CHAPTER 1

Introduction

1.1 Principle, Theory and Rationale/Hypothesis

Despite there are many treatment for cardiovascular disease, acute myocardial infarction (AMI) remains a common cause of death worldwide [1]. Myocardial reperfusion is a goal standard therapeutic intervention of myocardial infarction to prevent myocardial tissue damage and improve the long-term outcome of myocardial infarction (MI) patients. However, reperfusion after ischemia itself simultaneously causes irreversible myocardial damage and cell loss. This phenomenon, is known as myocardial ischemia/reperfusion (I/R) injury [2]. Therefore, myocardial I/R injury is considered a major concern in patients who receive a bypass surgery, balloon angioplasty, and thrombolytic drugs after AMI [3, 4]. Over the past decades, the enhance most of parasympathetic activity by electrical stimulation of the cervical vagus nerve has been shown to exert a therapeutic property for various conditions, including brain and heart disorder. Additionally, vagus nerve stimulation (VNS) has been shown to exert cardioprotection in both ischemic heart diseases and chronic heart failure [5-10]. Specifically, in ischemic hearts, VNS has been shown to decrease the infarct size, improve cardiac performance, attenuate cardiac remodeling, improve defibrillation efficacy, limit dispersion of repolarization and prevent reperfusion injury [11, 12]. These emerging data led us to hypothesize that VNS might be used as adjunctive myocardial salvage approach to current percutaneous coronary and pharmacological interventions designed to protect myocardium at risk of I/R injury. We previously demonstrated that intermittent VNS applied immediately at the onset of the ischemic period significantly decreased the infarct size and ventricular arrhythmia, and improved left ventricular (LV) function [13, 14]. Interestingly, stimulation only efferent direction of vagus nerve by stimulating distal part of the right cervical VNS, after cutting the vagus nerve in order to eliminate the effect of afferent vagal activity, also exert

cardioprotection in heart subjected to ischemia or/and reperfusion injury [15-18]. In addition, a previous study demonstrated that the activation of vagal afferent fibers during VNS decreased efferent parasympathetic tone to the heart by using both electrophysiological and hemodynamic parameters as an indicator [19]. Although VNS has been shown to exert cardioprotection against myocardial I/R injury in both preclinical and clinical studies, it is still remained unclear whether the cardioprotection of VNS is mainly due to direct vagal activation through its ipsilateral efferent vagal fibers (motor fiber) or indirect effects mediated by the afferent vagal fibers (sensory fiber). Therefore, the objectives of this study were to determine whether the cardioprotective effects against myocardial I/R injury of VNS were mainly due to direct ipsilateral efferent vagal fibers activation or indirect effects mediated by the afferent vagal fibers. Furthermore, roles of the contralateral efferent vagal fibers during VNS were also investigated. We hypothesized that VNS exerts cardioprotection against myocardial I/R injury predominantly through its ipsilateral efferent vagal fibers.

1.2 Literature Review

1.2.1 ischemia/reperfusion injury

Coronary heart disease (CHD) is a common cause of death with high morbidity and mortality rates. In 2008, WHO have reported that 7,254,000 peoples dead from CHD (12.8% of all deaths) [20]. The major cause of CHD is AMI. Restoration of blood flow with either thrombolytic agents or primary percutaneous coronary intervention (PCI) is the most effective therapeutic intervention to decrease myocardial cell loss, limit infarct size, preserve left ventricular (LV) function, prevent heart failure and improve the clinical outcome [21]. However, restoration of blood flow to the ischemic tissue may result in a devastating complication associated with the release of several toxic compounds [20, 21]. This phenomenon is known as myocardial I/R injury, which decreases the beneficial effects of myocardial reperfusion [2, 20]. Moreover, myocardial I/R injury can induce lethal myocardial reperfusion injury, a no-reflow phenomenon (microvascular obstruction), cardiac arrhythmias, myocardial stunning and hibernation [20]. Thus, myocardial I/R injury is a major concern in

patients with acute myocardial infraction who receives coronary artery bypass grafting (CABG) and transplantation [3, 4].

1.2.2 Mechanism of I/R injury

Following a coronary occlusion, loss of blood supply in myocardial is rendered ischemia. Ischemic cells switch aerobic respiration to anaerobic respiration, which is decrease efficient to produce adenosine triphosphate (ATP) and results in lactic acid accumulation. This induces the Na^+-H^+ exchanger to efflux H^+ and influx Na^+ leading to intracellular Na^+ overload. Intracellular Na^+ overload which activates reverse-mode of Na^+-Ca^{2+} exchanger leads to efflux Na^+ and influx Ca^{2+} leading to intracellular Ca^{2+} overload. Moreover, ATP depression causes Na^+-K^+ ATPase and K^+ ATPase loss of function during ischemia, exacerbating intracellular Na^+ overload and cell swelling. Moreover, lactic acid accumulation leads to reduction in intracellular pH prevented the opening of the mitochondrial permeability transition pore (MPTP) and cardiomyocyte hypercontracture at this time. Furthermore, ischemia also triggers the production of reactive oxygen species (ROS) which can disrupt the sarcolemma and mitochondrial function leading to cell death.

During reperfusion, blood supply in myocardial can reverse to aerobic metabolism. The electron transport chain is reactivated leading to a significant increased ROS generation. In addition, ROS can be generated from several other sources including endothelial cells (xanthine oxidase) and neutrophils (NADPH oxidase). Increasing of ROS production leads to MPTP opening, neutrophil accumulation and sarcoplasmic reticulum (SR) dysfunction. This contributes to intracellular Ca^{2+} overload and damages the cell membrane (lipid peroxidation), inducing enzyme denaturation and causing direct oxidative damage to deoxyribonucleic acid (DNA). Moreover, restoration of blood flow can washout H^+ and lactic acid in extracellular leading to a significant increased H^+ gradient between intracellular and extracellular. This induces the Na^+-H^+ exchanger to efflux H^+ and influx Na^+ leading to intracellular Na^+ overload. Intracellular Na^+ overload which activates reverse-mode of Na^+-Ca^{2+} exchanger leads to efflux Na^+ and influx Ca^{2+} leading to intracellular Ca^{2+} overload. Furthermore, washout of

H^+ and lactic acid restore physiological pH, which cancel the inhibitory effect on MPTP opening and cardiomyocyte hypercontracture. The opening of MPTP leads to cytochrome c release and induce apoptosis cascade. Several hours after the onset of reperfusion, neutrophils infiltrate in the ischemic tissue leading to the release chemoattractants, ROS, cytokines, and activated inflammatory processes (Figure 1) [22, 23].

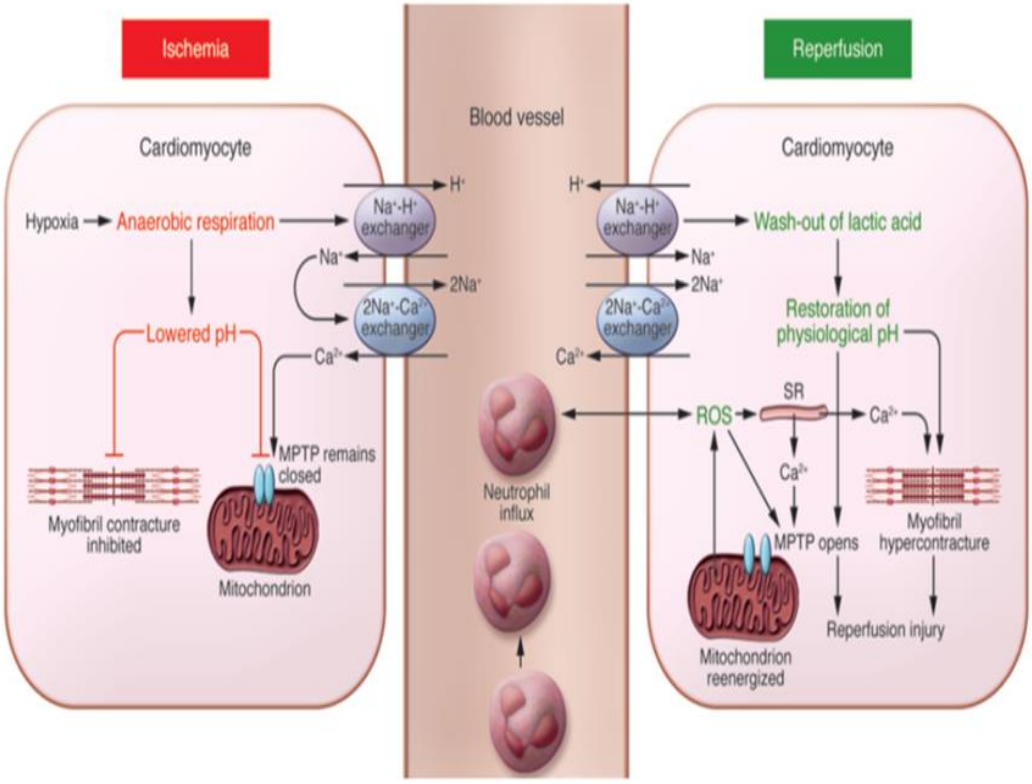

Figure 1-1: Summarize the mechanism of ischemia/reperfusion injury [20]

1.2.3 Mitochondrial function during I/R injury

Mitochondria are described as "cellular power house" because they generate most of the chemical energy in the form of adenosine triphosphate (ATP) to supply the whole cell. In the heart, mitochondria are highly abundant, constitute approximately 40% of total cardiomyocyte volume and produce more than 90% of the cell's energy [24].

Mitochondria are known to play an important role during myocardial I/R injury [25]. Previous studies reported that I/R injury leads to cardiac mitochondrial dysfunction indicated by increased ROS production, increased mitochondrial

depolarization and increased mitochondrial swelling [13, 14, 26]. Moreover, I/R injury leads to an increasing of NADPH oxidase, leading to increased oxygen peroxide (O^{2-}) level, which is the major source of ROS [27]. The increase of mitochondrial ROS levels activated the mitochondrial inner membrane anion channel (IMAC) opening and leads to the release of O^{2-} from mitochondria to cytosol [28]. Moreover, an increased mitochondrial ROS levels can activate mPTP opening and lead to the influx of K^+ from cytosol to mitochondrial matrix. Thus, the loss of negative ion and the increase of positive ion within mitochondrial matrix induce the mitochondrial depolarization during I/R injury [28]. The increased of mitochondrial ROS levels not only induce the influx of K^+ from cytosol to mitochondrial matrix but also the influx of water from cytosol to mitochondrial matrix, resulting in the mitochondrial swelling [26].

Moreover, I/R can also lead to the impairment of mitochondrial dynamics [29-33]. Mitochondrial dynamics is a quality control process of mitochondrial, which consists of mitochondrial fusion and fission [32]. For mitochondrial fusion, mitochondria fuse together to form a network, that able to communicate, integrate their matrix contents, and generate more ATP for cells when higher energy demands [31, 34]. In contrast, mitochondrial fission is the opposite of mitochondrial fusion, which mitochondria split into 2 fragments. This event generates smaller mitochondria responsible for decreased ATP demand and these segregated mitochondria are removed by mitophagy [30]. Numerous studies have shown that during the ischemic and reperfusion period mitochondria undergo fission and that there is an absence or reduction in mitochondrial fusion [33].

Furthermore, mitochondria can increase their individual mitochondrial mass and copy number to increase the production of ATP as a response to greater energy expenditure, this process call mitochondrial biogenesis [24]. The protein that plays an important role during mitochondrial biogenesis is peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) [35]. PGC1α is a transcriptional coactivator that regulates the genes involved in energy metabolism. In the cardiomyocyte PGC1α can bind with many receptors that play an important role with mitochindrial biogenesis [35]. Previous studies reported that the increased of PGC1α associated with increased fatty acid oxidation,

decreased glucose oxidation, increased OXPHOS activity. Finally, ATP production was increased. In addition, in many pathogenic conditions, PGC1α was decreased leading to the reduction of ATP production [35].

1.2.4 Treatment of I/R injury

Several cardioprotective strategies to prevented I/R injury have been characterized by a many of experimental studies with several pharmacological drugs that have shown great promise on the bench, but have lacked efficacy in the clinic. Furthermore, the field of endogenous cardioprotection is particularly appealing given that multiple clinical studies have documented feasibility and efficacy [36-39]. These strategies could be performed by one or several brief cycles of ischemia followed by reperfusion before ischemia (ischemic preconditioning), or after ischemia (ischemic postconditioning) and ischemia at other tissues or organs (remote conditioning) [40, 41]. Moreover, activation of endogenous cardioprotective signalings against I/R injury using an electrical stimulus such as spinal cord stimulation and VNS, as opposed to a pharmacological approach, is emerging as an attractive possibility for clinical application.

1.2.5 Autonomic nervous system

The autonomic nervous system (ANS) is a division of the peripheral nervous system that regulates cardiac performance, visceral activity, and glandular functions of the body. Specifically the ANS can control heart rate (HR), blood pressure (BP), rate of respiration (RR), body temperature, sweating, gastrointestinal motility and secretion, as well as other autonomic activities that maintain homeostasis. The ANS functions continuously without conscious effort. However, it is controlled by centers located in the spinal cord, brain stem, and hypothalamus [42].

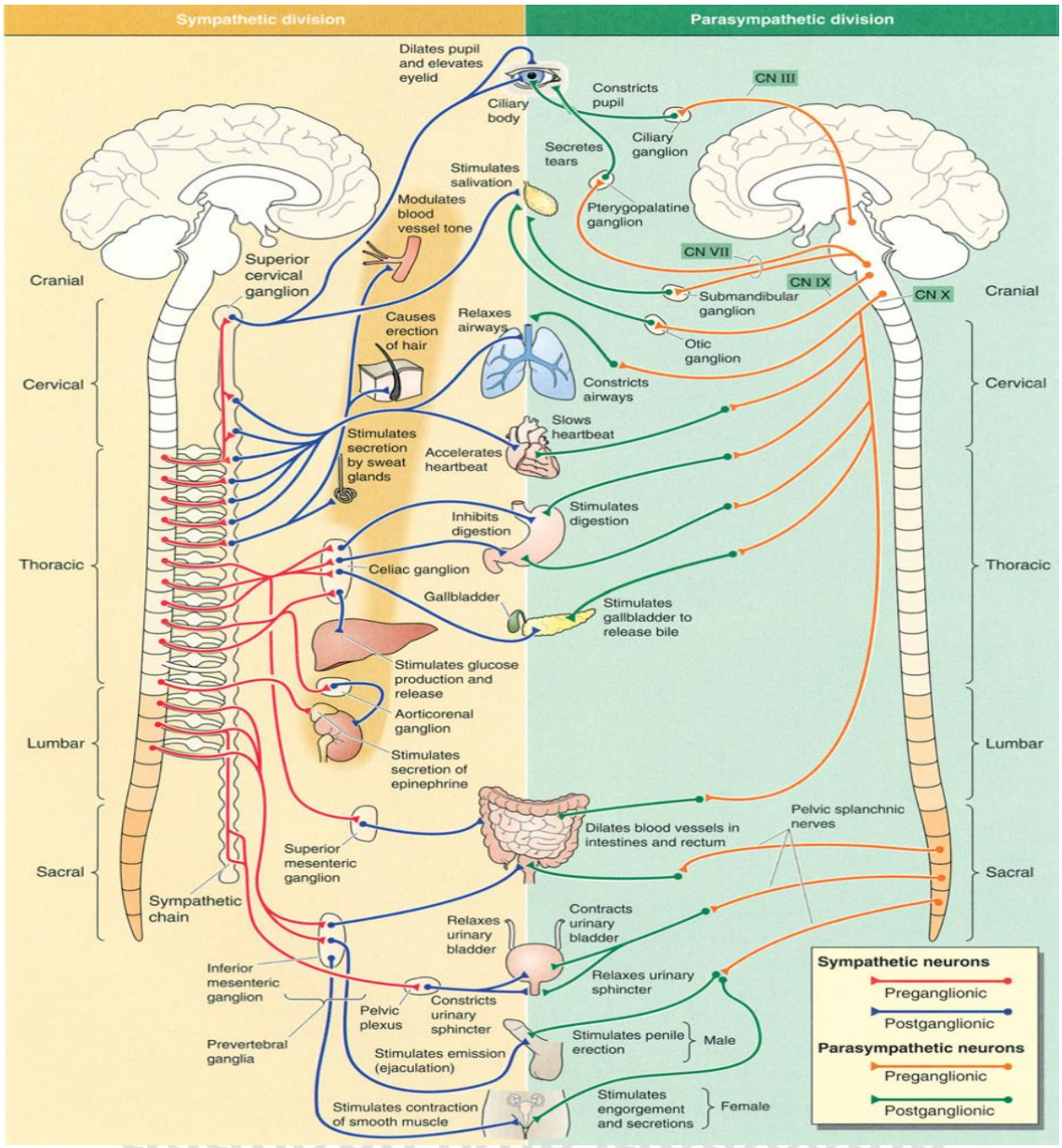

Figure 1-2: Schematic representation of the autonomic nervous system [43]

The ANS has three branches: the sympathetic nervous system, the parasympathetic nervous system and the enteric nervous system. Some textbooks do not include the enteric nervous system in the ANS. The sympathetic system promotes a fight-or-flight response, prepares the body for energy expenditure, emergency or stressful situations and inhibits digestion. In contrast, the parasympathetic system promote a "rest and digest" response, promotes calming of the nerves return to regular function, and enhancing digestion. The

sympathetic system (thoracolumbar division), these nerves originate from the thoracolumbar region of the spinal cord (T1-L2/3) and radiate out towards the target organs (Figure 1-2). The parasympathetic system originate within the midbrain, pons and medulla oblongata of the brain stem and part of these fibers originate in the sacral region (S2-S4) of the spinal cord. While sympathetic nerves contain a short preganglionic neuron followed by a relatively long postganglionic neuron, parasympathetic nerves have a much longer preganglionic neuron, followed by a short postganglionic neuron (e.g., the vagus nerve, which carries about 75 percent of all parasympathetic fibers) [42].

1.2.6 Vagus nerve anatomy

The vagus nerve (VN) is a mixed nerve with 80% afferent fibers (sensory) and 20% efferent fibers (motor). This nerve is the longest cranial nerve which innervates several organs throughout our body including heart (Figure 1-3). It is involved in autonomic, cardiovascular, respiratory, gastrointestinal, immune, and endocrine systems and has been called the "great wandering protector". The VN contains A-, B-, and C-fibers defined by their conduction velocities and sizes (Table 1-1). (1) A-fiber contain large myelinated A-fibers (Aα and Aβ) carry mostly somatic afferent and efferent information, and small myelinated A-fibers (Aδ) primarily transmit visceral afferent information; (2) B-fibers provide efferent sympathetic and parasympathetic preganglionic innervation; and (3) small unmyelinated C-fibers (the most VN fibers: 60-80%) primarily carry afferent visceral information [44].

VN fibers are primarily cholinergic neurotransmitter (ACh) but other non-cholinergic non-adrenergic neurotransmitters are also involved including nitric oxide (NO), vasoactive intestinal peptide (VIP), and calcitonin gene-related protein (CGRP). Moreover, VN have tyrosine hydroxylase, the enzyme responsible for dopamine and noradrenaline biosynthesis in cervical and thoracic vagal trunks, suggesting a potential cross-talk to the sympathetic system in VN function. Furthermore, the sympathetic fibers can be found in the VN (Figure 1-3) but their function and transmission direction is not clear.

Table 1-1: Fiber type and function of vagus nerve [44]

Fiber Type	Fiber size (mm)	Conduction velocity (m/s)	Main function	
			Afferent	Efferent
Aα	13-20	8-120	somatic; touch; pain, temperature	muscle
Aβ	6-12	35-75	somatic; touch	muscle, preganglionic
Aδ	1-5	3-30	visceral; pain, stretch, chemical, temperature	preganglionic
B	1-5	3-15	visceral	preganglionic
C	0.4-2	0.5-2	visceral; pain, stretch, chemical, temperature	preganglionic

The VN comprises four nuclei: dorsal motor nucleus (DMN), nucleus ambiguous (NA), nucleus of the solitary tract (NST) and spinal nucleus of the trigeminal nerve (SNT). Vagal afferents terminate in the SNT and NST with their neuron bodies inside the superior and inferior ganglia, respectively. The DMN and NA send vagal efferent to terminal ganglia (Figure 1-3) [44].

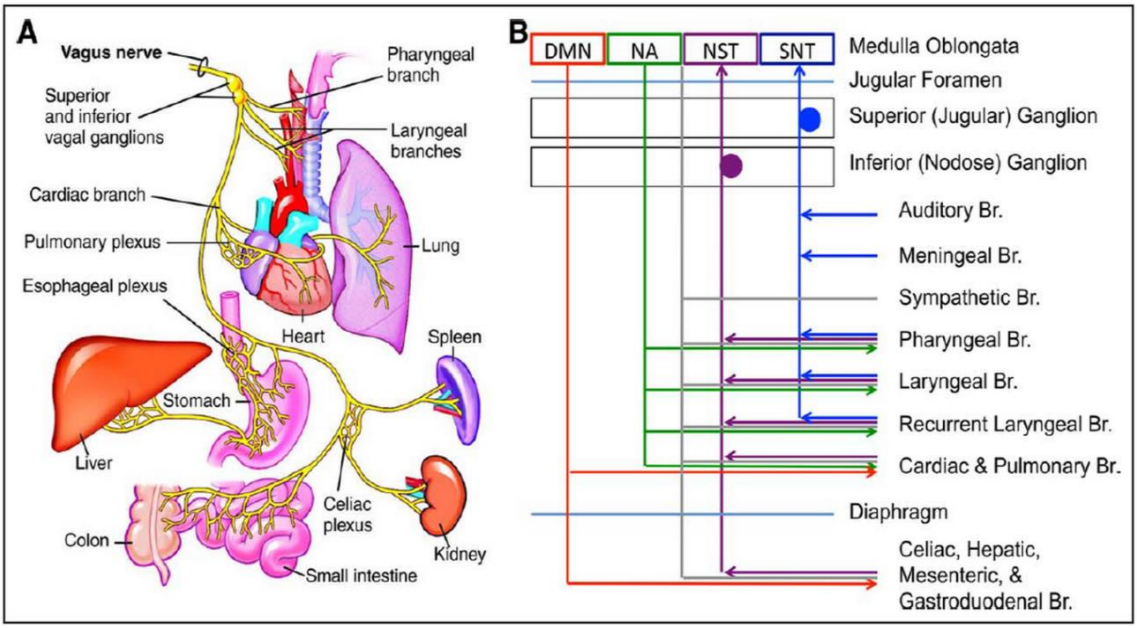

Figure 1-3: Illustration of the vagus nerve anatomy and vagal nuclei connections [44]

1.2.7 Alteration of autonomic control in ischemic heart disease

The autonomic nervous system plays an important role in the regulation of the mammalian heart [8, 45-48]. Normally, the autonomic nervous system tightly regulates cardiovascular function through the interaction of sympathetic and parasympathetic activities. A predominance of sympathetic activity increases heart rate (HR) and contractility [49]. In contrast, increased parasympathetic activity (vagal tone) decrease heart rate and contractility [49]. Impaired regulatory function of the autonomic nervous system are the characteristic autonomic phenotype associated with various forms of heart disease including myocardial infarction [50]. Previous study demonstrated that myocardial infarction increases sympathetic tone and decreases parasympathetic tone by a cardio-cardiac sympathetic reflex. This phenomenal is known as “autonomic imbalance or sympathovagal imbalance” [51]. Moreover, sympathovagal imbalance is a major cause of heart disease [52]. Data from a multicenter international prospective study, 1,284 MI patients (Autonomic Tone and Reflexes after Myocardial Infarction, ATRAMI) reduced vagal tone. This is a major cause for cardiac arrhythmia [52]. Reduced vagal activity accompanied by increased sympathetic activity is considered a known risk factor for MI and indicates a

potentially decreased survival rate following MI [53-56]. Interestingly, increased VN activity not only reduces cardiovascular disease risk factors, but also exerts anti-fibrillatory effects in both animal models and affected patients [57-62]. In addition, increased vagus nerve activity has been shown to be a positive indicator for recovery after MI [5]. Therefore, rebalanced autonomic activity by augmenting vagal activity by VNS may be a potential therapeutic intervention for the affected MI patients.

1.2.8 Vagus nerve stimulation (VNS) system

The VNS system composed of a pacemaker-like device (a generator), flexible wire (a lead) and electrodes. The electrodes consist of a positive electrode, negative electrode and anchor binding. The electrodes wrap around the vagus nerve and connect to the lead, which is attached to the pulse generator. A generator is implanted in the chest wall and is programmed by the scientist to stimulate the VN at cervical level. Thin wire is threaded under the skin and woven around the VN in the neck (figure 1-4).

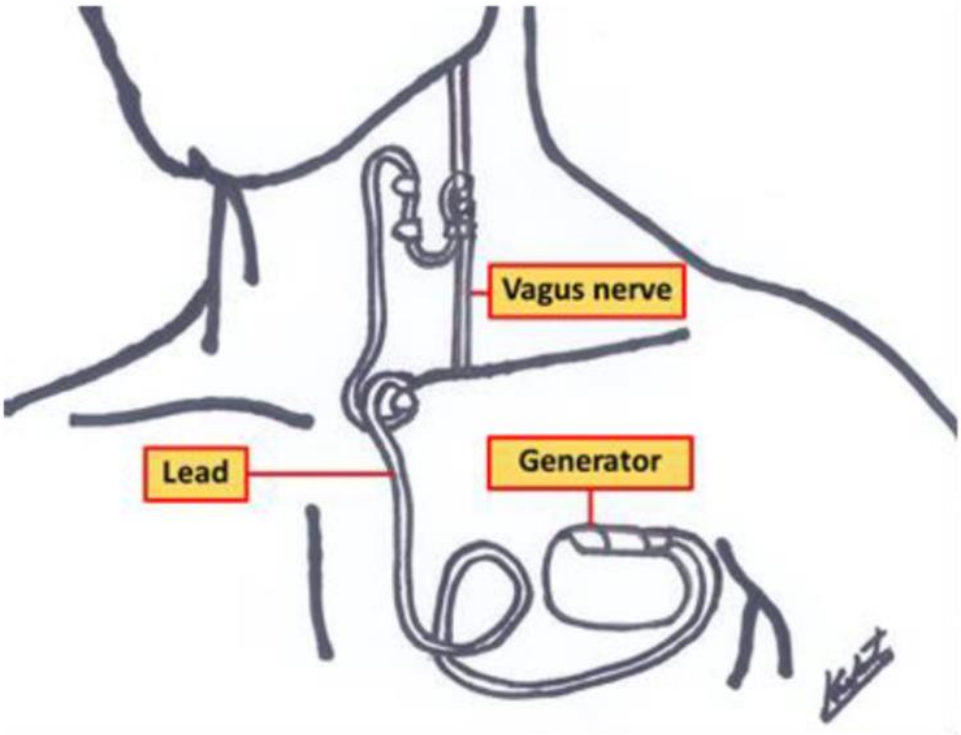

Figure 1-4: Illustration of the VNS system [63]

1.2.9 Effects of VNS on myocardial infarct size after I/R injury

In MI patients, the major determinant of the mortality is the infarct size [64]. Therefore, the best therapeutic strategy for these affected patients is to reduce the infarct size by revascularization of the occluded coronary artery as soon as possible. However, restoration of blood flow after ischemia itself simultaneously causes irreversible myocardial cell injury in a process called "myocardial ischemia reperfusion (I/R) injury" [2, 65]. The cause of myocardial damage in I/R injury can be divided into two categories: (1) myocardial damage as a result of the myocardial ischemic process itself; and (2) myocardial damage as a result of reperfusion, termed "reperfusion injury" (Figure 1-5) [20, 21].

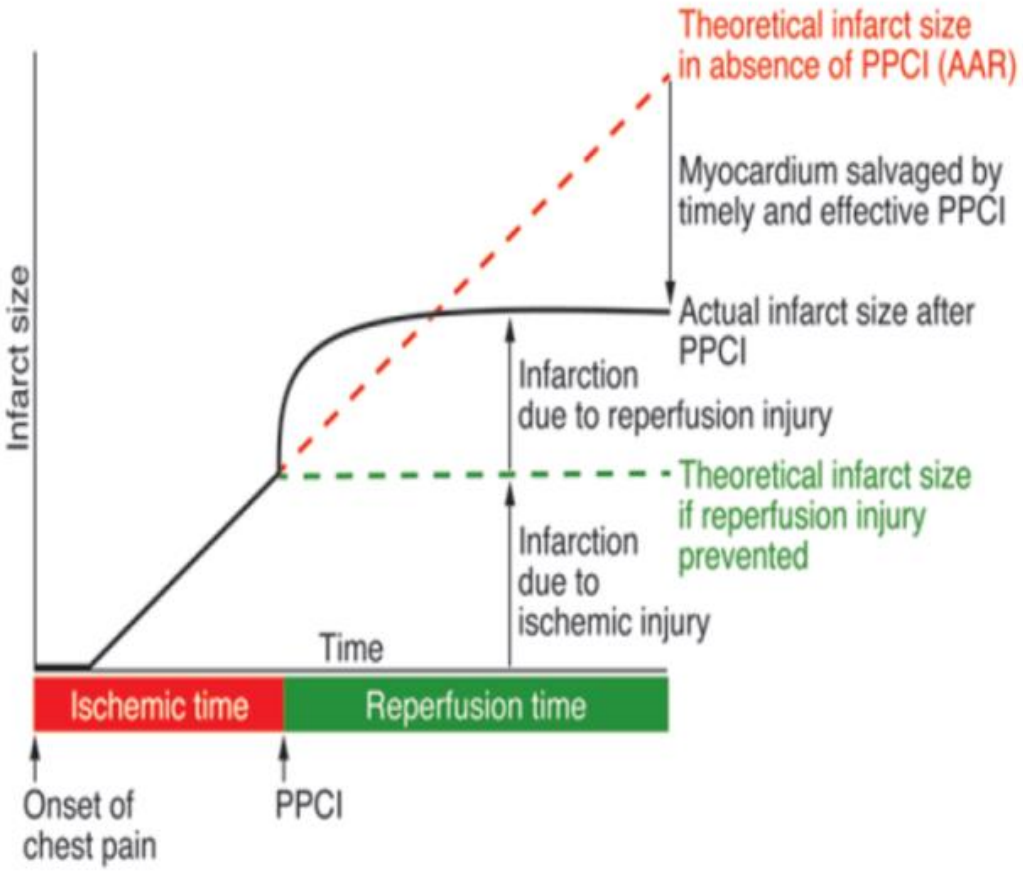

Figure 1-5: Illustration of myocardial damage in I/R injury [21]

Over several years, VNS has been shown to decrease the size of myocardial infarction in an experimental model. Right cervical VNS (intact and efferent portion) significantly decreased infarct size in rat subjected to left coronary artery (LCA) ligation [15, 17, 18, 66]. The protective effect of VNS against AMI is associated with increased hypoxia-inducible factor (HIF)-1α expression [15], regulated tumor necrosis factor (TNF) receptor subtypes [17, 66] and inhibited ROS production via the decreased NADPH oxidase (Nox) activity [18]. Moreover, in global I/R model (30-minutes of ischemia and 2-hours of reperfusion) with intact vagus nerve innervation, efferent VNS (5 minutes before ischemia) significantly decreased infarct size irrespective of HR when compared with non-stimulation (37±4% vs 43±5% at HR 250 bpm, 34±6% vs 85±9% at HR 300 bpm, and 39±4% vs 88±7% at HR 350 bpm) [16]. The protective affect was abolished after administration of Atractyloside (a permeability transition pore (PTP) opener). They concluded that VNS exerted a marked anti-infarct effect independent HR reduction via inhibition of PTP opening. In 2013, we recently reported that VNS start at onset of ischemia significantly decreased infarct size through prevention of mitochondrial dysfunction in swine model [13]. In this study, both continuous (C-VNS) and intermittent (I-VNS) attenuated cardiac mitochondrial ROS production, depolarization, and swelling, compared with the I/R group. Meanwhile, I-VNS produced the anti-infarct effects higher that C-VNS. Furthermore, we also found that VNS could provide significant cardioprotective effects even when initiated later during ischemia, but was not effective after reperfusion [14]. This finding indicates the importance of timing for VNS initiation, and warrants the potential clinical application of VNS in protecting the myocardium at risk of I/R injury. Therefore, to determine the optimal intervention time of the vagal stimulation (VS) attenuating myocardial I/R injury (IRI), Wang et al. (2014) randomly allocated rat into six groups: sham group, IRI group, the VS performed at 15 min of ischemia (VSI15) group, the VS performed immediately before reperfusion (VSR0) group, the VS performed at 30 min of reperfusion (VSR30) group, and the VS performed at 60 min of reperfusion (VSR60) group. They found that all groups treated with VNS significantly reduced infarct size when compared with IRI group. Interestingly,

VNS performed at 15 min of ischemia provides the best protection against myocardial IRI [67]. Not only in acute MI but VNS also decreased infarct size in chronic MI [68]. Uemura et al. (2010) have reported that, at 8 weeks after ischemia reperfusion in rabbit model, MI treated with VNS for 3 days showed significantly reduced infarct size, infarct wall thinning, and LV weight compared with MI rabbits. At 24 hours after I/R, MI-VNS rabbits showed significantly decreased myocardial infiltration of neutrophils and reduced myocardial expressions of TNF-α and matrix metalloproteinase-8 and -9, compared with MI rabbits. They conclude that, VNS attenuates LV remodeling after reperfused MI, which may be associated with suppression of acute inflammatory reactions. However, Buchholz and his college have reported that right continuous efferent VNS significantly increased infarct size in group subjected to right VN transection without HR constant. However, the VNS protocol applied in this study was too short (10 minutes) and did not cover either ischemic or reperfusion periods [69]. Zhang and colleagues have reported that VNS with very low intensity (50% of threshold) did not affect the area at risk, infarct size or the ratio of infarct size to area at risk in a canine model subjected to I/R injury [70]. However, this intensity significantly decreased LV arrhythmia through attenuated inflammation process. Moreover, Wu and colleagues have reported that right efferent VNS did not change myocardial infarct size in both adult and aging rat after LAD ligation 30 minutes [71]. These controversy findings might be due to various factors, including experimental protocols, VNS parameters, timing of VNS, animal model and methodological in each center.

1.2.10 Effects of VNS on anti-arrhythmic effects

Interestingly, VNS not only exhibits anti-infarct effects, but also has been shown to have anti-arrhythmic effects. In the feline regional I/R model (20-minutes of ischemia and 10-minutes of reperfusion), intact nerve and vagus nerve transection groups developed complete reperfusion arrhythmias. Surprisingly, in animals treated with efferent VNS and efferent VNS+pacing occurred arrhythmias only 60 and 72%, respectively. The incidence of VF was similar in intact (62%) and VN transection (58%) groups. However, it was lower in animals treated with VNS (7%), and VNS+pacing (48%). This data suggested that anti-arrhythmic

effects of VNS independent on HR reduction [72]. In 1991, Vanoli and colleague clearly demonstrated that enhanced VN activity by right VNS could prevent ventricular tachycardia (VT) in a model with treated MI, exercise testing and intermittent ischemia [5]. The anti-arrhythmic effects of VNS associated with suppress sympathetic tone and increase vagal tone. In 2005, Ando and colleague reported that right cervical efferent VNS protects the heart against ischemia-induced lethal arrhythmias by preserving connexin43 (Cx43) protein [73]. The result shown that, efferent VNS achieved an 87% reduction in the relative incidence ratio of VT (8 of 12 rats in the MI-SS group experienced VT, 1 of 11 in the MI-VS group developed VT during the 30-minute LCA ligation (67% versus 9%, $P = 0.005$). Atropine administration abolished the anti-arrhythmic effects of VNS; 4 of 6 rats developed VT. However, efferent VNS with constant HR (pacing) still provided these benefit. This data suggesting that, efferent VNS applied during ischemia may exert anti-arrhythmic effects by modulating the level of connexin43 phosphorylation independent of HR reduction. This result was consistent with Wu et al. (2011) [71]. They demonstrated that, right cervical efferent VNS significantly suppressed VT and VF in adult rats. However, VNS did not show anti-arrhythmic effects against ischemia-induced VT and VF in older rats (≥24 months). They found that the older rats reduced expression of Cx43 protein when compared with adult rats. This data suggested that Cx43 might be an important target for inhibiting ischemia-induced VT in adult patients but not in older patients. In the same way, we reported that intermittent VNS applied during ischemia protected the heart against reperfusion arrhythmias by increasing Cx43 phosphorylation. We found that VNS significantly decreased number of PVC, VT/VF and time of the T-wave peak to end (Tpe). However, QRS interval was not changed. This data suggested that VNS mainly improved ventricular repolarization, but not depolarization [13, 14]. Wang et al. (2012) have reported that right continuous VNS decreased incidence of ventricular fibrillation and decreased arrhythmia score during reperfusion period after rat subjected to ischemia-30 minutes and reperfusion 120 minutes [74]. These results were comparable with present of infarct size and associated with attenuate local and systemic inflammation. Interestingly, low level VNS (LLVNS: 80% of

threshold) significantly reduced the episodes of VPC and VT during ischemic and reperfusion periods when compared with non-stimulation [75]. The mean duration of VT in the ischemic and reperfusion periods was lower in LL-VNS as well. The number of VF episodes was significantly decreased in the LL-VNS group compared with the I/R group. The protective effects of VNS were associated with increasing the vagal tone against ventricular arrhythmias observed by increased the HF power, reduced the LF power and decreased the LF/HF ratio. Moreover, in canine subjected to I/R injury (ischemia-1 hour and reperfusion-2 hours), bilateral continuous VNS (50% of threshold) significantly decreased number of PVC, Salvo, VT and percent of animal with VF when compared with untreated group [70]. The cardioprotective effect was associated with attenuate local and systemic inflammation. In contrast, we found that at onset of ischemia continuous VNS was not significantly decreased number of VT/VF when compared with intermittent VNS [13]. Moreover, intermittent VNS treated heart after reperfusion (15 minutes) was not significantly decreased number of VT/VF when compared with VNS treated heart during ischemia [14]. The results are similar to Wang et al. (2014). They found that continuous VNS after reperfusion (30 and 60 minutes) was not decreased incidence of VF and arrhythmia score in rat subjected to I/R injury [67]. Furthermore, Uemura and colleagues [68] reported that 8 weeks after I/R, the mortality rate from arrhythmia at MI induction was comparable between the MI and MI with VNS. In this study suggesting that VNS did not afford any survival benefit. Therefore, the anti-arrhythmic effects of VNS against I/R injury is not clearly understood.

1.2.11 Effects of VNS on LV function and hemodynamic parameters

For the heart, excitation of vagal activity, either spontaneously or via electrical stimulation, exerts negative chronotropic, dromotropic and inotropic effects. When, increased vagal activity is well known to induce bradycardia which can improve the demand-supply mismatch, and therefore its cardioprotective effects might be exerted through this bradycardia effect [63]. Continuous right cervical VNS markedly reduced HR but had no significant effect on MAP in normal rat. However, in a rat subjected to AMI by 4-hours of LAD ligation, mean arterial pressure (MAP) was markedly reduced after AMI but was

partially restored by VNS treatment. Characteristic impairments in contractility ($\pm dP/dt_{max}$), systolic and diastolic function (LVSP and LVEDP) after AMI were also significantly improved in AMI rats treated with VNS. The protective effects were associated with anti-infarct effects. Inhibition of a TNF-α mediated signaling pathway was the potential mechanism [66]. These result consistent with another rat model [16, 76-78]. In ex vivo, efferent VNS treat LV showed significantly high performance indicated by significantly increased LVDP and decreased LVEDP in global ischemia reperfusion heart [16, 76]. Although, heart rate constant by pacing, efferent VNS also exert cardioprotective effects [76]. In vivo study, Zhao et al. (2013) have reported that efferent VNS significantly increased LVSP, decreased LVEDP and increased LV dP/dt after ischemia 1 hour follow by reperfusion 2 hours [77]. Xue et al. (2016) have reported that efferent VNS significantly increased LVDP and increased LV ±dP/dt after induced global ischemia heart by administration of isoproterenol [78]. These findings are also consistent with experiments performed in subjected to regional I/R injury [72]. In the swine model, we demonstrated that intermittent VNS applied during ischemia but not at the onset of reperfusion preserved LV functional performance during ischemia and reperfusion periods. In the control group, the stroke volume (SV) and ejection fraction (%EF) were significantly decreased and the end-diastolic pressure (EDP) was significantly increased during the I/R period compared with the baseline period. In addition, stroke volume (SV), ejection fraction (%EF) and the end-diastolic pressure (EDP) was not significantly difference when compared with baseline in VNS applied during ischemia but not at the onset of reperfusion group [14]. The cardioprotection was association with reducing infarct size and via its action on cardiac mitochondrial integrity through its anti-apoptotic and anti-inflammatory effects. These results have been consistented with wang et al. (2014) and Uitterdijk et al. (2015). Wang et al. (2014) have reported that VNS significantly decreased HR when compared with non-stimulation. As compared to the baselines, MAP and rate pressure product (RPP) at the onset of ischemia significantly decreased in the IRI, VSI15, VSR0, VSR30 and VSR60 groups. At 10 min after ischemia, MAP and RPP returned to the baselines. However, only VSI15 significantly decreased RPP when compared with another groups [67].

These results suggest that VNS performed at 15 min of ischemia provides the best protection against myocardial IRI. In the same way, VNS was started 5 min prior to reperfusion and continued until 15 min of reperfusion, decreased mean aortic and LV peak systolic pressure at 45 min of CAO. VNS did not alter LV dP/dt_{P40}, LV end diastolic pressure, or regional systolic and post-systolic segment shortening. This results suggested that VNS started 5 min prior to reperfusion did not affect global or regional LV function [79]. Moreover, efferent VNS did not prevent contractile dysfunction after I/R in rabbits subjected to 30 minutes of ischemia and 180 minutes of reperfusion [80]. In this study, it has been shown that efferent VNS and I/R with VNS groups, HR decreased significantly compared with sham and I/R values at 60 and 180 minutes. In I/R and I/R with VNS groups, fractional shortening (FS) was depressed during ischemia with only partial recovery after reperfusion. Moreover, there was no significant difference in LV dP/dt_{max} and FS between the I/R and I/R with VNS groups by sonomicrometry technique.

VNS not effect only acute phase, but have effected in chronic condition after ischemia and reperfusion injury [7, 68, 81, 82]. In VNS induced HR reduction, Li et al. (2004) have reported that the difference in heart rate between untreated and treated chronic heart failure (CHF) rats reached 40 beats per minute at the end of treatment [7]. CHF rats had significantly lower blood pressure, but the vagal stimulation did not affect blood pressure during the 6-week treatment period. CHF rats had low blood pressure, high LVEDP, a depressed LV $+dp/dt_{max}$, and an increased heart weight. In contrast, CHF rats treated with VNS had significantly lower LVEDP and higher LV $+dp/dt_{max}$ than untreated CHF rats. Improvement of pumping function in treated CHF rats was accompanied by a significant decrease in normalized biventricular weigh. They concluded that vagal nerve stimulation markedly improved the long-term survival of CHF rats through prevention of the progression of pumping failure and cardiac remodeling. In VNS not induced bradycardia effect. Uemura et al. (2010) have reported that there were no significant differences in HR between the MI and MI-VNS groups at baseline, after 30 minutes of coronary occlusion, and at 3 days after coronary reperfusion [68]. However, at 8 weeks after reperfusion, VNS treated heart 3 days

significantly increased FS, decreased LV end systolic and diastolic diameter, and decreased LVEDP when compared with MI group. They concluded that in the chronic phase of reperfusion, VNS markedly attenuated LV dysfunction even through VNS was limited to a short period early after MI. Beaumont et al. (2015) have reported that At 90 days post-MI, LVESV was increased significantly by 30%, whereas LVEF was reduced by 6.5%. VNS prevented these MI-induced changes in LVESV and LVEF [81]. Moreover, there was no significant difference in heart weight or lung weight (wet and dry) as percentage to body weight among all treated groups. Zhang et al. (2016) have reported that 4 weeks after ligation of the LAD, rats showed a significant reduction in LVEF, indicative of impaired LV systolic function [82]. Correspondingly, the LVESD and LVEDD were markedly increased in rats with post-MI. Furthermore, cardiac systolic function was improved by 8 weeks of VNS as the LVEF was significantly increased. The LVESD but not LVEDD was dramatically reduced after VNS treated heart. Meanwhile, VNS these level no significantly reduced HR. They concluded that chronic VNS could improve the cardiac systolic function.

1.2.12 Clinical application of VNS on cardiovascular diseases

Zamotrinsky et al. (1997) have reported that, in 10 patients with coronary artery disease (CAD) who were classified as Canadian Cardiovascular Society class IV, low-frequency electroneurostimulation (ENS) of the ear afferent vagus endings and brainstem structures via transauricular electroacupuncture improved the patients' preoperative clinical course, producing a reduction in their angina during treatment [83]. At the end of course (10 days), patients no longer developed angina either at rest or after moderate physical activity. Moreover, ENS significantly decreased patient treated with vasodilator, decreased HSP70i and ATP content in atrial tissue. The result concluded that ENS had a central vagotonic/sympatholytic influence on the heart (increases the parasympathetic tone of the autonomic nervous system), leading to relief of angina symptoms, diminution of some biochemical myocardial signs of the disease, and an increase in the heart's tolerance of operative reperfusion damage. In chronic heart failure (CHF) patient (New York Heart Association (NYHA) class II–IV) De Ferrari et al. (2010) have reported that right cervical VNS implant (total 6 months)

significantly increased NYHA class, distant of 6-min walk test and improve LV function indicated by increased LVEF and LVSV when compared with control group [9]. The effects of VNS were maintained at 1 year. They concluded that chronic VNS in CHF patients with severe systolic dysfunction may be safe and tolerable and may improve quality of life and LV function. These findings are also consistent with another CHF patient [84-87]. In heart failure preserve ejection fraction patient, chronic right cervical VNS implantation (total 6 months) improved LV function, HRV and clinical outcome when compared with non-stimulation group [84]. However, chronic VNS failed to improve cardiac remodelling and functional capacity in heart failure patient, but still improved quality-of-life in this patient [85]. In ANTHEM patient (NYHA class II/III), Libbus et al. (2016) have reported that chronic left and right cervical VNS (total 1 year) exert anti-arrhythmic effect indicated by decreasing number of VT [86]. The anti-arrhythmic effect was associated with decreased peak of T wave alternans. Moreover, VNS significantly improved HRV and HRT when compared with control group. Interestingly, Yu L et al. (2017) have reported non-invasive VNS at tragus in the right ear (2-3 hours) reduces myocardial ischemia-reperfusion injury in patients with STEMI [87]. 24 hours after reperfusion (balloon dilation) VNS significantly decreased total ventricular premature beats (VPBs), isolated VPBs, coupled VPBs and VT when compared with non-stimulation group. Plasma inflammatory markers were significantly reduced after 24 hours of reperfusion. Plasma CK-MB and plasma myoglobin was significantly reduced after 72 hours of reperfusion. VNS improved LV function indicated by increased LVEF and decreased wall motion index after 7 days of reperfusion. Therefore, they conclude that non-invasive VNS reduces myocardial I/R injury in patients with STEMI via attenuated inflammation process. Furthermore, 2 ongoing trials investigating the effects of VNS therapy have progressed to phase III clinical trials, which are currently ongoing [10, 88]. Furthermore, In healthy subject who have no cardiovascular disease, diabetes and hypertension, non-invasive VNS with transcutaneous VNS (tVNS) pace on the inner and outer surface of the tragus of the ear significantly decreased LF/HF ratio and frequency and incidence of muscle sympathetic nerve activity when compared with non-

stimulation subject [89]. In this intensity, tVNS significantly decreased HR but not changed MAP. They conclude that tVNS can increase HRV and reduce sympathetic nerve outflow in healthy subject.

1.3 Purposes of the study

Aim1: to determine whether the cardioprotective effects against myocardial I/R injury of VNS were mainly due to direct ipsilateral efferent vagal fibers activation or indirect effects mediated by the afferent vagal fibers.

Aim 2: to determine the roles of the contralateral efferent vagal fibers during VNS on the cardioprotective effects against myocardial I/R injury.

1.4 Hypotheses of the study

Hypothesis 1: VNS exerts cardioprotection against myocardial I/R injury predominantly through its ipsilateral efferent vagal fibers via attenuating mitochondrial dysfunction, modulating mitochondrial dynamics and improving mitochondrial biogenesis by shifting cardiac fatty acid metabolism toward beta oxidation.

Hypothesis 2: The cardioprotection of VNS requires both ipsilateral and contralateral efferent vagal activity to exert its full benefit.