CHAPTER 4

Discussion and Conclusions

In this present study, we sought to investigate 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 by using myocardial infarct size as the primary endpoint. Furthermore, roles of the contralateral efferent vagal fibers during VNS were also investigated. The major findings of this study are as followed: (1) VNS exerted cardioprotection against myocardial I/R injury via attenuation of mitochondrial dysfunction, improved mitochondrial dynamics and shifted cardiac fatty acid metabolism toward beta oxidation; (2) LC-VNS and LtVNX produced more profound cardioprotection, particularly infarct size reduction, decreased arrhythmia score, oxidative stress and apoptosis and attenuated mitochondrial dysfunction compared to RtVNX; (3) VNS required both ipsilateral and contralateral efferent vagal activities to fully provide its cardioprotection. These findings suggest that selective efferent VNS may potentially be effective in attenuating myocardial I/R injury.

Impact of VNS during intact, after ipsilateral and contralateral vagus nerve transection on reperfusion arrhythmia and myocardial infarct size

Since both reperfusion arrhythmia [99] and infarct size [64] are the potential serious complications after myocardial reperfusion, strategies to limit these two components of I/R injury have significant therapeutic potential. In the present study, we found that LC-VNS prevented cardiac arrhythmias during I/R as indicated by decreasing the total number of PVC, VT/VF incidence, arrhythmia score, Tpe and preserving Tpe/QT ratio, suggesting that LC-VNS decreased heterogeneity of ventricular repolarization. It is well recognized that increased myocardial infarct size and decreased phosphorylation of connexin43 play an important role in the development

of cardiac arrhythmias, including VT/VF, during I/R [73]. Thus, the anti-arrhythmic effects of VNS might be due to its potential to decrease the arrhythmogenic substrates during I/R by reducing myocardial infarct size and increasing the phosphorylation of connexin43 at the serine 368 residue. In the present study, we found that VNS significantly reduced myocardial infarct size and increased phosphorylation of connexin43 compared with the I/R group, which are consistent with our previous studies in swine model [14] and other studies in rat model [71, 73]. Furthermore, LC-VNS also preserved LV function in the heart subjected to I/R injury when compared with baseline, which might be due to its ability to reduce myocardial infarct size. Because the vagal trunk consists primarily of afferent fibers (80%) [44], the role of these fibers, particularly during VNS, needs to be clearly assessed. Previous study reported that vagal afferent fibers are activated during VNS and decrease efferent parasympathetic electrophysiological and hemodynamic effects of electrical stimulation [19]. However, it is still remained unclear whether the cardioprotection against myocardial I/R injury of VNS is mainly due to direct vagal activation through its ipsilateral efferent vagal fibers (motor) or indirect effects mediated by the afferent vagal fibers (sensory). Thus, roles of the ipsitralateral afferent vagal fibers during VNS were also investigated by LtVNX 2 cm above the stimulation probe. Interestingly, we found that LtVNX exerted the anti-infarct and anti-arrhythmic effects similar to LC-VNS, suggesting that the anti-infarct effect of VNS were driven primarily through its efferent vagal fibers, rather than the indirect afferent vagal activation in the ipsilateral vagal trunk.

Although all VNS treated groups exerted cardioprotection against myocardial I/R injury, LC-VNS and LtVNX produced more profound cardioprotection, particularly infarct size reduction (by 89% for LC-VNS and 84% for LtVNX), compared to RtVNX (by 63% reduction). Moreover, for the anti-arrhythmic effect, RtVNX did not significantly decrease the total number of PVC and arrhythmia score, but significantly decreased VT/VF incidence when compared with I/R and atropine groups. Interestingly, RtVNX also preserved LV function similar to the LC-VNS and LtVNX groups. These beneficial effects of VNS were abolished by atropine. Our findings suggest that selective efferent VNS may potentially be effective in attenuating

myocardial I/R injury. Moreover, VNS also required the contralateral efferent vagal activities to fully provide its cardioprotection.

Impact of VNS during intact, after ipsilateral and contralateral vagus nerve transection on myocardial apoptosis during I/R

In the present study, we found that, all VNS treated groups markedly decreased the expression of Bax (a pro-apoptotic molecule), Claved caspase-3/Pro caspase-3 ratio, and % TUNEL positive cells. However, only LC-VNS and LtVNX significantly increased the expression of Bcl-2 (an anti-apoptotic molecule) when compared with other groups. Moreover, % TUNEL positive cell of RtVNX significantly increased when compared with LC-VNS (both intact and LtVNX), which also consistent with myocardial infract size. Myocardial I/R injury leads to the activation of program cell deaths, including cell apoptosis and necrosis [100]. Specifically, the decrease in an anti-apoptosis Bcl-2 and the increase in a pro-apoptosis Bax expression are the underling of myocardial ischemia-induced apoptosis [16]. Moreover, a recent study demonstrated that overexpression of cardiac specific caspase-3, a key molecule in the execution of apoptosis, decreased cardiac function and caused abnormality of ultrastructural damage to the nucleus as measured by the TUNEL staining method [98]. These results suggested that the anti-apoptosis Bcl-2 molecule could be responsible for the reduction of % TUNEL positive cells observed in both LC-VNS and LtVNX groups. Previous study in rat model demonstrated that VNS prevents downregulation of the anti-apoptotic protein Bcl-2 [16], which consistent with our finding. Thus, our results suggested that VNS reduced myocardial infarct size through the anti-apoptotic effect. Moreover, VNS also required the contralateral efferent vagal activities to fully provide its cardioprotection.

Impact of VNS during intact, after ipsilateral and contralateral vagus nerve transection on cardiac mitochondrial function and inflammation during I/R

Furthermore, accumulating evidence has demonstrated that myocardial ischemia and post-ischemic reperfusion cause a wide array of functional and structural alterations of cardiac mitochondria [13, 14, 25, 26, 101]. Our previous studies have shown that one potential possible mechanism underlying the pronounced cardioprotection of VNS against I/R injury is through the prevention of cardiac mitochondrial dysfunction [13,

14]. Increasing ROS production and the oscillation of mitochondrial membrane potential have been shown to play an important role in the genesis of cardiac arrhythmias and myocardial infarction [26]. In the present study, all VNS treated groups significantly reduced cardiac mitochondrial ROS production and prevented depolarization of mitochondrial membrane potential. These results could be responsible for the anti-infarct, anti-arrhythmia and the preservation of cardiac function of VNS in the heart subjected to myocardial I/R. Interestingly, we found that both LC-VNS and LtVNX, but not RtVNX, could prevent mitochondrial swelling after I/R injury. This result might explain why LC-VNS and LtVNX have higher efficiency on infarct size and prevention of cardiac arrhythmia than RtVNX.

In addition to cardiac mitochondrial dysfunction during I/R, inflammatory processes have been shown to play a critical role during myocardial I/R injury [102]. In the present study, only LC-VNS significantly decreased the expression level of myocardial TNF-α (a pro-inflammation marker) after I/R injury. However, the level of tissue TNF-α was not changed after vagus nerve transection, suggesting that VNS required both ipsilateral and contralateral efferent vagal activities to fully exert the anti-inflammation. Thus, it is reasonable to speculate that LC-VNS, both efferent vagal fibers are intact, provides the vagal tone that high enough to reach the activation threshold of the anti-inflammatory signaling pathway. However, the expression level of myocardial IL-10 (anti-inflammation) tended to increase in all VNS treatments after I/R injury, but did not reach the statistically significant level. Additionally, both LC-VNS and LtVNX significantly decreased the oxidative stress level as shown by the reduction in myocardial MDA levels after I/R when compared with RtVNX and atropine groups. Again, this result suggests that both efferent vagal fibers are required for VNS to provide the vagal tone that high enough to reach the activation threshold of the anti-oxidative effect.

Impact of VNS during intact, after ipsilateral and contralateral vagus nerve transection on cardiac myocardial dynamics and and fatty acid oxidation during I/R

Mitochondria are dynamic organelles that continually undergo fusion and fission [30-32, 78, 98]. Generally, mitochondrial outer and inner membrane fusion events are mediated by MFN1/2 and OPA1 protein [34]. In contrast, phosphorylation of DRP1 on Ser 616 promotes mitochondrial fission and phosphorylation of DRP1 on Ser 637 inhibits mitochondrial fission [30]. A growing body of literature has shown that enhancing mitochondrial dynamics and reducing mitochondrial oxidative stress have emerged as crucial therapeutic strategies to ameliorate myocardial I/R injury [103, 104]. In the present study, LC-VNS and LtVNX, but not RtVNX, significantly increased mitochondrial fusion protein (MFN2 and OPA1) expression, increased phosphorylation of DRP1 on Ser 637, and decreased the phosphorylation of DRP1 on Ser 616 when compared with I/R group.

Furthermore, it has been shown that pathological stressors for the heart, such as ischemia, are associated with a downregulation of mitochondrial biogenesis via PGC1α activity [29], and the impairment of the PGC1α-mediated mitochondrial biogenesis increased heart vulnerability to myocardial I/R injury [104]. Accordingly, upregulation of the PGC1α pathway has been shown to confer protection against simulated I/R in cardiomyoblast cells [103]. In the present study, LC-VNS and LtVNX, but not RtVNX, significantly increased the expression of PGC1α. Moreover, VNS significantly increased CPT1 expression in the heart subjected to I/R injury and this effect was abolished after the administration of atropine. The elevation of PGC1α and CPT1 expression suggests that cardiac fatty acid metabolism is shifted toward mitochondrial beta oxidation. In summary, we have demonstrated that the mechanism underlying the cardioprotection of VNS were associated with anti-apoptosis, anti-oxidative stress, anti-inflammation, prevent cardiac mitochondrial dysfunction, improved mitochondrial dynamic (increased mitochondrial fusion and decreased mitochondrial fission), improved mitochondrial biogenesis and shifted cardiac fatty acid metabolism toward beta oxidation. The summary of the molecular mechanism of I/R injury are depicted in Figure 4-1. Moreover, the results shown that the effects of LC-VNS was equivalent with LtVNX. The summary of the cardioprotection of VNS against I/R injury are

depicted in Figure 4-2. This finding suggests that VNS exerts cardioprotection against I/R injury predominantly through it efferent direction. In addition, LC-VNS and LtVNX have higher efficiency than RtVNX, which observed by the increasing of Bcl-2, P-DRP1 (ser 637) and PGC1α protein expression and the decreasing of TUNEL positive cells, MDA level, and mitochondrial swelling. The summary of the molecular mechanism underlying the cardioprotection of LC-VNS, LtVNX and RtVNX against I/R injury are depicted in Figure 4 -3 (yellow box and red font represent the higher efficiency of LC-VNS and LtVNX than RtVNX). Finally, VNS required both ipsilateral and contralateral efferent vagal activities to fully provide its cardioprotection against myocardial I/R injury. These findings may be associated with the vagal activity in each condition which is depicted in Figure 4-4.

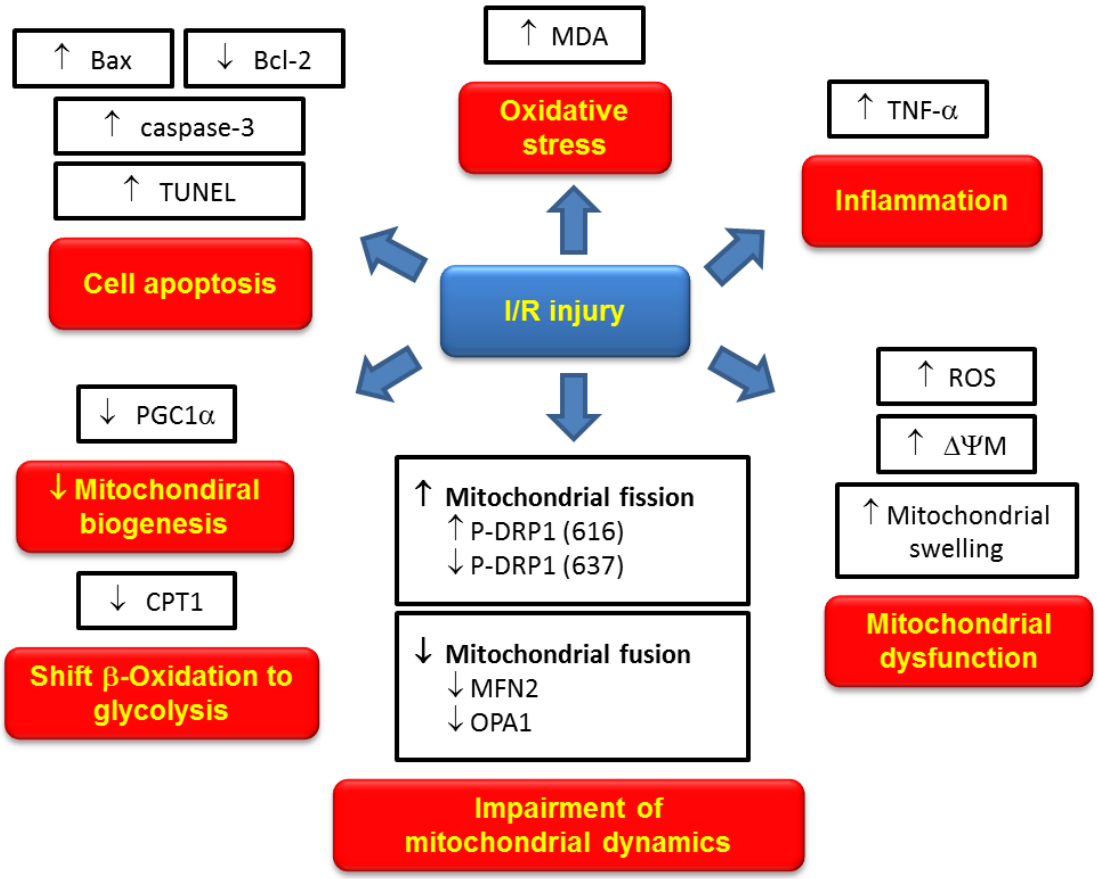

Figure 4-1: Summary of the molecular mechanism of cardiac I/R injury.

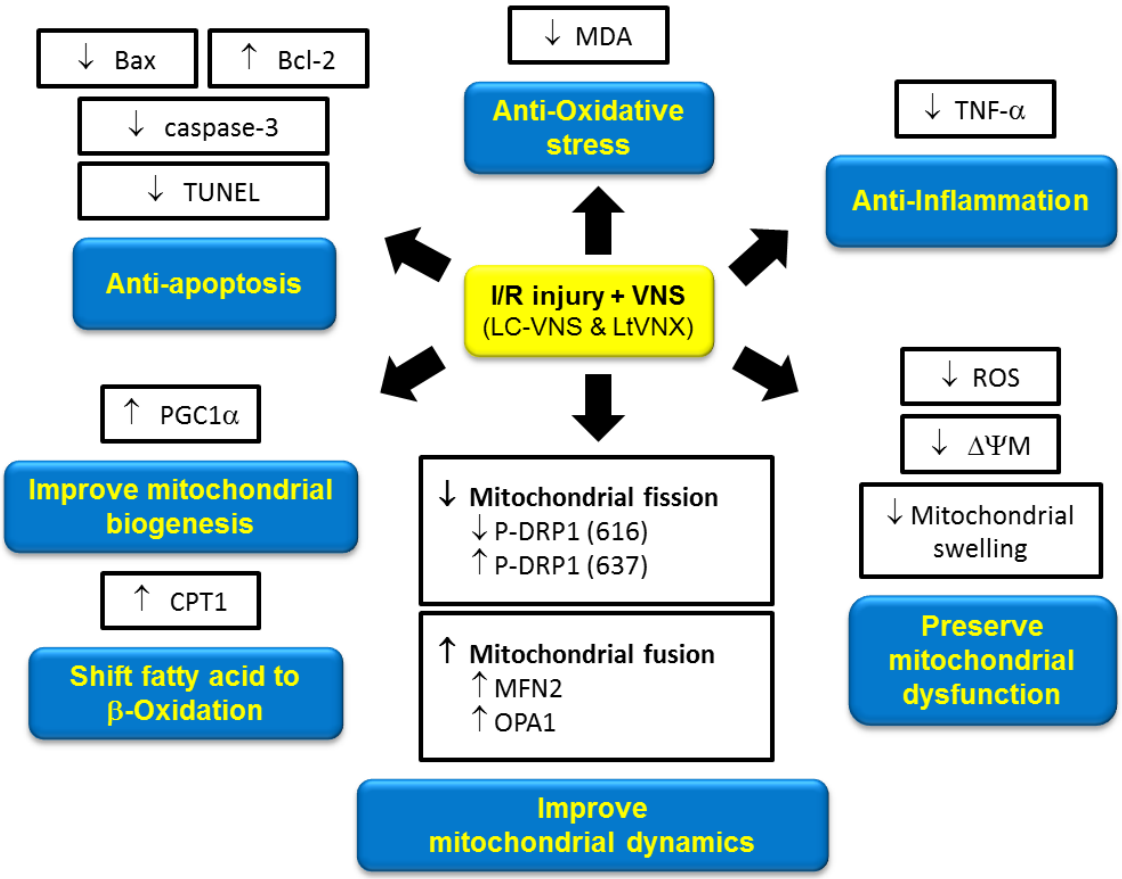

Figure 4-2: Summary of the cardioprotection of VNS against I/R injury.

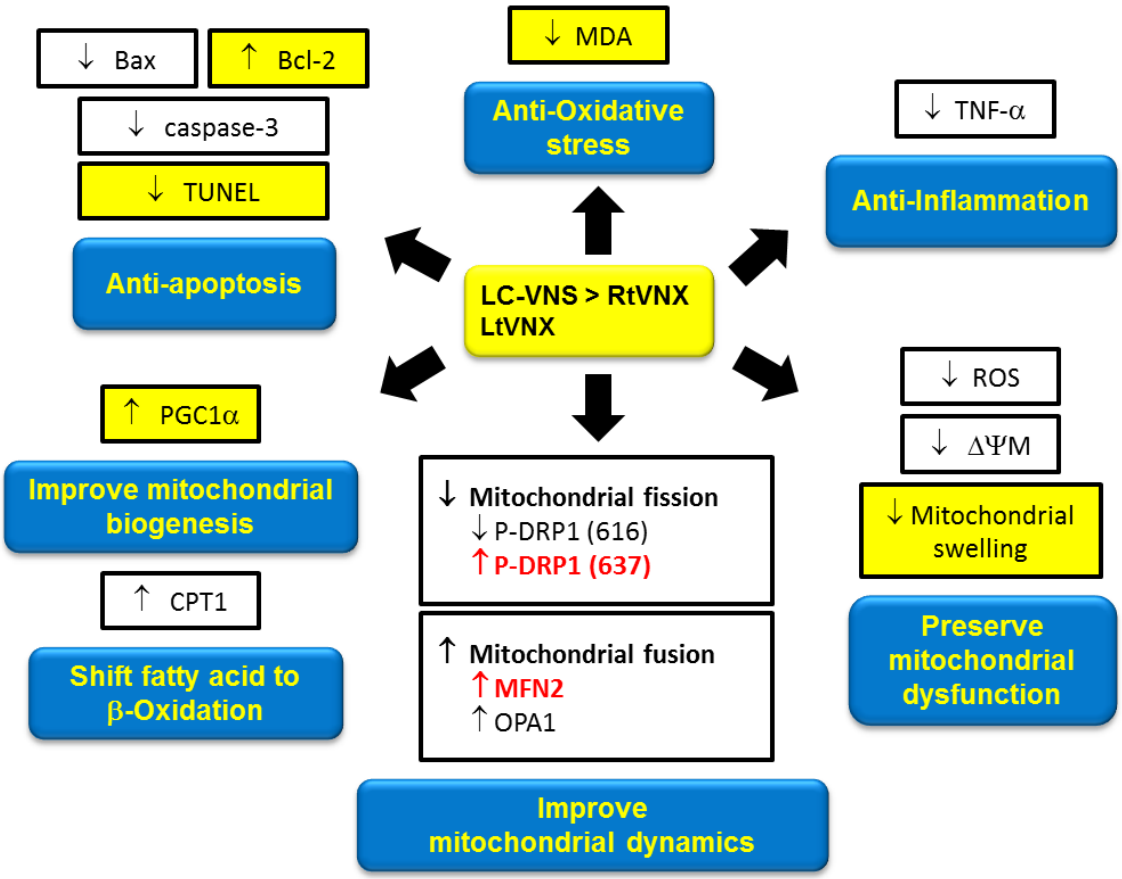

Figure 4-3: Summary of the molecular mechanism underlying the cardioprotection of LC-VNS, LtVNX and RtVNX against I/R injury.

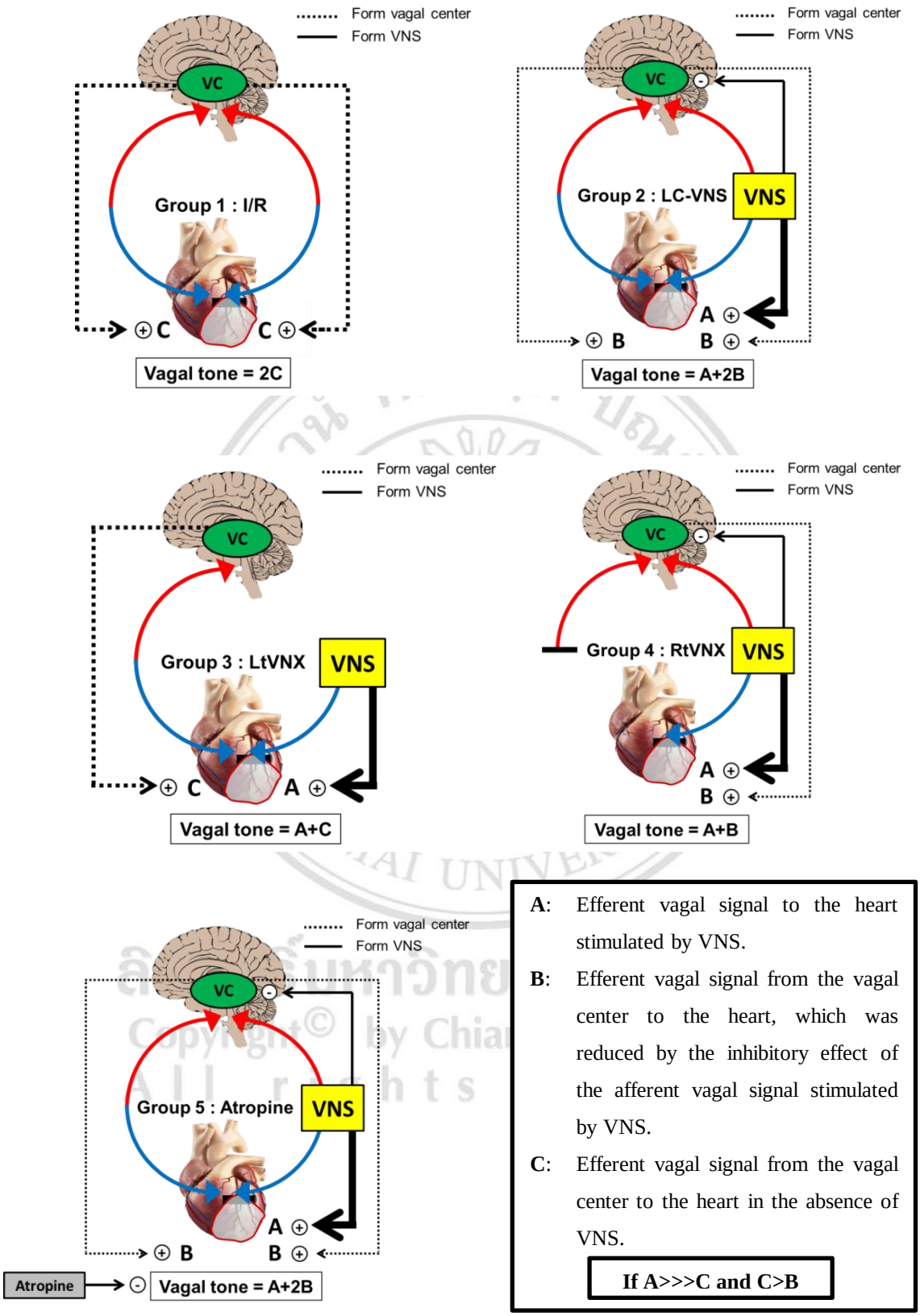

Figure 4-4: Summary of the vagal activity to the heart in the presence or absence of VNS

Conclusions and clinical implications

Our study has shown that VNS exerted cardioprotection against myocardial I/R injury via attenuation of mitochondrial dysfunction, increased mitochondrial fusion, decreased mitochondrial fission and shifted cardiac fatty acid metabolism toward beta oxidation. However, LC-VNS and LtVNX produced more profound cardioprotection, particularly infarct size reduction, decreased arrhythmia score and apoptosis and attenuated mitochondrial dysfunction compared to RtVNX. Our findings suggest that selective efferent VNS may potentially be effective in attenuating myocardial I/R injury. Moreover, VNS also required the contralateral efferent vagal activities to fully provide its cardioprotection. Thus, if it is possible to design stimulating devices that selectively stimulate efferent vagal nerve fibers, VNS may potentially be an attractive potential adjuvant therapy to limit reperfusion injury in patients with acute MI.