CHAPTER 1

INTRODUCTION

1.1 Statement and significant of the problem

According to the World Health Organization (WHO), heart disease is the leading cause of death worldwide. Unfortunately, this percentage is continuously increasing (WHO, 2011). The most lethal form of heart disease is ventricular fibrillation (VF) which causes sudden death in human with all ages (Bunch et al., 2005; McRae et al., 2001). Disordered, rapid, and ineffective contraction of the ventricle caused by the disorganization of cardiac electrical activity leads to a loss of cardiac output, organ dysfunctions, irreversible brain damage and possibly brain dead (McRae et al., 2001). Currently, the most effective strategy for VF termination is an electrical defibrillation delivers directly to the heart (Cevik et al., 2009; Chen and Guo, 2003; Kelly et al., 1988; Lee, 2011; Patel et al., 2009). The lowest electrical energy or voltage that required for successful defibrillation is defined as a defibrillation threshold (DFT) (Chapman et al., 1987; Chattipakorn et al., 2004). To prevent sudden death from VF, cardiopulmonary resuscitation (CPR) combined with electrical defibrillation must be performed without delay. Previous study suggested that the survival rate was decreased by 10% each minute of delayed in defibrillation (Marenco et al., 2001). Therefore, to overcome this limitation, the automated external defibrillator (AED) was placed in the location where the probability of sudden cardiac arrest is high (Figure1-1) in order to increase survival rate by the early defibrillation.

Figure 1-1: The automated external defibrillator (AED).

(Marenco et al., 2001).

However, high-energy shocks are not benign for human bodies. Several studies have shown the potential for myocardial damage with higher-energy shocks during cardiac defibrillation (Berg et al., 2008; Epstein et al., 1998; Nakagawa et al., 2012). Thus, there are significantly clinical interests in reduction of the shock strength required for defibrillation. The improvement of defibrillation efficacy has been studied in attempt to reduce the DFT, by implementation of medical devices (Neuzner et al., 1997; Yamanouchi et al., 1999), pharmacological interventions (Chattipakorn and Ideker, 2003; Murakawa et al., 1997; Qi et al., 1999; Tsagalou et al., 2004), or introducing a novel therapeutic strategy such as vagal nerve stimulation (VNS) (Murakawa et al., 1997).

Although VNS has been known as a therapeutic tool for epilepsy and drug resistant depression (George et al., 2000; Panescu, 2005; Rizzo et al., 2003), growing evidence from both animals and clinical studies strongly confirmed the potential of VNS as an effective therapeutic strategy for heart disease (De Ferrari et al., 2011; Li et al., 2004; Olshansky et al., 2008; Sabbah et al., 2011; Zhang et al., 2009). Moreover, VNS has been shown the improvement benefits on sinus arrhythmias and atrial fibrillation (Kobrin, 1991; Kolman et al., 1975; Murakawa et al., 2003; Zhang and Mazgalev, 2011). Furthermore, the study by Murakawa and colleagues has shown that VNS applied before the defibrillation onset (the pre-shock period) could decrease the DFT in canine model (Murakawa et al., 2003). However, previous studies reported that rapid cardiac electrical activities occurring after shock (post-shock activation) could be responsible for failed defibrillation (Chattipakorn et al., 2001; Chattipakorn et al., 2000a; Chattipakorn et al., 2000b). Thus, the radiofrequency ablation was performed at the arrhythmogenic region to interrupt the post-shock activation leading to the DFT reduction (Chattipakorn et al., 2000c). Therefore, we hypothesized that VNS applied continuously at the VF onset until post-shock period can interrupt the post-shock activation and reduce the DFT more than VNS applied at the pre-shock period only.

Moreover, in the present study we sought to investigate the impact of timing of VNS and determine the effects of various VNS stimulation frequencies and stimulus training duration on the DFT in a swine model.

1.2 Literature review

1.2.1 Principle of cardiac electrophysiology

During normal sinus rhythm, a synchronize contraction of heart can be achieved by the propagation of an electrical action potential throughout the heart via a low electrical resistance communicating junction known as gap junction (Spray and Burt, 1990). The electrical activity started from sinoatrial (SA) node. Then, the internodal pathway will conduct the electrical action potential to atrioventricular (AV) node which delays the impulse transmission from the atria to the ventricles. After that, the electrical impulse conduct via Bundle of His (A-V Bundle) throughout the complex network conducting fibers known as Purkinje fibers which conduct cardiac electrical impulses to all part of the ventricles (Guyton AC, 2000). The cardiac electrical impulse conductive system is shown in Figure 1-2.

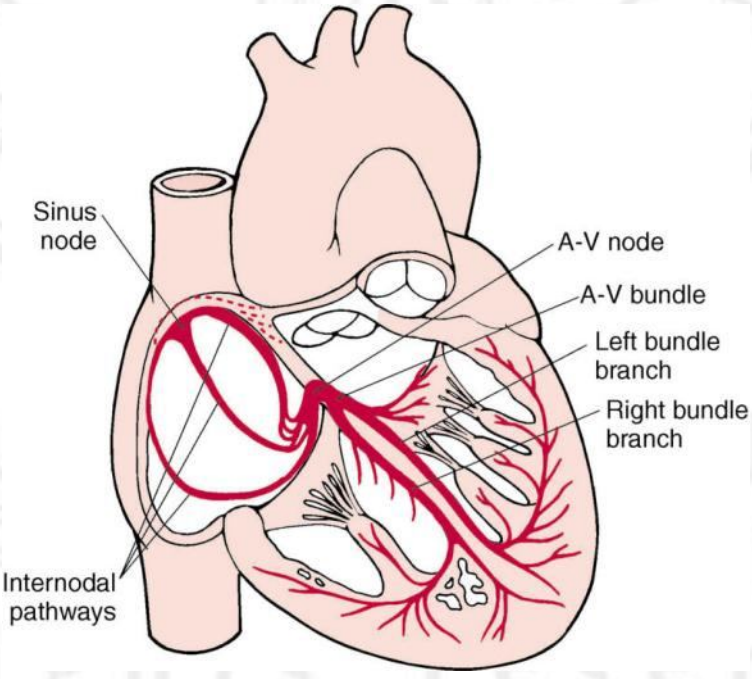

Figure 1-2: Cardiac electrical impulse conductive system.

(**Ref.**Textbook of medical physiology.10th W.B. Saunders @ 2000.)

The electrical activities of the whole heart known as electrocardiogram (ECG) can be recorded by electrocardiograph. ECG of normal heartbeat consists of P wave, QRS complex, and T wave as shown in Figure 1-3. The normal pacemaker, SA node, located in the right atrium which is the first part of the electrical activation (Wagner, 2000). After the activation of SA node, the generation of P wave occurs by the atrial depolarization leading to the contraction of atria. Then, the electrical conducting system transfers the electrical impulses to the ventricles. Then, the ventricular depolarization was occurred which represents as the QRS complex on the ECG before ventricular contraction. After the recovering from depolarization state of the ventricles (ventricular repolarization) which represents as the T wave on the ECG and also relaxation period of ventricles (Guyton AC, 2000; Wagner, 2000).

The intervals between each ECG components represent the cardiac electrical activities. For example, the P-R interval, the time from the onset of the P wave to the onset of the QRS complex, represents the time of the electrical conduction from the atria to the ventricles through the AV node. The R-R interval is the interval between consecutive R waves (at the peak of QRS complexes) used for the heart rate determination (Wagner, 2000). Considering the importance of the normal sequence of activation, the alteration of the cardiac conduction system due to the underlying heart disease can result in the sudden cardiac death. During VF, waveforms of the ECG are changed from normal sinus rhythm to irregular form (Cobbe S.M., 2010; Guyton AC, 2000; Porth, 2005.)as shown in Figure 1-3.

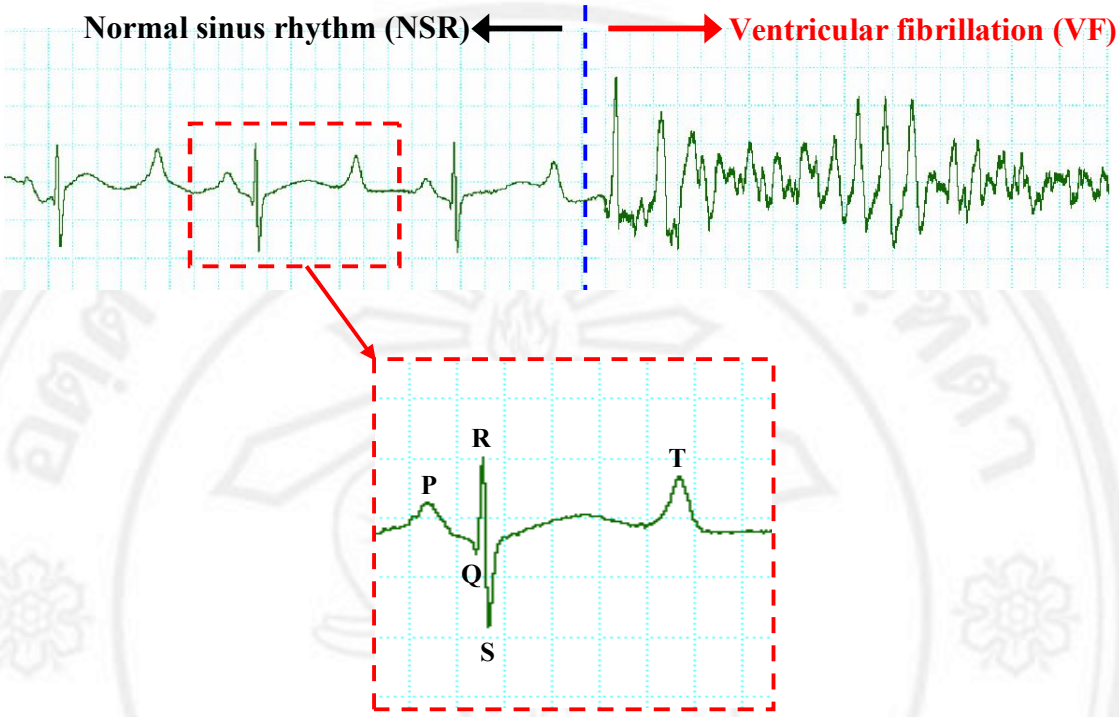

Figure 1-3: ECG waveform changes from NSR to VF.

1.2.2 Ventricular fibrillation and defibrillation

Physiologically, heart pumps out the blood under the cooperation of the electrical and the mechanical activities. The electrical activity generated from SA node and followed by heart contraction rapidly. Nevertheless, during VF condition, the coordinated regular contraction of the heart is overthrown by a state of the mechanical and the electrical anarchy (ten Tusscher et al., 2009). Thus, the heart cannot pump out enough blood for the body supply causing the death in a few minutes. Currently, the most effective therapeutic for VF termination is delivered an electrical shock to the heart known as defibrillation (Irnich, 1990). At this moment, the defibrillation performed by the defibrillator that could be classified into 2 main types; the external defibrillator and the implantable cardioverter defibrillator (ICD) as shown in Figure 1-4.

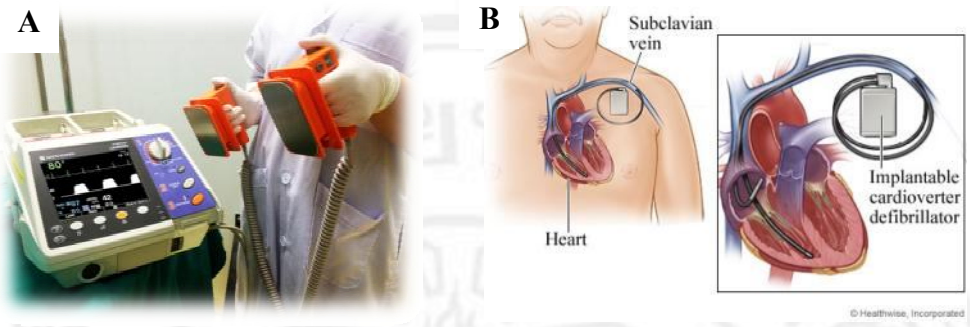

Figure 1-4: Defibrillator. **A:** External Defibrillator.

B: Implantable Cardioverter Defibrillator (ICD) (Healthwise)

For defibrillation by the external defibrillator, the defibrillation paddles are positioned at the ventricular apex and the right infraclavicular area. Then, high electrical shocks are delivered directly to the skin until achieving a successful defibrillation (Chattipakorn et al., 2000a). However, high strength electrical shocks delivered to the heart also damaged the surrounding tissue. Even the patients survive from VF, they will face the pain from the defibrillation paddle burning on the skin. The relation of the delivered energy dose on the percentage of defibrillation efficacy, damage, and death is shown in Figure 1-5. The maximum percentage of defibrillation efficacy, the delivered energy causes at least 10% damage to the animal models (Babbs et al., 1980). Thus, in case of successful defibrillation, the tissue damage could occur. Currently, the recommended clinical therapy for VF termination is the ICD (Kelly et al., 1988). ICD is more effective compared with the external defibrillator (Cevik et al., 2009). This device is implanted subcutaneously to the patient's chest and delivers electrical shock directly to the heart during VF when receiving the abnormal cardiac electrical signal.

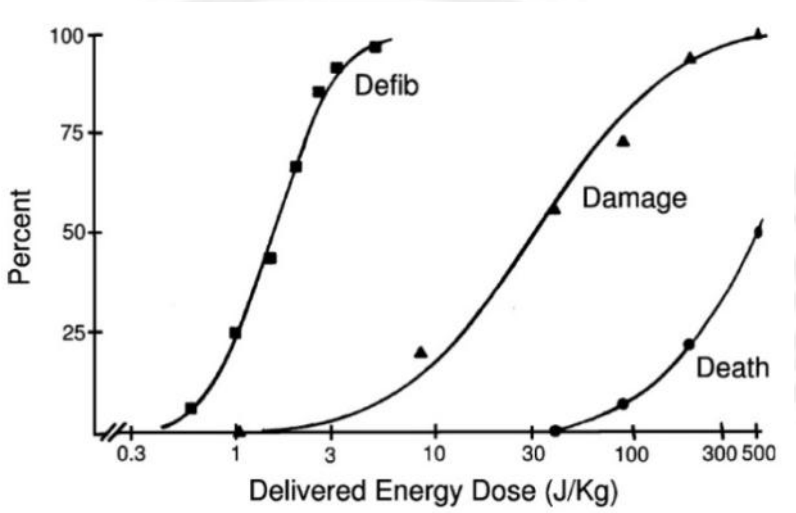

Figure 1-5: The relation of the delivered energy dose on the percentage of defibrillation efficacy, damage, and death in a series of dogs receiving various sized transthoracic monophasic defibrillation shocks (Babbs et al., 1980)

ICD consists of two main parts as shown in Figure 1-6. The first part is the ICD pocket (generator) that contains electrical circuit inside the pocket with the programming for detection of the abnormal cardiac electrical activities, which could generate VF, and delivers high amount of the electrical shock from the electrical capacitor to terminate VF. The second part is the catheter that delivers the electrical shock from the ICD pocket to the heart. The wire and coil of this electrode are made of low electrical resistance materials (such as Titanium, Tantalum, Platinum or Stainless), covered with silicone insulator for the wire.

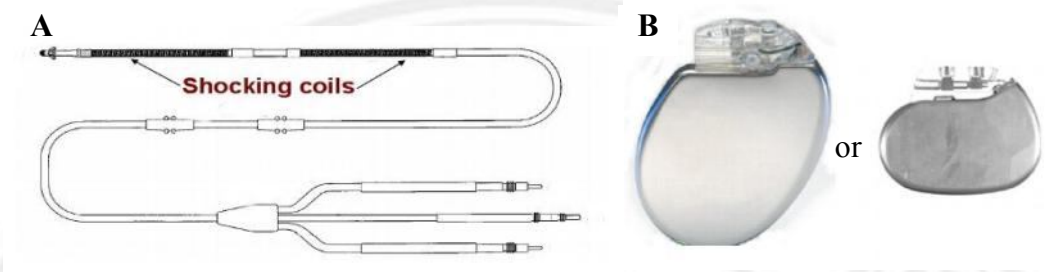

Figure 1-6: Implantable Cardioverter Defibrillator (ICD) components.

A: ICD catheter. **B:** ICD pocket (generator)

(Washington Heart Rhythm Associates)

However, when defibrillation is attempted, the outcome is not always successful. In some cases, multiple shocks with increasing energy must be delivered to successfully defibrillate the patient (Chattipakorn et al., 2001). Because of the high energy of the electrical defibrillation not benign to human, the improvement of defibrillation efficacy is required. There are many approaches to reduce DFT and/or improve the defibrillation efficacy such as catheter ablation therapy with/without pharmacological intervention (Patel et al., 2009).

1.2.3 Defibrillation threshold reduction

The implementation of medical device was another approach to reduce DFT. In 1997, Neuzner and colleagues demonstrated that the abdominally placement of an additional hot Can electrode (an electrical returning electrode) equipped with single defibrillation lead result in a lower DFT than only single lead (Neuzner et al., 1997). In 1999, the biventricular leads which the defibrillation leads were inserted into the left and the right ventricular chambers combined with Can electrode, can improve

defibrillation efficacy compared with a single lead with Can electrode (Yamanouchi et al., 1999). Not only the implementation of medical device that caused the DFT reduction but pharmaceutical treatments also reduced the DFT. A nonspecific K^+ channel blocker (MS-551) has been shown the capability to improve the defibrillation efficacy when infused during VF (Murakawa et al., 1997). Moreover, the injection of Azimilide (drug with class III antiarrhythmic properties) significantly decreased the DFT (Qi et al., 1999). Furthermore, the intravenously injection of flunarizine, a delayed after depolarization (DAD) inhibitor, has shown significant improve the defibrillation efficacy by decreasing the delivered voltage and energy for DFT (Chattipakorn and Ideker, 2003). Not only DFT reduction, some drugs administration can also increase the ventricular fibrillation threshold (VFT) without any side effect on the DFT (Tsagalou et al., 2004). The VFT is the minimum electrical strength that able to trigger the ventricular fibrillation (Valentinuzzi et al., 1984). However, previous studies, which improve defibrillation efficacy by targeting directly to the heart, might cause some serious long-term side effects. For example, the cardiac myocardial injuries caused by the additional medical device to heart chamber (Epstein et al., 1998) or other organs dysfunction after long term pharmaceutical intervention (Costache and Aprotosoaie, 2013). Thus, we intend to use a novel therapeutic strategy such as VNS, to improve the defibrillation efficacy and improve clinical outcomes.

1.2.4 Vagal nerve activation

The vagus nerve is the longest cranial nerve (CN) in the parasympathetic nervous system. It extends from the brain stem to the abdomen and innervates various organs

including the heart, esophagus and lungs. The vagus nerve controls many autonomic functions and also plays an important role in heart function such as the modulation of the heart rate and conduction velocity of AV node (George et al., 2000; Guyton AC, 2000). The vagus nerve (CN X) has four components. The first is the branchial motor (special visceral efferent), the second is the visceral motor (general visceral efferent), the third is the visceral sensory (visceral afferent), and the fourth is the general sensory (general somatic afferent) (Thomas R. Van De Water, 2006). The vagus nerve innervates organs from the pharyngeal level down to colon (proximal portion) as shown in Figure 1-7.

The mammalian heart is innervated by sympathetic and parasympathetic nerves which their actions often oppose each other. The parasympathetic innervation of the heart is controlled by the vagus nerve which releases acetylcholine (ACh) from post ganglion. ACh binds to muscarinic (M2) receptor on the cell membrane of cardiomyocytes (Dhein et al., 2001). M2 receptor couple with G protein-gated ion (Gi) channel that activates the opening of inward rectifying potassium (K^+) channel and increases K^+ efflux leading to hyper-polarization in cardiac resting membrane potential (DiFrancesco et al., 1989). It decreases heart rate (negative chronotropy), decreases force of contraction (negative inotropy) and decreases electrical conduction from SA node to AV node (negative dromotropy) (Tsuboi et al., 2000). The right vagus nerve hyper-stimulation predisposes those affected individuals to bradyarrhythmia. Whereas, the hyper-stimulation of the left vagus nerve predisposes the heart to AV block (Tsuboi et al., 2000). The cardiac innervation of the autonomic nervous system has been shown in Figure 1-8.

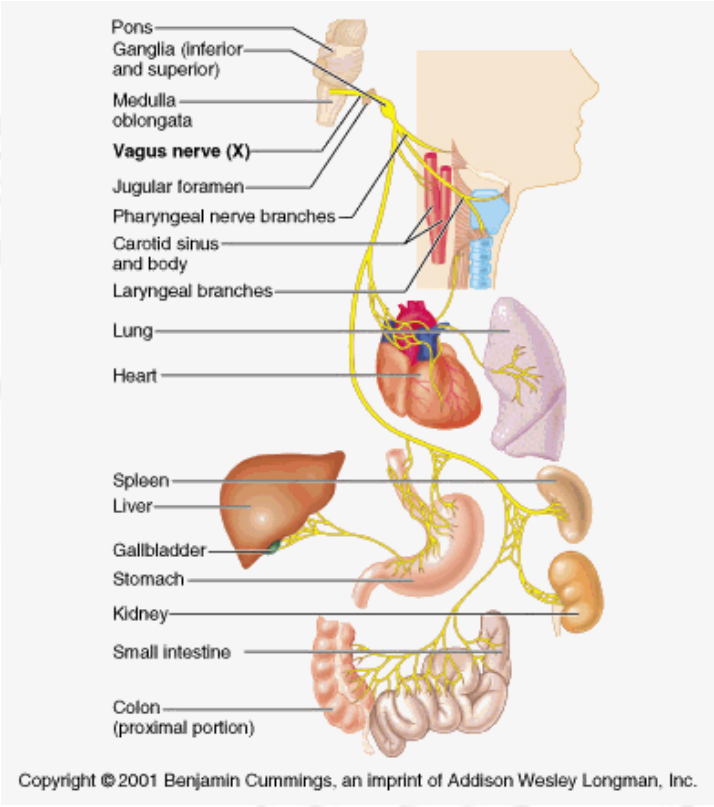

Figure 1-7: Vagus nerve and its responsible organs

(**Ref.** Addison Wesley Longman)

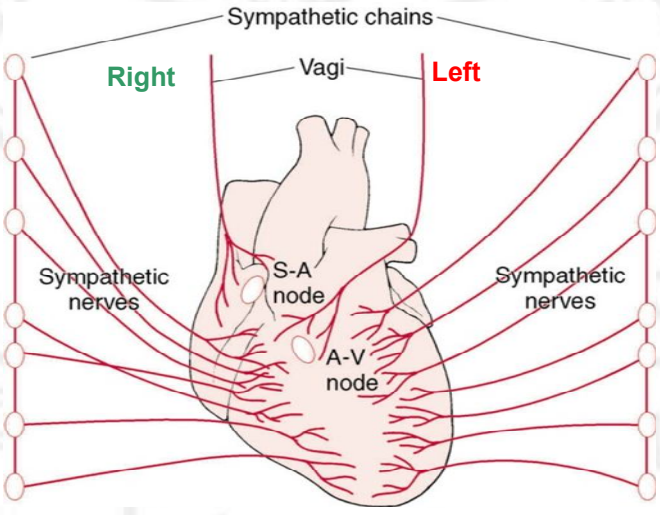

Figure 1-8: Cardiac nerve (Vagi: Vagus nerve which is parasympathetic nerve).

(**Modified from** Textbook of medical physiology. 10th W.B. Saunders @ 2000.)

1.2.5 Vagal nerve stimulation

Currently, VNS is the therapeutic tool for epilepsy and drug resistant depression (Rizzo et al., 2003). In 1988, the first human implantable VNS was performed in epileptic patient (George et al., 2000). The mild electrical stimulation from the pulse generator is delivered through the VNS lead as shown in Figure 1-9. The programming of VNS pulse generator allows the physician to conduct functional assessments and data suit for each patient (Panescu, 2005). The right vagus nerve directly innervates the SA node, which is a normal pacemaker of the heart. Although the stimulation on the right vagus nerve is effective for epilepsy and depression as same as the stimulation on the left, VNS treatment performs on left vagus nerve should avoid the direct effect on the heart rate (Krahl et al., 2003). Furthermore, the VNS has been proposed to exert a cardioprotective effect against cardiac arrhythmias and be an effective therapeutic strategy for heart disease (Zhang et al., 2009).

Figure 1-9: Vagal nerve stimulation (VNS) coponents

(**Ref.**Cyberonics, Inc.)

1.2.6 The improvement of cardiac function by VNS

In the study of chronic heart failure rats, after 2-week survival from the left coronary artery ligation has shown that the VNS significantly improved long-term survival by prevented pumping failure and cardiac remodeling (Li et al., 2004). Moreover, VNS treatment has been shown to have the anti-inflammatory effects and strongly improves cardiac autonomic control and attenuates the development of heart failure (Zhang et al., 2009). Both animals and clinical studies strongly confirm the efficacy of chronic VNS. Therefore, VNS could be an effective therapeutic strategy for heart failure treatment (De Ferrari et al., 2011; Olshansky et al., 2008; Sabbah et al., 2011). Furthermore, left cervical (LC) VNS either applied intermittently or continuously provide significant cardioprotective effects. VNS could reduced infarct size, improved ventricular function, decreased ventricular fibrillation episodes, and attenuated cardiac mitochondrial reactive oxygen species production, depolarization, and swelling, compared with the control group (Shinlapawittayatorn et al., 2013).

Effects of VNS on cardiac electrophysiology and cardiac arrhythmia depend on the condition of VNS. The relation between arrhythmias and VNS had shown the benefits of VNS on sinus arrhythmias, atrial electrophysiology, atrial fibrillation induction, AV node electrophysiology, and ventricular arrhythmias (Zhang and Mazgalev, 2011). Moreover, Kolman and colleagues found that the effect of VNS during concurrent left stellate ganglion stimulation (LSGS) significantly increased VFT by the precondition of sympathetic tone (Kolman et al., 1975). In canine model, VNS with 10-mA current strength significantly reduced the DFT (compared with 1, 3, and 6 mA), and also significantly reduced the DFT, pre-shock 8-s VNS with 10-mA current, compared with 2 and 4 s pre-shock (Murakawa et al., 2003).

To date, as mention before, the only effective treatment for VF termination is an electrical shock known as defibrillation. The conditions of pre-shock and post-shock activations were attended as a defibrillation outcome predictor. In 2000, the study by Chattipakorn and colleagues reported that the activations of the cardiac electrical activity after shocked (post-shock activation) can predict the defibrillation outcome either successful or failed defibrillation (Chattipakorn et al., 2000b). To study how important of post-shock activation on defibrillation, there is the study of VF induction after the successful defibrillation by rapid electrical pacing on left ventricle (LV), the number and rapidity of electrical pacing (post-shock activation) may be used to predict the defibrillation outcome. It has been shown that, at least three rapid electrical pacing after a successful defibrillation can reinitiate VF (Chattipakorn et al., 2000a). Thus, even the defibrillation outcome tend to successful, it can be disturbed by another electrical pacing and turning the successful to fail defibrillate(Chattipakorn et al., 2000a). Under the optical mapping, the first few activations appeared focally on the epicardiumat almost the same site at the LV apex. So, the radiofrequency ablation was performed at the arrhythmogenic region to interrupt the early post-shock activation. It could decrease the shock strength required for the successful defibrillation (Chattipakorn et al., 2000c). Even the study of Murakawa and colleague has shown that the VNS cause a DFT reduction in canine model, VNS of their study was performed only at the period before the defibrillation shock onset (pre-shock period) (Murakawa et al., 2003).

As mention previously, the repolarization pattern at post shock period can cause either successful or failed defibrillation (Chattipakorn et al., 2001) and this early repolarization that caused failed defibrillation could interrupted by the ablation at the

arrhythmogenic region (Chattipakorn et al., 2000c). Thus, in the present study, we sought to investigate the impact of timing of VNS both pre-shock and post-shock, on the DFT in swine. We hypothesized that VNS could interrupt the early post-shock activation. So, VNS applied continuously at the VF onset until the post-shock period could improve the defibrillation efficacy by decreasing the DFT. We also determined the effects of various VNS stimulation frequencies and stimulus training duration on the DFT. Moreover, lower electrical shock for VF termination would reduce the damaged heart and improve the patient outcomes.

1.2.7 VNS block by local anesthetic agent

The VNS effect on ECG morphology and DFT should be confirmed that the occurring effect was the direct effect of VNS. For VNS pathway confirmation, the local anesthetic agent as Mepivacaine Hydrochloride was used for nerve block (Pawlowski et al., 2012). The local anesthetic agents have been known as nerve blockade which blocked the nerve conduction (Bainton and Strichartz, 1994; McLure and Rubin, 2005). Mepivacaine is a local anesthetic amide group, with a short onset action (Porto et al., 2007). It has been used to study a VNS blockade which strongly confirmed the blocking effect (Zheng et al., 2006). Thus, the VNS efficacy on ECG morphology and DFT should be abolished after mepivacaine application.

1.3 Objectives of this study

Aim 1 To confirm VNS effects on the heart and determine the effects of VNS frequency and duration on normal sinus rhythm (NSR)using ECG morphology (R-R interval, P-R interval, QRS complex and Q-T interval).

It is known that the vagal nerve activation causes the changed on cardiac electrical activities. The delay of electrical conduction from the SA node to AV node causes a prolonged P-R interval and also prolonged R-R interval (Fairchild et al., 2011; Tsuboi et al., 2000). Thus, the hypothesis of this aim was that LC VNS could prolong the P-R and the R-R interval. However, it was unclear how difference would occurron the ECG morphology if we applied the different amount of VNS. Therefore, the result of the effect of different amount of VNS frequencies and durations on NSR from this thesis would be one of the databases for the future studies.

Aim 2 To determine the effects of VNS frequency and duration on the DFT

Previous study demonstrated that VNS caused a DFT reduction in canine model (Murakawa et al., 2003). However, the VNS was performed during the pre-shock period with only one VNS frequency. Previous studies suggested that the post-shock activation is the important parameter for successful defibrillation, leading to our research question. If VNS applied before the defibrillation shock should reduced the DFT, VNS applied continuously at the VF onset until the post-shock period may better reduce the DFT. Thus, we hypothesized that VNS applied continuously at the VF onset until the post-shock period could better improve the defibrillation efficacy by decreasing the DFT.

Aim 3 To determine the VNS pathway by local nerve block

The electrical stimulation on vagus nerve conducts to the heart via nerve fibers. The electrical conduction in nerve fiber can be blocked by local anesthetic agent (Ikeda et al., 1996). Previous studies reported the effect of VNS on NSR and DFT that VNS reduced heart rate, prolonged P-R and R-R intervals(Fairchild et al., 2011; George et al., 2000; Guyton AC, 2000; Tsuboi et al., 2000) and also reduced the DFT (Murakawa et al., 2003). Thus, we hypothesized that the application of local anesthetic agent at the position below the VNS electrode (caudal side) could block the effect of VNS on NSR and DFT.