CHAPTER 4

DISCUSSION AND CONCLUSIONS

4.1 Discussion

The major findings of our study are as follows 4.1.1) VNS induced the P-R and the R-R intervals prolongation, 4.1.2) VNS applied continuously at the VF onset until the post-shock period could improve defibrillation efficacy by reducing the DFT, and 4.1.3) Direct LC VNS effects on the heart were confirmed by results from local nerve block.

4.1.1 VNS induced the P-R and the R-R intervals prolongation

Previous study, the P-R and R-R intervals of swine model with the weight 22-32 kg were 125±21 ms and 861±272, respectively (Stubhan et al., 2008). In our study, the average baseline P-R and R-R intervals were approximately 132±9 ms and 689±45 ms, respectively. The hyper-stimulation of the left vagus nerve predisposes the heart to AV block (Tsuboi et al., 2000). The AV block or heart block can be classified in to 3 groups : first degree, second degree, and third degree AV block (Ganong, 1995). The form of first degree AV block, all of electrical impulses that generated from SA node pass through AV node and reach the ventricle but slower than normal condition (Ganong, 1995). Leading to P-R interval prolongation as shown in Figure 4-1A. In the second degree AV block, some of electrical impulses are conducted to ventricle (Ganong, 1995). The dropped beats of the ventricles (the QRS complex disappear) were shown on ECG known as second degree AV block

(Figure 4-1B). The first and second degree AV block known as incomplete heart block. If the conduction of electrical impulses from SA node to ventricle completely interrupted, it is a third degree AV block or complete heart block as shown in Figure 4-1C (Ganong, 1995). In our study, the 5 Hz VNS caused first degree AV block at all duration, indicated by the P-R interval prolongation. For the 20 Hz VNS, the second or third degree AV block were observed in all durations. These findings suggested that the LC VNS could induce higher degree of AV block if the higher frequency were used. At the molecular level, the increasing of VNS frequency increases the releasing of ACh at the synaptic cleft as shown by Akiyama and Yamazaki (Akiyama and Yamazaki, 2001). They found that at 20 Hz, ACh was released from post-ganglionic vagal nerve terminals in the left ventricular myocardium more than 10 Hz of VNS (Akiyama and Yamazaki, 2001). It was consistent with our study that 20 Hz VNS had more negative dromotopic effect than 5 Hz that was shown as second or third degree AV block. Thus, results also confirmed that VNS was able to modify vagal activity by causing marked prolongation of the R-R or the P-R intervals or the advanced AV block.

Although we performed VNS on LC vagus nerve, the prolonged R-R interval effect was observed in the animal model leading to slower heart rate when compared with baseline. The bradycardia effect of LC VNS might be explained by the complexity of the distribution of the cardiac parasympathetic nerve fiber in both epicardium and endocardium (Ulphani et al., 2010). The enrich cholinergic parasympathetic nerve innervations pattern in four chambers of swine heart has been demonstrated after staining by a histochemical method (Ulphani et al., 2010) as shown in Figure 4-2.

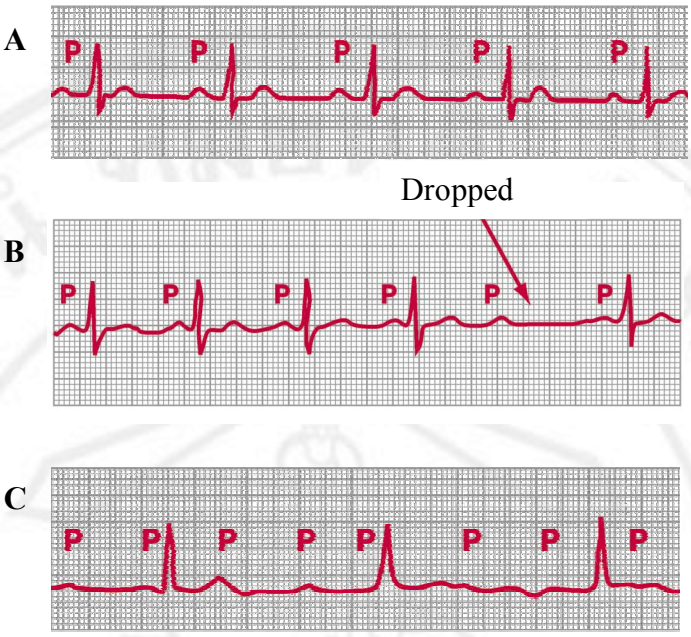

Figure 4-1: Heart block or AV block **A:** First degree AV block **B:** Second degree AV block **C:** Third degree AV block (complete heart block) (Guyton AC, 2000)

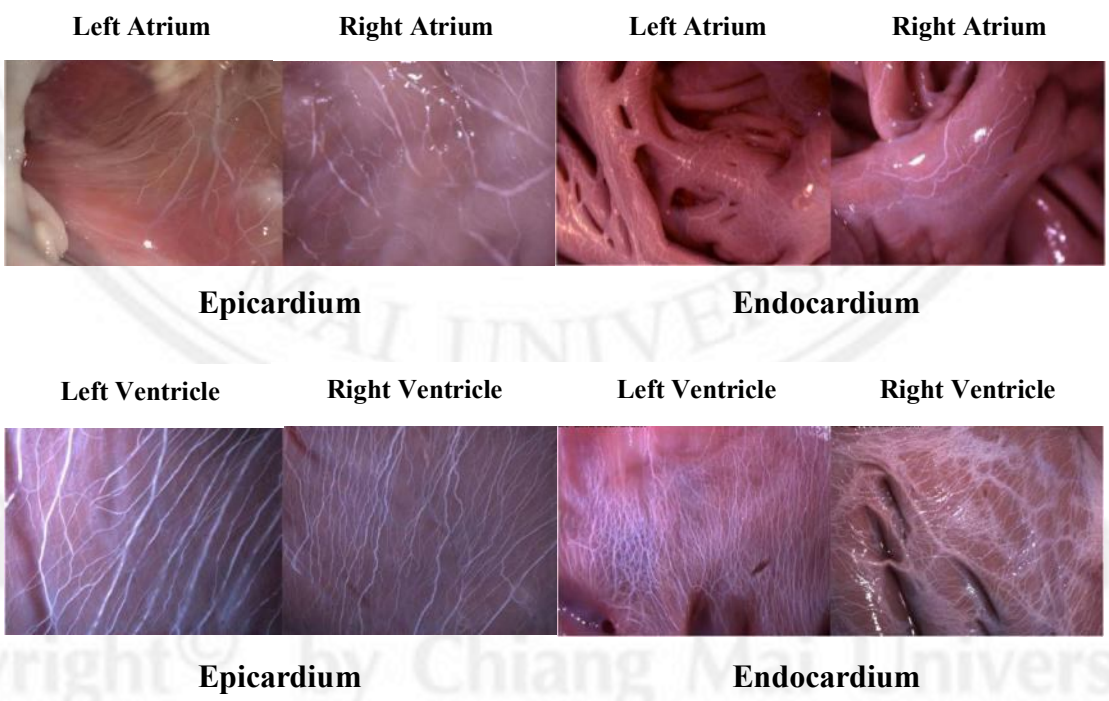

Figure 4-2: The complexity of the distribution of the cardiac parasympathetic nerve fiber in both epicardium and endocardium after staining by a histochemical method

Modified from (Ulphani et al., 2010)

This finding suggested an existence of parasympathetic nerve innervations in both atrias and ventricles. Thus, during LC VNS, the electrical stimulation might be transferred from the LC vagus nerve fiber branch to SA node directly, resulting in prolonged P-R and R-R intervals in different degrees depending on VNS frequencies. Nevertheless, both VNS frequencies did not alter the Q-T interval and the duration of QRS complex.

4.1.2 VNS applied continuously at the VF onset until the post-shock period could improved defibrillation efficacy by reducing DFT

Previous study showed that VNS, applied 8 s before the defibrillation onset, could decrease the defibrillation energy in dog model (Murakawa et al., 2003). In the present study, our results provided additional findings regarding the duration of VNS and its effect on the DFT. The effective VNS parameter in our study was 5 Hz with 15 and 20 s of VNS duration which applied continuously from the VF onset until post-shock period. Our study strongly confirms that VNS applied continuously at the VF onset until the post-shock period could improved defibrillation efficacy by reducing the DFT. Previous studies demonstrated that rapid cardiac electrical activities occurring after defibrillation (post-shock activation) could be responsible for failed or successful defibrillation (Chattipakorn et al., 2001; Chattipakorn et al., 2000a; Chattipakorn et al., 2000b). The radio frequency ablation was performed at the arrhythmogenic region to interrupt the early post-shock activation and it was shown to decrease the DFT (Chattipakorn et al., 2000c). Therefore, the DFT reduction by VNS might be explained by the interruption of the early post-shock electrical activation. Moreover, the parasympathetic nervous system related to

cardiac action potential. The VNS was induced the releasing of ACh from post-ganglionic vagal nerve terminals (Akiyama and Yamazaki, 2001). At the molecular level, 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). The hyper-polarization lead to longer took to reach depolarization threshold. Thus, the rapid activation post-shock was preventing by the hyper-polarization condition. Interestingly, the effect of parasympathetic modulation observed in our present study also consistent with the previous experimental studies (Morillo et al., 1996; Wang et al., 1992). Another study in a swine model showed that parasympathomimetic drugs (both methacholine and carbacol) decreased the DFT (Morillo et al., 1996). VNS not only decreased the DFT, but also increased the ventricular fibrillation threshold (VFT) (Brack et al., 2011; Morillo et al., 1996). The increasing of the VFT occurs via post-ganglionic efferent fibers which is a direct action of VNS on the ventricle (Brack et al., 2011).

The DFT reduction of 20 Hz VNS did not relate to 5 Hz VNS. The 20 Hz VNS did not decrease the DFT when VNS applied continuously at the VF onset until the post-shock period. Due to the nerve fatigue, the 20 Hz VNS applied continuously at the VF onset until the post-shock period could not decrease the DFT. Parasympathetic nerve (Pelvic) fatigue occurred after increased the stimulation duration from 10 to 15 s with the same stimulation frequency (50 Hz) (Gillespie and Mackenna, 1961). Previous study presented that stimulation parameter such as pulse amplitude, shape, and width, and train frequency, directionality, polarity, and duration exert unique

neurobiological effects (de Kroon et al., 2005; Peterchev et al., 2010). Thus, the effects on the DFT of 20 Hz VNS at 15 and 20 s were abolished because of the nerve fatigue that occurred after increased the stimulation duration.

4.1.3 Direct LC VNS effects on the heart were confirmed by the results from local nerve block

In our study, LC VNS could increase defibrillation efficacy in the swine model. Therefore, we would like to confirm the VNS effect that occurred throughout our study by blocking the vagus nerve locally at the portion below the VNS electrode. The local anesthetic agent was used in our study. Normally, it was used for pain prevention by blocking electrical conduction in the nerve fiber (Ikeda et al., 1996). A previous study demonstrated that the local amide anesthetic agent such as lidocaine can be used as a VNS blockade (Zheng et al., 2006). Lidocaine and mepivacaine are local amide anesthetic agents that have similar effect when applied with the same concentration (Berini-Aytes, 2000; Porto et al., 2007). Takenami and colleague suggested that mepivacaine is less neurotoxic than lidocaine (Takenami et al., 2004). Thus, we performed local nerve block by using mepivacaine.

The electrical conduction in vagus nerve fiber was blocked by local anesthetic agent (Ikeda et al., 1996). In our present study, Mepivacine demonstrated the nerve block ability. Prior to the application of the mepivacaine at the portion below VNS electrode, VNS caused the prolongation of the P-R and the R-R interval, and reduced the DFT. Interestingly, after we applied the mepivacaine, VNS did not make any changed on the P-R, the R-R interval and the DFT when compared with baseline.

Thus, this finding not only confirmed the effects of VNS, but also confirmed the effective stimulation direction. We suggested that the electrical impulse that conducted from the stimulation point to the heart was blocked by mepivacaine. Thus, the effects of LC VNS were directly from the stimulation point to the heart.

4.2 Conclusions and study limitation

LC VNS can significantly reduce the DFT in the swine model. In the present study, the most effective parameters of VNS are 10 mA, 5 Hz for 20 s. As we hypothesized, VNS applied continuously at the VF onset until the post-shock period could improve the defibrillation efficacy by reducing the DFT. Moreover, the reduction of the DFT might be due to the post-shock activity interruption by VNS. This finding strongly confirmed the importance of post-shock activation effects on the DFT.

Our findings also strongly confirmed anti-arrhythmogenic effect of VNS. Therefore, VNS might be used to enhance the therapeutic performance during defibrillation. However, in the present study, VNS treatment was conducted immediately after VF. The time schedule of treatment in this experimental study might be different from the clinical condition.

Future investigation including the effects of delayed VNS treatment on defibrillation is required. Moreover, the combination of implantable VNS and ICD should be considered to be used in the patient in the very near future.