al., 1992).

In the neurogenic relaxation of rat thoracic aorta, it was found that the NANC nerve system mediated vasodilatation by sensory nerves that with ATP acts as a neurotransmitter. This neurotransmitter activates P_2Y-purinoceptors located on the endothelium and stimulates the NO/cyclic GMP pathway, resulting in vasodilatation (Park et al., 2000).

1.2.3 The autonomic nervous system

The cardiovascular system is mainly controlled by the autonomic nervous system, sympathetic and parasympathetic nervous systems (Randall and Ardell, 1988; Zipes and Inoue, 1988). Both systems contain preganglionic neurons that originate in the central nervous system releasing acetylcholine to act on cholinergic nicotinic receptors on the postganglionic fiber. The sympathetic postganglionic neurons that terminate in the effector organs release the neurotransmitter norepinephrine, whereas the parasympathetic postganglionic neurons release the neurotransmitter acetylcholine (Figure 6).

In addition, the adrenal medulla is also a component of the sympathetic nervous system that is analogous to postganglionic sympathetic nerve fibers. Instead of norepinephrine, the adrenal medulla releases epinephrine upon the activation of the preganglionic sympathetic nerve that innervates this tissue. This event normally occurs during times of stress: exercise, heart failure, hemorrhage, emotional stress or excitement and pain (Hoffman and Taylor, 2001). The

target organs of the sympathetic nervous system contain receptors for norepinephrine and epinephrine that are known as adrenergic receptors, while the receptors of acetylcholine are muscarinic receptors (Johnson et al., 1998).
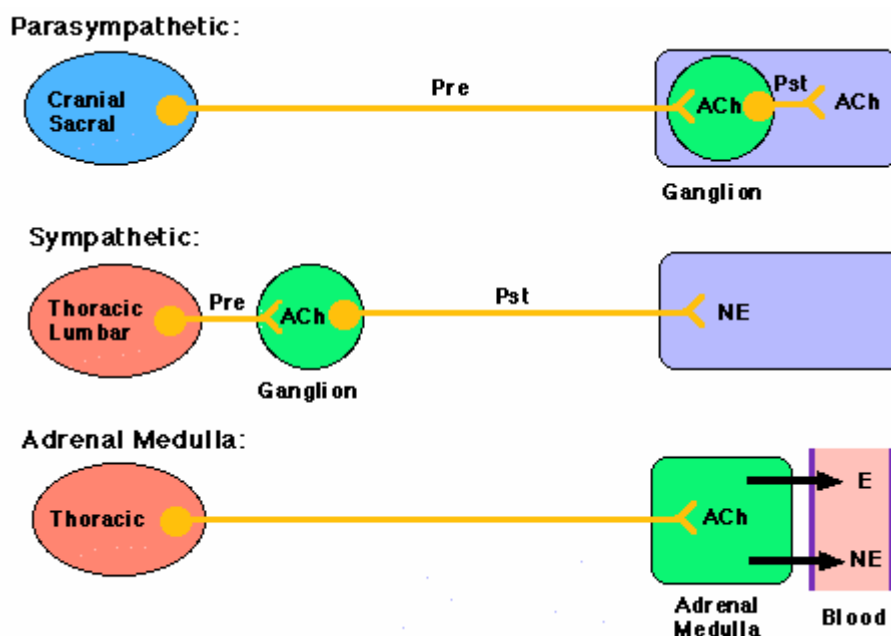

Figure 6 Neurotransmitter releases from pre-and post-ganglionic neurons of the
sympathetic and parasympathetic nervous systems and the adrenal medulla.
Pre=preganglionic nerve; Pst=postganglionic nerve; ACh=acetylcholine;
E=epinephrine; NE=norepinephrine.

Sympathetic nervous system at the heart and blood vessels

The sympathetic nervous system is able to release its neurotransmitters, norepinephrine and epinephrine from the sympathetic nerve terminals and the adrenal medulla (Levy and Martin, 1989). As demonstrated in Figure 7, the sympathetic nerves stimulate the heart by acting via the right stellate ganglion to increase the release of norepinephrine to areas of the SA and AV nodes ①, the left stellate ganglion to increase the release of norepinephrine to the left ventricle ②, and the adrenal medulla releasing epinephrine to all parts of the heart and also to the ③

arterioles   . The cardiac receptors, stimulated by norepinephrine and epinephrine, are mainly the

☐-adrenergic receptors. This causes an increased heart rate, by activating the rate of conduction

of the electrical impulse through the AV node and the conducting system, as well as causing an

increased force of contraction (Opie, 2004).

Blood vessels are innervated by sympathetic nerves that traverse in the

adventitial layer and which have the varicosities that release norepinephrine as a neurotransmitter.

Norepinephrine acts on the underlying smooth muscle and endothelial cells to regulate vascular

tone (Kanagy, 2005) and their vasoconstrictor effect by acting via ☐$_1$-adrenergic receptors. In the

adrenal medulla, on the other hand, some sympathetic postganglionic fibers also reach to

stimulate epinephrine release from chromaffin cells of the adrenal grand (Levick, 2000). Both

catecholamines act by binding to ☐$_1$-, ☐$_2$ or ☐$_2$-adrenergic receptors on the vascular smooth
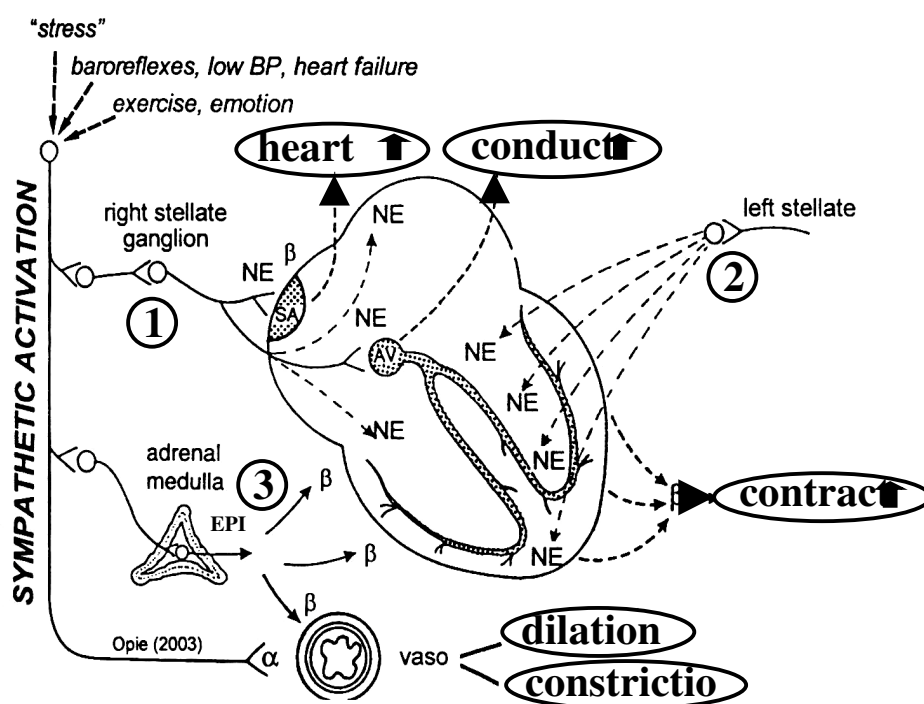
muscle.

Figure 7 Mechanisms of sympathetic stimulation in the heart (modified from Opie, 2004)

Synthesis, release and inactivation of norepinephrine at the sympathetic nerve terminal

In noradrenergic neurons containing the sympathetic nerve terminals, an increase in sympathetic nerve activity is associated with an acceleration of norepinephrine biosynthesis that plays a critical role in the maintenance of the adrenergic stores of this amine (Carson and Robertson, 2002). The norepinephrine biosynthetic pathway begins with the amino acid tyrosine (Figure 8). L-Tyrosine is transported into the neuronal varicosity where it is converted to L-3,4-dihydroxyphenylalanine (DOPA). This is the rate-limiting step in the synthesis of all catecholamines that is catalyzed by tyrosine hydroxylase. The newly synthesis DOPA is rapidly decarboxylated to dopamine by aromatic L-amino acid decarboxylase, also known as dopa decarboxylase. Then, dopamine is actively transported into synaptic vesicles, where the enzyme $\square$-hydroxylase is located. This enzyme catalyzes the conversion of dopamine to norepinephrine. When synthesized, norepinephrine is bound to ATP and stored within the vesicle until released (Brody et al., 1994).

The release of norepinephrine from a varicosity of noradrenergic nerve occurs when the nerve action potential arrives at the varicosity (Figure 8). The depolarization of the presynaptic membrane activates voltage-sensitive Ca^{2+} channels triggering a rapid but transient influx of Ca^{2+} into the nerve terminal ①. The increase in free Ca^{2+} concentration leads to the fusion of the vesicles at the presynaptic membrane ② and subsequent release of norepinephrine into the neuroeffector junction by a process of exocytosis ③. Norepinephrine binds to postsynaptic adrenergic receptors ($\square_1$-, $\square_1$- or $\square_2$-receptor) ④ (Brody et al., 1994; Rhoades and Pflanzer, 2003).

The amount of norepinephrine release is controlled by a process of feedback inhibition and excitation that act via the autoreceptors, $\square_2$- and $\square_2$-adrenergic receptors ⑤, located on the membrane of the presynaptic terminal (Opie, 2004). When the amount of norepinephrine in the neuroeffector junction is increased, more $\square_2$-autoreceptors become activated to inhibit the release of norepinephrine from the presynaptic nerve terminal (Langer, 1997; Starke, 2001). Stimulation of the $\square_2$-adrenergic receptors lowers intracellular Ca^{2+} by: (1) activation of $G_{\square i}$-proteins, (2) inhibition of adenylase cyclase, (3) activation of sarcolemmal

inwardly rectifying K^+-channels, and (4) inhibition of the L-type Ca^{2+}-channels. This event results in lowering of the Ca^{2+}-dependent norepinephrine release from the presynaptic transmitter vesicles (Zaugg and Schaub, 2004). In contrast, the activation of ☐ ☐$_2$-adrenergic receptors increase the norepinephrine release in a process called feedback excitation.

Actions of norepinephrine at the sympathetic nerve endings are terminated after uncoupling of norepinephrine from its receptors by amine uptake pumps (Figure 8). The major uptake of norepinephrine is by a neuronal uptake-1 or NET (norepinephrine transporter) that removes approximately 80% of the norepinephrine from the synaptic cleft into presynaptic neurons (Brody et al., 1994; Rhoades and Pflanzer, 2003) ⑥ . The extraneuronal uptake-2 or EMT (extraneuronal monoamine transporter) can transfer norepinephrine into postsynaptic neurons by passive diffusion (Axelrod, 1970) ⑦ . The remaining norepinephrine inside the noradrenergic cells and in the circulation is degraded by the process of oxidative deamination with an intra-neuronal enzyme, MAO (monoamine oxidase) and through the *O*-methylation by an extra-neuronal enzyme COMT (catechol-*O*-methyl transferase) (Carson and Robertson, 2002).

Elevation and depletion of norepinephrine in sympathetic nerve terminal

Drugs can interfere with sympathetic neuronal function by: (1) inhibition of synthesis of norepinephrine, e.g. metyrosine and carbidopa, (2) disruption of vesicle storage of norepinephrine by behaving as a vesicle monoamine transporter (VMT) inhibitor, e.g. **reserpine**, (3) inhibition of norepinephrine release, e.g. guanethidine, and (4) blockades of ☐- and ☐-adrenergic receptors by antagonists, e.g. phentolamine and propranolol (Katzung, 1998). However, reuptake mechanisms and synthesis are of major importance in the conservation and maintenance of the adrenergic stores of norepinephrine. In contrast, the indirectly acting sympathomimetic amines, such as **tyramine**, ephedrine and amphetamine, that are taken up into sympathetic nerve terminals by the amine uptake pump cause displacement of norepinephrine from storage sites in vesicles, or from other binding sites (Hoffman and Taylor, 2001).
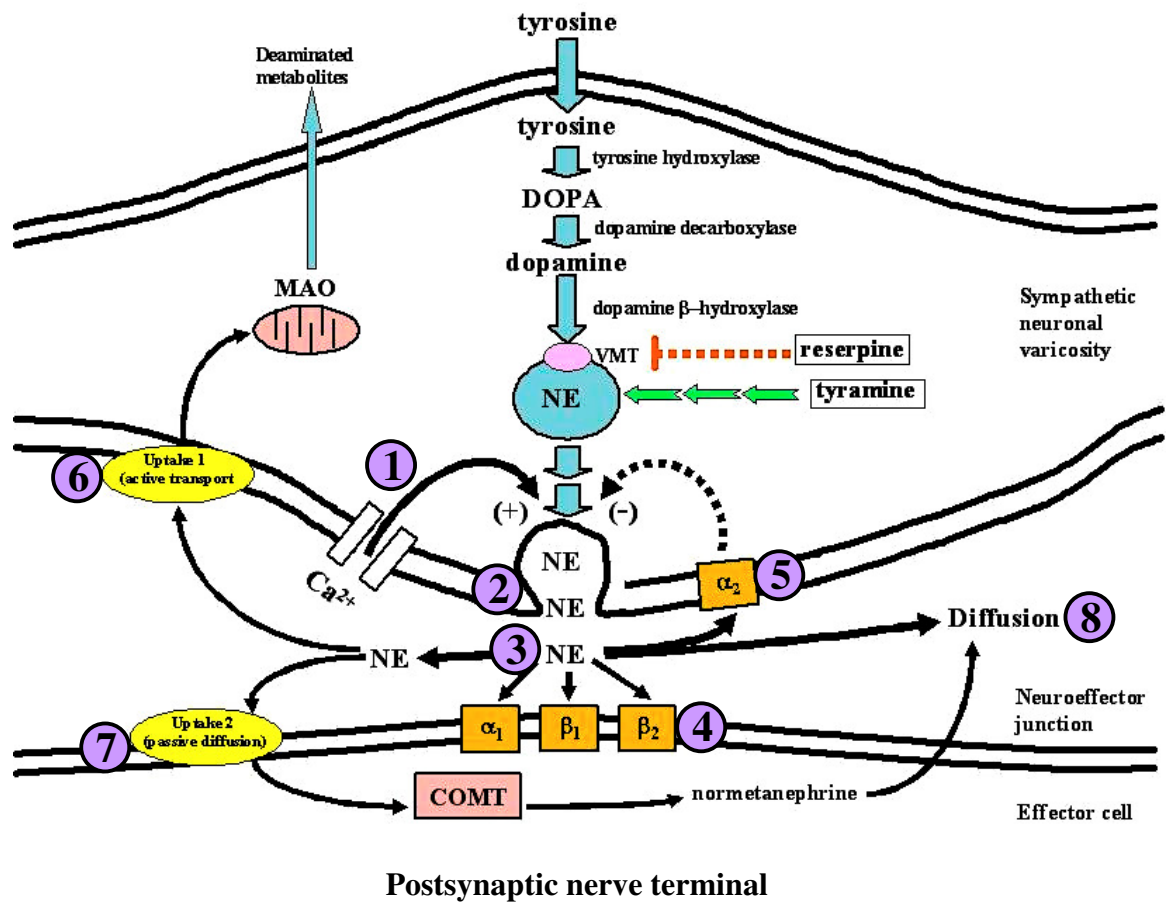
Postsynaptic nerve terminal

Figure 8 Synthesis, storage, release and inactivation of norepinephrine (NE). (modified

   from Brody et al., 1994).

Epinephrine

Epinephrine or adrenaline is a hormone that is one of several structurally related
compounds in the body called catecholamines. Epinephrine is generated from norepinephrine by
N‑methylation, a process catalyzed by phenylethanolamine *N*‑methyltransferase (PNMT)
(Axelrod, 1961) in chromaffin cells of the adrenal medulla using the methyl donor *S*-
adenosylmethionine as co‑substrate (Wong, 2006). The synthesis of epinephrine is controlled by

the central sympathetic neurons through preganglionic splanchnic nerves (Axelrod and Reisine, 1984). Splanchnic nerve endings make synaptic-like contacts with chromaffin cells and release acetylcholine as a neurotransmitter to evoke catecholamine release from chromaffin cells via the cholinergic receptor (Holman et al., 1994).

Epinephrine is called an emergency hormone, because its synthesis is elevated during physiological stress. Epinephrine is normally secreted in a small amount from the adrenal medulla into the circulation to activate cardiovascular and metabolic responses (Hoffman and Taylor, 2001). Stratton et al. (1985) found that physiological increases in plasma epinephrine cause only a modest change in heart rate, systolic blood pressure, and mean blood pressure in humans. Even, at high infusion levels, epinephrine leads to only a 20-30% increase in heart rate and systolic pressure and a 5-10% decrease in mean arterial pressure.

Effects of epinephrine and norepinephrine on the cardiovascular system

Epinephrine and norepinephrine show both similar and different effects on the cardiovascular system. Epinephrine is a potent stimulant of $\square_1$-, $\square_1$- and $\square_2$-adrenergic receptors, whereas norepinephrine can activate $\square_1$-and $\square_1$- adrenergic receptors but has relatively little action on $\square_2$-receptor (Brody et al., 1994). On the $\square$-receptors of most organs, however, norepinephrine is somewhat less potent than epinephrine (Hoffman and Taylor, 2001).

Both epinephrine and norepinephrine increase the heart rate and cardiac contractility by stimulation of the cardiac $\square_\square$-adrenergic receptor. In vascular smooth muscle, epinephrine especially at high concentrations and norepinephrine cause vasoconstriction in all tissues by acting via $\square_1$-adrenergic receptors (Levick, 2000). Epinephrine at physiological concentrations causes vasodilatation in three tissues, skeletal muscle, myocardium and liver. This effect is due to the abundance of $\square_2$-adrenergic receptors coupled with the high affinity of epinephrine. Norepinephrine, on the other hand, normally causes vasoconstriction because it has a higher affinity for $\square$-adrenergic receptors than for $\square$-receptors (Hoffman and Taylor, 2001). This raises the peripheral resistance and blood pressure, which then elicits a baroreceptor reflex to reduce the sympathetic drive to the heart and increases the parasympathetic drive, resulting in decreases of heart rate and cardiac output (Figure 9). In contrast, intravenous administration of

epinephrine reduces the total peripheral resistance slightly, because of its vasodilatory effect. Mean blood pressure therefore changes little, and the direct stimulation of the heart by circulating epinephrine proceeds without significant opposition by the baroreflex (Levick, 2000).
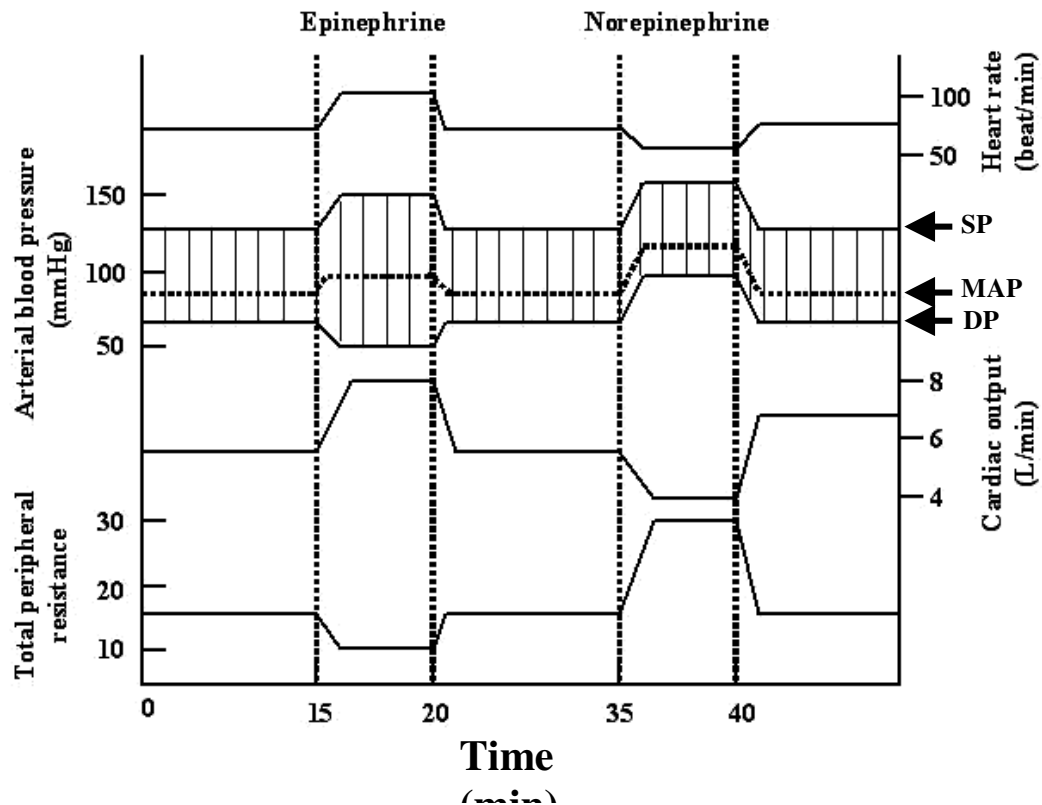
Figure 9 Comparison of the effects of intravenous epinephrine and norepinephrine in man.

     SP=systolic blood pressure; DP=diastolic blood pressure; MAP=mean arterial

     blood pressure, (modified from Levick, 2000).

        Ahlquist (1948) studied the effects of catecholamines, epinephrine and norepinephrine, compared to isoproterenol, on a variety of physiological responses and found that isoproterenol (ISO), a non-specific $\square$-adrenergic receptor agonist, and norepinephrine (NE) caused smooth muscle relaxation and contraction respectively, while epinephrine (E) could cause

both contraction and relaxation of smooth muscle. These effects were mediated by two distinct receptors, □- and □-adrenergic receptors. □-adrenergic receptors were defined by the catecholamine potency series of ISO > E > NE, whereas □- adrenergic receptors were defined by the series of E = NE > ISO.

In addition, a short-term infusion of epinephrine was followed by a sustained increase in heart rate and/or blood pressure. It probably is due to epinephrine, released from the adrenal medulla, being taken up into sympathetic nerve terminals and then rereleased with norepinephrine as a cotransmitter. Both epinephrine and norepinephrine act on postsynaptic □-adrenergic receptors and in this way amplifies and prolongs sympathetic responses (Majewski, 1981; Floras, 1992).

Adrenergic receptors at the heart

At least nine adrenergic receptor subtypes: $\Box_{1A}$, $\Box_{1B}$, $\Box$ $\Box_{1D}$, $\Box_{2A}$, $\Box_{2B}$, $\Box_{2C}$ $\Box$ $\Box_1$, $\Box_2$ and $\Box_3$, exist in the human heart. These receptors are involved in the regulation of contractility and heart rate (Brodde and Michel, 1999). In human heart, the density of □-adrenergic receptors was higher than that of $\Box_1$- adrenergic receptors (Bristow et al., 1988; Steinfath et al., 1992b). In a direct comparative study, Steinfath et al. (1992a) found that the human right and left ventricles had the smallest $\Box_1$- adrenergic receptor density, whereas rats had the highest $\Box_1$- adrenergic receptor density by at least 5-fold among seven species, including guinea-pig, mouse, rabbit, pig, and calf.

Cardiac □-adrenergic receptors are mainly of the $\Box_1$-adrenergic receptor subtype, approximately 60-70% in the human atria and 70-80% in the human ventricle, whereas the rest are the $\Box_2$-subtype (Lands et al., 1967; Brodde, 1991). In the SA nodes, $\Box_1$-adrenergic receptors are predominately about 3-fold higher than in the adjacent atrial myocardium (Rodefeld et al., 1996). The $\Box_2$-adrenergic receptors are primarily expressed in cells other than cardiac myocytes, e.g. endothelial cells, fibroblasts, and vascular smooth cells. However this receptor can mediate functional responses in the cardiac myocytes and becomes more prominent in heart failure (Kiuchi et al., 1993).

Norepinephrine, released from the sympathetic nerve terminals, binds to $\square_1$-, $\square_2$-, and $\square_1$-adrenergic receptors in the heart causing the positive chronotropic effect, positive inotropic effect, and positive dromotropic effect (Klabunde, 2004). Skomedal et al. (1988) found that only 25% of the inotropic response to norepinephrine is mediated through $\square$ the $\square_1$-adrenergic receptors, but 75% via the $\square$-adrenergic receptors. Similarly, responses to the endogenous norepinephrine, released by tyramine administration in the human heart, occurred via $\square_1$- and $\square$-adrenergic receptors at 14% and 86% respectively (Borthne et al., 1995).

Inotropic effect

The sequence of events of cardiac contraction (inotropic effect) induced by the $\square\square$-adrenergic receptor agonists catecholamines, norepinephrine or epinephrine, is demonstrated in Figure 10. Epinephrine or norepinephrine stimulates $\square$-adrenergic receptors coupling to $G_{\square s}$ protein ① to activate adenylyl cyclase (AC) causing formation of cAMP from ATP ②. The increased intracellular level of cAMP leads to an activation of PKA and this ③ phosphorylates L-type Ca^{2+} channels to promote the entry of Ca^{2+} through the cell membrane . The Ca^{2+} influx ④ can trigger a large Ca^{2+} release from SR via the RyR through the Ca^{2+}-induced ⑤ Ca^{2+} release mechanism resulting in a large increase of intracellular Ca^{2+}. The Ca^{2+}-binding TnC switches- on a cross-bridge cycle causing the cardiac contraction (Bers, 2002).
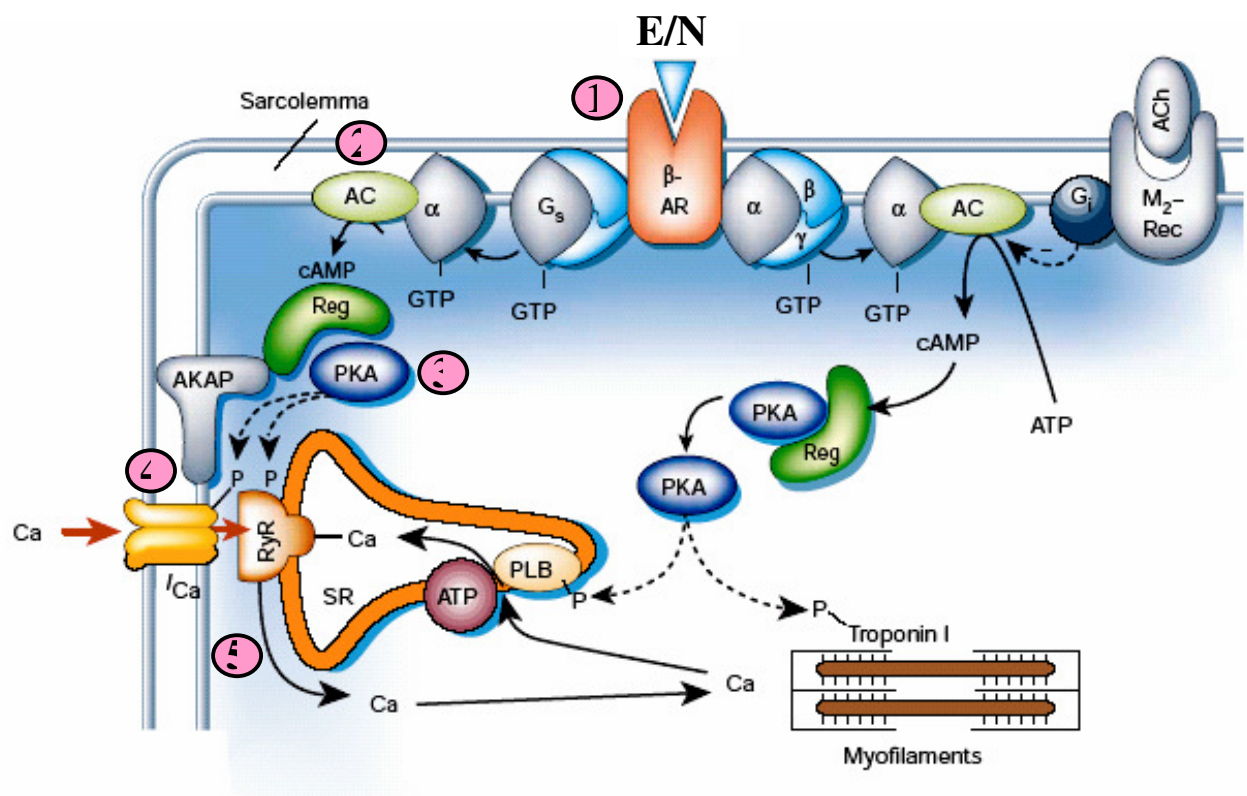
Figure 10 Sequence of events of the cardiac contraction induced by □-adrenergic receptor

agonists, epinephrine and norepinephrine (E/NE) (modified from Bers, 2002).

An interesting aspect of the catecholamine-induced inotropic response is that it passes off rapidly within minutes. This response results from the increased cAMP during the □-adrenergic receptor stimulation in turn activating an enzyme phosphodiesterase that promotes the rate of cAMP breakdown resulting in the rapid reduction in the inotropic response (Opie, 2004). In addition, the intense stimulation of □-adrenergic receptors also leads to the activation of a □-adrenergic receptor kinase that phosphorylates the □-adrenergic receptors to uncouple them from the G_s protein (Choi et al., 1997).

In contrast to the □-adrenergic receptors, $□_1$-adrenergic receptor stimulation does not increase cyclic AMP and this indicates that it does not involve the G_S/adenylyl cyclase/cyclic AMP system (Brodde et al., 2001). All three $□_1$- adrenergic receptors interact with the pertussis toxin-insensitive $G_{□q}$ component that follows the main signaling route via PLC. This leads to DAG inducing the activation of PLC and IP_3 for the liberation of Ca^{2+} from the SR, resulting in increases in the force of contraction (Zaugg and Schaub, 2004).

In addition, the cardiac $□_1$-adrenergic receptor can couple to numerous intracellular signal transductions response-not only PLC but also various Ca^{2+} and K^+ currents. Moreover, the Na^+/H^+ exchanger and Na^+, K^+-ATPase can be activated (Endoh et al., 1991).

Lusitropic effect

The □-adrenergic receptor-induced cardiac relaxation (lusitropic effect) is mainly mediated by the PKA-dependent phosphorylation of phospholamban by dual protein kinases: PKA activated by an increase of cAMP ①, and Ca^{2+}-calmodulin kinase stimulated by an elevation of intracellular Ca^{2+} concentration ② (Figure 11) (Le Peuch et al., 1979; Cory et al., 1994). The phosphorylation of phospholamban promotes the liberation of Ca^{2+} from TnC by activating the SR-Ca^{2+} pump causing a greater and faster Ca^{2+} uptake into the SR and a decreased

③

intracellular Ca^{2+}. In addition, the phosphorylation of troponin I by the PKA activation also promotes a decrease in the sensitivity of the Ca^{2+} contractile system that causes a decreased intracellular Ca^{2+}-myofilament interaction and an increased rate of crossbridge detachment. These events enhance the velocity of cellular relaxation (Barros et al., 1999; Opie, 2004).
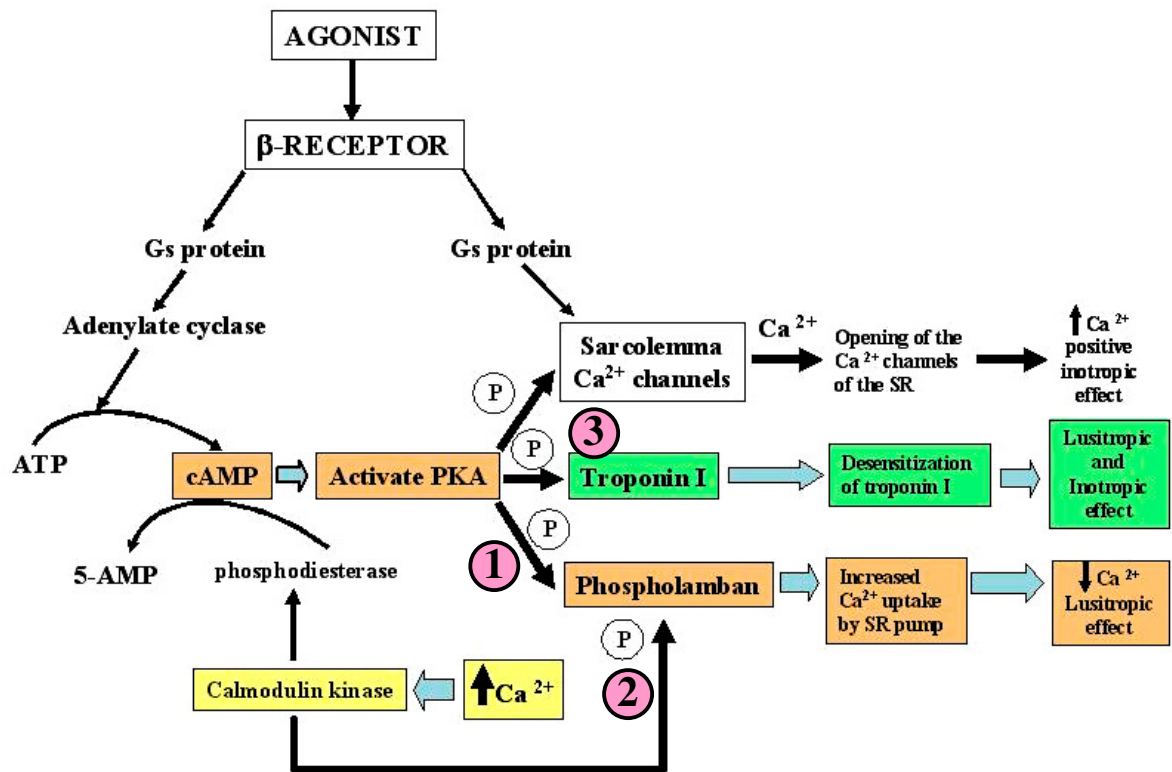
Figure 11 Mechanisms of □-adrenergic receptor agonist mediated the inotropic and

lusitropic effects (modified from Barros et al., 1999).

Chronotropic effect

An increase of the heart rate (positive chronotropic effect) is promoted when □-adrenergic action is exerted on the pacemaker SA node. This causes increases in both the rate of diastolic depolarization and the SA node pacemaker firing. The rapid firing of the SA node occurs by an increased opening of the inward current that is evoked by the hyperpolarization, and the long-lasting inward calcium current (Opie, 2004).

Dromotropic effect

An increase in the velocity of impulse conduction (dromotropic effect) occurs when the impulse is conducted more rapidly down the AV node, His bundle, and Purkinje fibers. The conduction velocity through the AV node is enhanced, probably as a result of stimulation of the slow calcium channel in the AV nodal cells (Opie, 2004).

Adrenergic receptors in the blood vessels

□ -Adrenergic receptors in blood vessels

The □ □$_1$-adrenergic receptors play a crucial role in the regulation of vascular tone. They are primarily responsible for the contractile responses of the arteries, aorta and mesenteric artery that in the rat, □$_{1A}$- and □$_{1D}$- adrenergic receptor subtypes regulate the larger vessels, whereas □$_{1B}$- adrenergic receptors control the small resistance vessels (Leech and Faber, 1996; Gisbert et al., 2000). At the postsynaptic □-adrenergic receptors of the rat aorta, the sensitivity to □ □$_1$-adrenergic receptors was higher than to □ the □ □$_2$-adrenergic receptors by about 12-200 fold (Macia et al., 1984).

All three □$_1$-subtypes produce a vascular contraction by activating phosphoinositide turnover and calcium signaling (Hieble et al., 1995) as demonstrated in Figure 12. Norepinephrine or epinephrine binds to □$_1$-adrenergic receptors (□$_1$-ADR) that interact with G-proteins: pertussis toxin-sensitive G-proteins (Perez et al., 1993) and G-proteins of the G$_{q/11}$ family (Wu et al., 1992) in the vascular smooth muscle cell ①. This allows the operation of two mechanisms. One mechanism is the opening of the receptor-operated Ca^{2+} channels (ROC) causing extracellular Ca^{2+} to flow into the cell ②. The second mechanism involves a chain of biochemical reactions that involves activation of the □$_1$-adrenergic receptors leading to the dissociation of the □ and □③□ subunits of G-proteins that, in turn, activate PLC . This enzyme hydrolyses PIP$_2$ into IP$_3$ and DAG (Zhong and Minneman, 1999). IP$_3$ bind④ on the SR to release stored intracellular Ca^{2+} . In addition, when the cell depolarizes, the extracellular Ca^{2+} ions enter through the vol⑤ge-sensitive Ca^{2+} channels (VSCC) . These mechanisms lead to a ⑥ large increase of intracellular Ca^{2+}

, which then binds to calmodulin to form the calcium-calmodulin complex. This complex activates myosin light chain (MLC) kinase to phosphorylate MLC causing crossbridge formation and contraction. On the other hand, DAG activates protein kinase C ⑦ rising in sensitivity to Ca^{2+}. This inhibits MLC phosphatase to promote vascular smooth muscle contraction (Levick, 2000).
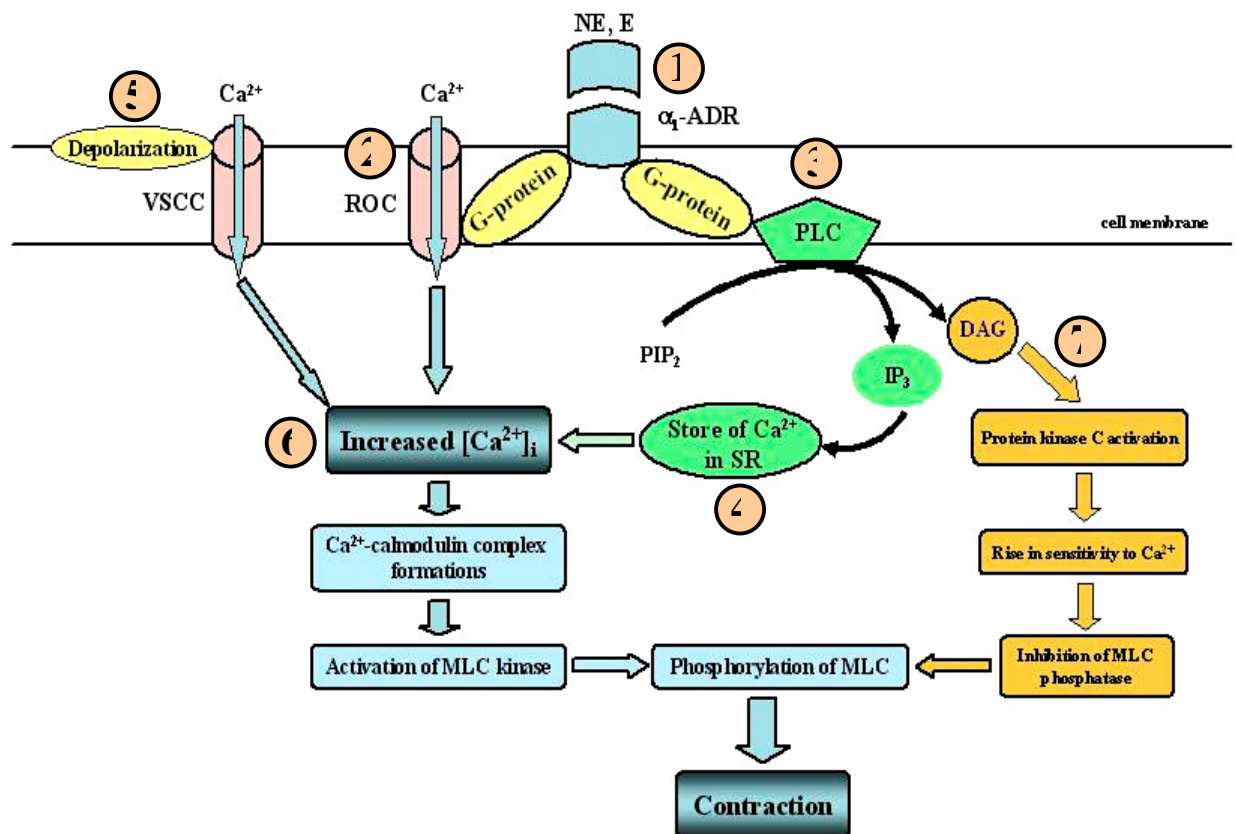
Figure 12　Mechanisms of α_1-adrenergic receptor mediated vasoconstriction (modified from Levick, 2000).

In blood vessels, the roles of α_2-adrenergic receptors include presynaptic inhibition of neurotransmitter release, diminished sympathetic efferent traffic, vasodilatation and vasoconstriction (Carrier and White, 1985; Bockman et al., 1996; Kanagy, 1997; Figueroa et al., 2001). The increase or decrease in vascular tone induced by these receptors depends on the kind of blood vessel and also on the location of the receptors. Endothelial α_2-adrenergic receptors are proposed to be involved in the vasorelaxant response to norepinephrine and epinephrine (Vanhoutte and Miller, 1989; Vanhoutte, 2001), whereas α_2-adrenergic receptors located in vascular smooth muscle have been characterized as vasoconstrictor receptors physiologically activated by cathecolamines (Docherty, 1998; Aantaa et al., 1995). However, the physiological role of the extrajunctional α_2-adrenergic receptors is not fully understood. Since these receptors are located some distance away from adrenergic nerve terminals, they would not interact with

neurally released norepinephrine but rather respond to circulating epinephrine that would directly regulate vessel tone (Langer and Shenpperson, 1982).

$\square$-Adrenergic receptors in blood vessels

Activation of $\square$-adrenergic receptors in the peripheral vasculature leads to vascular smooth muscle relaxation, manifested as a hypotensive blood pressure response in both humans and animals.

Epinephrine mediates vasodilatation by acting via $\square_2$-adrenergic receptor as shown in Figure 13. Epinephrine binds to $\square_2$-adrenergic receptors that couple to G_S-protein ①. It activates adenylyl cyclase to convert ATP to cAMP ②. The cAMP induces PKA activation and causes vasodilatation by chiefly three actions. First, the cytosolic Ca^{2+} concentration falls due to stimulation of the Ca^{2+}-ATPase pumps in the surface membrane and SR ③. Second, a decrease in cytosolic Ca^{2+} concentration is promoted by a hyperpolarization that is brought about by ④ phosphorylation of K^+ channels by PKA ⑤. This hyperpolarization can reduce the open probability of voltage-sensitive channels permeable to Ca^{2+} (VSCC) ⑥. Third, the sensitivity of the contractile process to Ca^{2+} is attenuated, because PKA phosphorylates MLCK to inhibit its ⑦ action (Levick, 2000).

In addition, the relaxant responses of the rat aorta to a non-specific $\square$-adrenergic receptor agonist, isoproterenol, could be inhibited by methylene blue and hemoglobin, indicating that the endothelium-dependent NO/cGMP system may be activated by stimulation of $\square$-adrenergic receptors (Gray and Marshall, 1992; Iranami et al., 1996).
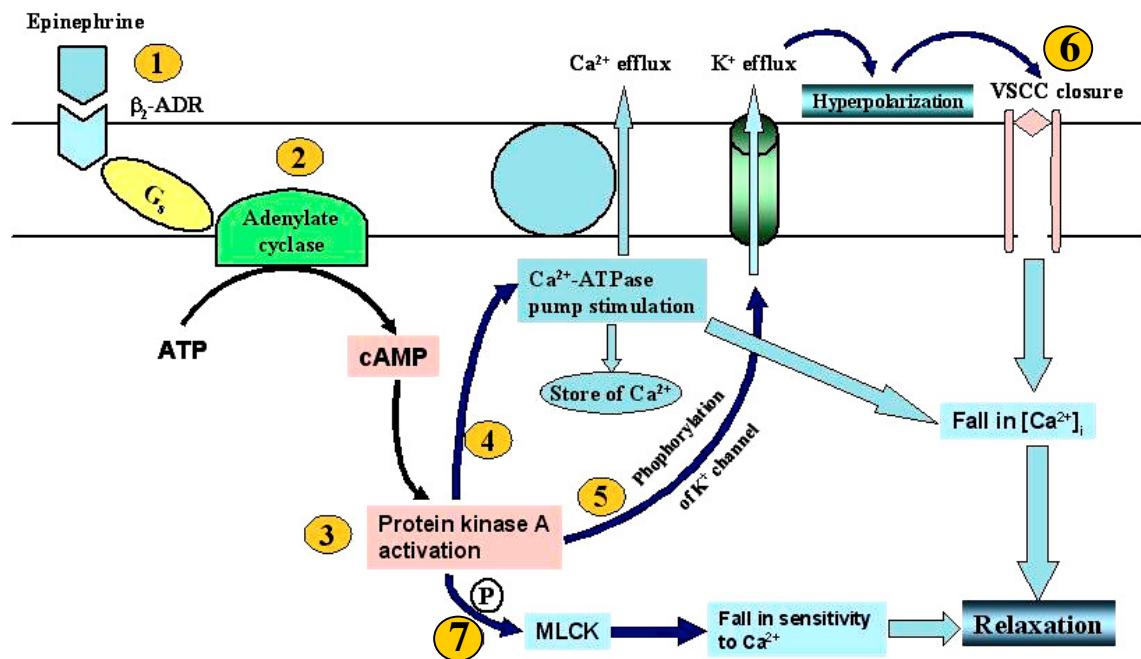
Figure 13 Mechanisms of epinephrine inducing the vasodilatation via the ☐$_2$-adrenergic

receptor. G$_S$=G-protein; VSCC=voltage-sensitive channel permeable to Ca^{2+}

(modified from Levick, 2000)

Parasympathetic nervous system of the heart and blood vessels

Parasympathetic preganglionic neurons originate in the brain stem (Figure 14), from which they run in the vagal nerves to essentially innervate the heart, being limited to the SA node and the AV junction. There is little or no innervation of the peripheral nervous system on cardiac ventricles. On blood vessels, only a small proportion of the resistance vessels, arterioles, of the body receive parasympathetic fibers. The parasympathetic postganglionic neurons release the neurotransmitter, acetylcholine, acting on cholinergic muscarinic (M) receptors. In the heart, acetylcholine binds to the cholinergic M$_2$ receptor causing a decrease in heart rate, whereas in the vascular endothelium, acetylcholine acts via cholinergic M$_3$ receptors

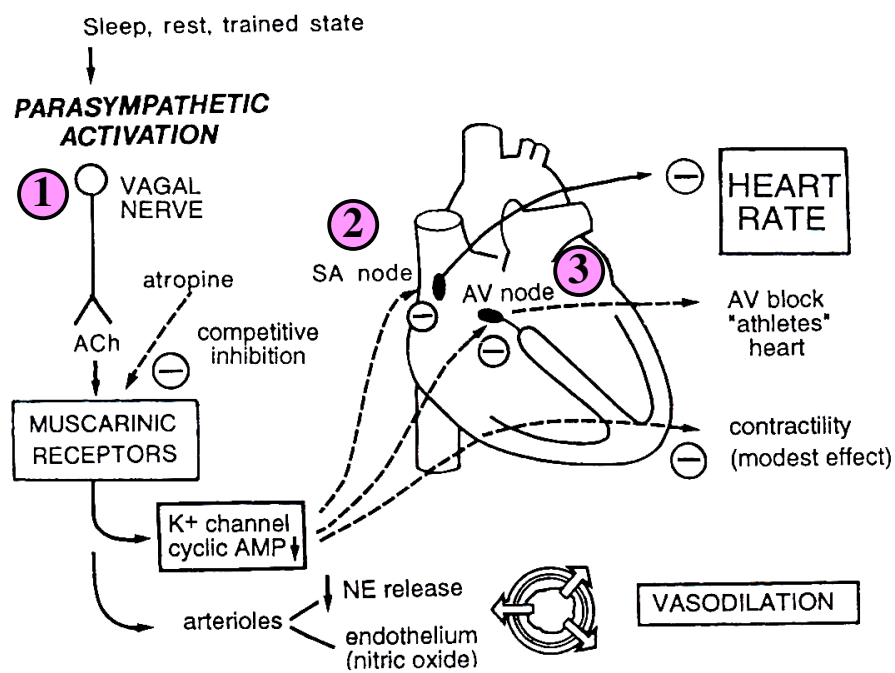causing the release of endothelium derived relaxing factor that dilates vascular smooth muscle (Opie, 2004).

Figure 14 Parasympathetic cholinergic system at the heart and blood vessels (modified from Opie, 2004).

Muscarinic receptors in the heart

Muscarinic receptors in the human heart are predominantly M_2-receptors. The number of M_2-receptors is significantly higher in the atria than in the ventricular myocardium approximately by up to 2.5 fold (Giraldo et al., 1988; Hulme et al., 1990; Caulfield, 1993; Giessler et al., 1999).

In human atria, the stimulation of muscarinic receptors causes direct negative chronotropic and inotropic effects, whereas it causes only the indirect effects in the human ventricle (Brodde and Michel, 1999). The indirect negative inotropic effects by the muscarinic receptor agonists, acetylcholine or carbachol, were demonstrated only when the basal force of contraction had been enhanced in advance by the cAMP-elevating agents such as isoproterenol or forskolin (Brodde et al., 1995; Caulfield, 1993; Dhein et al., 2001). In contrast, the muscarinic receptor antagonist, atropine, has no effect on the resting force of contraction, but it causes an increase in the resting heart rate (Landzberg et al., 1994; Poller et al., 1997).

The activation of M_2 receptors in the heart that couples to a pertussis toxin-sensitive G protein (G_i/G_o) leads to the inhibition of adenylyl cyclase and hence inhibits the increased intracellular cAMP (Felder, 1995). It causes a reduction of the L-type Ca^{2+} current that appears to be the predominant mechanism that inhibits the force of contraction enhanced by the cAMP-elevating agents (Mery et al., 1997) (Figure 10).

The stimulation of the muscarinic receptors in the heart can also elevate cGMP levels (Brodde and Michel, 1999) by the stimulation of cGMP-stimulated cAMP phosphodiesterase (Mery et al., 1997) or cGMP-dependent protein kinase (Lohmann et al., 1997). The increased cGMP levels cause a decrease in the intracellular Ca^{2+} concentration to promote cardiac relaxation.

In addition, in the myocytes of the human atria, acetylcholine additionally opens an inwardly rectifying K^+ channel ($I_{K,ACh}$) through direct effects of the G protein $\square\square$-subunits (Yamada et al., 1998) that result in the hyperpolarization, slowing of heart rate, shortening of the action potential duration, abbreviation of the L-type Ca^{2+} current and reduction of force of the contraction (Belardinelli and Isenberg, 1983).

Muscarinic receptors in blood vessels

The vasodilator effect of the muscarinic receptor agonist, acetylcholine, is endothelium-dependent. It acts via M_3 receptors on the surface of the endothelial cells causing an increase of intracellular Ca^{2+}. The Ca^{2+} activates the enzyme NO synthase to produce NO causing vascular smooth muscle relaxation (Eglen et al., 1996; Caulfield and Birdsall, 1998).