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REVIEW

Novel strategies and underlying protective mechanisms of modulation of vagal activity in cardiovascular diseases

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Cardiovascular disease remains a major cause of disability and death worldwide. Autonomic imbalance, characterized by suppressed vagal (parasympathetic) activity and increased sympathetic activity, correlates with various pathological conditions, including heart failure, arrhythmia, ischaemia/reperfusion injury and hypertension. Conventionally, pharmacological interventions, such as β-blocker treatment, have primarily targeted suppressing sympathetic over-activation, while vagal modulation has always been neglected. Emerging evidence has documented the improvement of cardiac and vascular function mediated by the vagal nerve. Many investigators have tried to explore the effective ways to enhance vagal tone and normalize the autonomic nervous system. In this review, we attempt to give an overview of these therapeutic strategies, including direct vagal activation (electrical vagal stimulation, ACh administration and ACh receptor activation), pharmacological modulation (adenosine, cholinesterase inhibitors, statins) and exercise training. This overview provides valuable information for combination therapy, contributing to establishment of a comprehensive system on vagal modulation from the aspects of clinical application and lifestyle improvement. In addition, the mechanisms contributing to the benefits of enhancing vagal tone are diverse and have not yet been fully defined. We endeavour to outline the recent findings that advance our knowledge regarding the many favourable effects exerted by vagal activation: anti-inflammatory pathways, modulation of NOS and NO signalling, regulation of redox state, improvement of mitochondrial biogenesis and function, and potential calcium regulation. This review may help to develop novel therapeutic strategies targeting enhancing vagal activity for the treatment of cardiovascular diseases.

LINKED ARTICLES

This article is part of a themed section on Chinese Innovation in Cardiovascular Drug Discovery. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2015.172.issue-23

Abbreviations

AMPK, adenosine monophosphate-activated protein kinase; H/R, hypoxia/reoxygenation; I/R, ischaemia/reperfusion; Nox, NADPH oxidase; nACh receptors, nicotinic acetylcholine receptors; ROS, reactive oxygen species

Tables of Links

TARGETS	
GPCRs[a]	**Enzymes**[c]
Adenosine A_1 receptors	AChE, acetylcholinesterase
α_1-adrenoceptors	Akt
β-adrenoceptors	AMPK, AMP-activated protein kinase
Muscarinic M_1 receptors	eNOS
Muscarinic M_2 receptors	iNOS
Muscarinic M_3 receptors	nNOS
Ligand-gated ion channels[b]	p38
α7nACh receptor, nicotinic acetylcholine receptors	PI3K
	PKC

LIGANDS
Adenosine
Angiotensin II
Atorvastatin
Carvedilol
Choline
Donepezil
Losartan
Pravastatin
Rosuvastatin
Simvastatin
TNFα

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c]Alexander *et al.*, 2013a,b,c).

Introduction

Cardiovascular disease remains a major cause of disability and death worldwide. The autonomic nervous system exerts a major role in the pathogenesis and progression of cardiovascular disease. Substantial evidence has accumulated that the impaired cardiovascular functions and the elevated morbidity and mortality are closely related to autonomic imbalance characterized by vagal (parasympathetic) withdrawal and sympathetic predominance in various cardiovascular diseases, including heart failure, arrhythmia, ischaemia/reperfusion (I/R) injury and hypertension (Abboud, 2010; Abboud *et al.*, 2012; Dicarlo *et al.*, 2013; Pal *et al.*, 2013). The clinical importance and prognostic implications of exaggerated sympathetic nerve activity are well-documented (Floras, 2009). Pharmacological therapies have primarily aimed at decreasing sympathetic over-activation and β-adrenoceptor antagonists are a well-established treatment for heart failure and cardiac ischaemia (Liggett *et al.*, 2008), while the possibility of enhancing vagal tone has been neglected. More recently, an increasing amount of attention has been focused on the importance of elevating vagal activity. A series of animal studies illustrated the pronounced protection afforded by vagal stimulation in the setting of heart failure, and recent clinical studies evaluated vagal stimulation as a potentially novel and useful therapeutic modality for chronic heart failure (De Ferrari *et al.*, 2011; Schwartz, 2011; Dicarlo *et al.*, 2013). Vagal stimulation also elicits cardiovascular protective effects in ischaemia and/or reperfusion injury (Calvillo *et al.*, 2011; Zhao *et al.*, 2013a).

In this review, we have summarized recent experimental and clinical findings highlighting the role of vagal nerve modulation in a variety of cardiovascular diseases. We also focus on the new and established therapeutic strategies targeting the modulation of vagal activity, including non-pharmacological approaches (electrical vagal nerve stimulation), pharmacological treatments (using adenosine, cholinesterase inhibitors and statins) or exercise training. Importantly, we also summarize and discuss the potential mechanisms involved in the cardiovascular protective effects of improved vagal activity. Finally, identification of the optimal vagal stimulation parameters, the possibility of rational use for combination of the therapeutic intervention and future applications of vagal stimulation in clinical settings are also discussed.

Vagal nerve modulation and cardiovascular diseases

The autonomic nervous system consists of two main branches, the sympathetic and parasympathetic nervous systems. The majority of the parasympathetic system comprises the vagal nerve and its branches. The sympathetic nervous system has many cardiovascular actions, including increasing cardiac contractility and accelerating the heart rate, via the binding of noradrenaline to β-adrenoceptors (Floras, 2009; Triposkiadis *et al.*, 2009). In addition, noradrenaline-mediated constriction of resistance vessels is mediated not only by β-adrenoceptors but also substantially by α_1-adrenoceptors (Woodman, 1989). On the contrary, the vagal nerve regulates the CVS by slowing down heart rate through the binding of ACh to muscarinic receptors (Schwartz, 2011; Harvey, 2012). The activities of the two branches are normally in dynamic balance, and the complex interplay between sympathetic and vagal nerves plays a critical role in the homeostasis of the CVS. In general, sympathetic overactivity has been depicted as 'bad' and vagal activity as 'good', in the context of the CVS (Abboud, 2010). There is growing evidence for a significant association between autonomic dys-regulation or imbalance, characterized by a hyperactive sympathetic system and a hypoactive vagal nerve system, and a range of conditions such as heart failure, I/R injury and hypertension. Reduced vagal tone as

shown by depressed heart rate variability and blunted baroreflex sensitivity is closely related to disease progression and poor clinical outcomes in chronic heart failure patients or myocardial infarction patients (La Rovere *et al.*, 2009; 2013; Wu *et al.*, 2014). Thus, depression of vagal modulation characterizes cardiovascular pathology and the therapeutic potential of restoring or enhancement of vagal nerve activity is very promising.

New therapeutic options targeting modulation of vagal activity

Vagal stimulation, ACh and ACh receptor activation

Many investigators have tried to explore ways of regulating the autonomic nervous system for the purpose of correcting autonomic imbalance in which, typically, cardiac vagal tone is diminished. Vagal stimulation appears to be the most direct and effective way to increase the vagal tone. In animal models with acute myocardial injury such as acute myocardial infarction or I/R injury, bilateral cervical vagal nerves were identified and then cut in the region of the neck. To rule out the influence of the vagal afferents, the cardiac distal end of the right vagal nerve was stimulated with an isolated constant voltage stimulator (Kong *et al.*, 2012b; Zhao *et al.*, 2013a). Hamann *et al.* (2013) also stimulated the right vagal nerve of dogs with chronic heart failure for 6 months with an implantable electrical stimulator. The results showed that vagal stimulation markedly attenuated left ventricular dysfunction, prevented left ventricular dilation and positively influenced the apoptotic, inflammatory and NO biomarkers. Vagal stimulation therapy has also been shown to favourably affect the left atrial function and structure in heart failure dogs (Kusunose *et al.*, 2014). Our previous studies have suggested that vagal stimulation improved cardiac pump function, reduced infarct size and decreased serum myocardial enzyme activities in a rat model of acute myocardial infarction (Kong *et al.*, 2011; 2012a). Calvillo *et al.* (2011) demonstrated a decrease in inflammatory markers and infarct size afforded by vagal stimulation in myocardial I/R injury in rat and this protective effect was independent of heart rate changes. Other studies also demonstrated the marked protection of vagal stimulation against myocardial I/R injury (Katare *et al.*, 2009; Uemura *et al.*, 2010). These recent animal studies reveal that vagal stimulation exerts profound favourable effects, not only in chronic heart failure, but also in ischaemia and/or reperfusion injury. Intriguingly, these compelling data also indicate that a reduction in heart rate is not always an essential prerequisite for the protective effects of vagal stimulation.

These encouraging results of experimental studies are a result of clinical trials in patients. Chronic vagal stimulation in 32 heart failure patients yield the apparent protective and long-lasting effects shown by improvement in life quality and New York Heart Association class, and a marked decrease in left ventricular end-systolic volume (Schwartz *et al.*, 2008). In this study, the vagal nerve was simulated by CardioFit, an implantable neuronal electrical stimulator system. This system can control the applied parameters remotely via a wireless link. This study represents the first piece of evidence to indicate that this novel strategy for treatment of patients with heart failure is well tolerated, applicable and safe. This initial study was followed by a growing number of clinical trials in heart failure patients (De Ferrari *et al.*, 2011; Dicarlo *et al.*, 2013). Recently, vagal stimulation has been reported to be a new therapeutic intervention for other vascular conditions, such as intermittent claudication (Payrits *et al.*, 2011). This new therapeutic strategy appears promising and warrants further investigation.

ACh is the main neurotransmitter in the vagal nerve. *In vivo* microdialysis studies demonstrated that vagal stimulation not only increased the myocardial ACh content during I/R (Kawada *et al.*, 2009) but also increased ACh concentration in the mesenteric circulation, suggesting that the ACh release from the vagal nerve is transported into the mesenteric circulation and has effects on endothelial cells and vascular smooth muscle cells (Zhao *et al.*, 2013a). Recent studies in our laboratory have demonstrated that ACh protected cardiomyocytes from hypoxic or reoxygenation injury (Li *et al.*, 2011; Miao *et al.*, 2013; Sun *et al.*, 2013; Zhao *et al.*, 2013b).

ACh is the endogenous agonist at two main classes of cholinoceptor, the muscarinic and nicotinic receptors. There are five distinct subtypes of muscarinic ACh receptors (M_{1-5}) and their importance is increasingly being appreciated as novel targets for drug development (Kruse *et al.*, 2014). Our recent studies have demonstrated that M_2 receptor antagonists (Li *et al.*, 2011) or knockdown of these receptors by siRNA (Miao *et al.*, 2013) abolished the effects of ACh-induced protection in cardiomyocytes, further supporting the functional role of M_2 receptors. In addition, emerging evidence argues for a critical role of M_3 receptors in the modulation of cardiovascular physiology. Pharmacological activation of M_3 receptors by choline was cardioprotective in ischaemia-induced arrhythmias and I/R injury (Zhao *et al.*, 2010; Wang *et al.*, 2012a). Furthermore, overexpression of M_3 receptors decreased the incidence of arrhythmias in a mouse myocardial I/R injury model (Liu *et al.*, 2011). In addition, M_3 receptors mediated the vasorelaxation of most blood vessels induced by ACh (Lamping *et al.*, 2004; Beny *et al.*, 2008; Gericke *et al.*, 2011).

The nicotinic ACh receptors (nACh receptors) have also been identified in many cell types, for instance, vascular smooth muscle cells and endothelial cells (Cooke and Ghebremariam, 2008; Wessler and Kirkpatrick, 2008). Calvillo *et al.* (2011) suggested that the nicotinic pathway is involved in the cardioprotection conferred by vagal nerve stimulation. In particular, the predominant function of the α7nACh receptor in mediating this anti-inflammatory action is now well accepted. Recent work showed that the α7nACh receptors blocked inflammasome complex activation through suppressing mitochondrial DNA release (Lu *et al.*, 2014). Xiong *et al.* (2012) demonstrated that α7nACh receptor agonists showed pronounced beneficial anti-inflammatory effects in the context of myocardial I/R injury. Vagal stimulation protected against myocardial I/R-induced remote vascular dysfunction, through the cholinergic anti-inflammatory pathway, which is dependent upon α7nACh receptors (Zhao *et al.*, 2013a). Several recent findings have also disclosed a critical role of α7nACh receptor in angiogen-

esis (Ni *et al.*, 2010) and a α7nACh receptor agonist up-regulated the capillary density and enhanced neovascularization in ischaemic tissues of rats with myocardial infarction (Li and Wang, 2006). Therefore, the α7nACh receptors must be seen as a potentially new therapeutic target in cardiovascular diseases (Liu and Su, 2012).

Drug targets to mimic vagal activation

In view of the favourable outcome related to vagal stimulation, there has been revived interest in drug development aiming to enhance vagal nerve function. We attempt to summarize some established and novel pharmacological approaches that might augment vagal activity and thereby favourably affect the clinical symptoms and/or prognosis in cardiovascular diseases.

Adenosine. There is accumulating evidence documenting a functional link between adenosine and the vagal nerve. Adenosine potentiated vagal activity in dog isolated atria (Tsuboi and Chiba, 2003) and might also enhance ACh release from motor neurons (Searl and Silinsky, 2003). Our recent study put forward a possible functional interaction between muscarinic M_2 receptors and A_1 adenosine receptors in I/R myocardium and it appears that NOS serves as the link between these two sets of receptors (Sun *et al.*, 2011a). Importantly, adenosine has beneficial effect on M_2 receptors, thus contributing to the improved cardiac function. These data have delineated a possible new mechanism underlying the cardioprotection afforded by adenosine. Another study conducted by our group also suggested that adenine sulfate, a precursor of adenosine, produced cardioprotective effects by enhancing cholinergic nerve density and expression of M_2 receptors (Sun *et al.*, 2011b).

Cholinesterase inhibitors. The cholinesterase inhibitor pyridostigmine increases levels of synaptic ACh by decreasing the degradation of ACh and this inhibitor favourably affected vagal tone and cardiac function in a rat model of heart failure (Lataro *et al.*, 2013). More recent studies have demonstrated important roles for pyridostigmine in preserving the autonomic balance (Durand *et al.*, 2014; Gavioli *et al.*, 2014). Our studies also suggested that pyridostigmine restored baroreflex sensitivity and improved heart rate variability, thus ameliorating cardiac remodelling and improving peripheral vascular endothelial function in rats with myocardial infarction (Lu *et al.*, 2014; Qin *et al.*, 2014). These data support the notion that enhancement of vagal tone by pyridostigmine is beneficial in cardiovascular diseases.

Statins. In animal models with chronic heart failure, simvastatin treatment partially restored vagal activity, as seen by the reversal of depressed heart rate variability (Pliquett *et al.*, 2003). In I/R injury, atorvastatin enhanced serum ACh level and baroreflex sensitivity (Bi *et al.*, 2013). In a human study, atorvastatin had a beneficial impact on vagal activity as measured by improvement in heart rate variability and might reduce the risk for arrhythmias in heart failure patients (Vrtovec *et al.*, 2005). The detailed underlying mechanisms for the effects of statins on cholinergic systems are not fully clarified. Welzig *et al.* (2003) demonstrated that lipid lowering by pravastatin could modulate the expression of $G\alpha_{i2}$, which is a molecular marker of myocardial vagal responsiveness. In addition, rosuvastatin treatment reduced the expression of caveolin-1 and induced NOS improvement along with enhancement of heart rate variability (Pelat *et al.*, 2003), potentially reflecting the pleiotropic effects of statins in cardiovascular autonomic modulation.

β-Adrenoceptor antagonists (β-blockers). The β-blockers improve prognosis in heart failure but their effects are not entirely explained by direct blockade of the sympathetic nervous system. In an earlier study, carvedilol, a β-blocker, up-regulated expression of M_2 receptors in adriamycin-injured myocardium, suggesting that up-regulation of these muscarinic receptors might be partly responsible for the effects exerted by carvedilol in heart failure (Xu *et al.*, 2006). In human studies, chronic carvedilol treatment in patients with heart failure partly normalized autonomic balance, as shown by improvement in heart rate variability (Bullinga *et al.*, 2005). Other studies also demonstrated that administration of carvedilol improved important surrogate markers of vagal tone, such as baroreflex sensitivity and heart rate variability, and haemodynamic function in heart failure patients, suggesting that carvedilol did have effects on restoring autonomic balance and increasing vagal activity (Mortara *et al.*, 2000; Piccirillo *et al.*, 2000).

Of note, in the studies of vagomimetic effects of pharmacological agents, most measures such as heart rate variability are indices or substitutes of vagal function, and it is hard to distinguish between direct pharmacological actions on the vagal nervous system and secondary phenomena. Potential mechanisms of action of these drugs await further elucidation.

Exercise favourably influences the vagal activity

Exercise has received much attention as a key component of a healthy lifestyle, and it plays a salutary role in the promotion of health and prevention and cure of diseases including cardiovascular disease. There is emerging evidence that the favourable and therapeutic outcomes of exercise training are partly associated with its regulatory effects on the autonomic nervous system (Joyner and Green, 2009). Autonomic adaptation induced by physical training has been assessed in several experimental studies. The significant beneficial effects of aerobic exercise by running were partly attributable to the enhanced expression of M_2 receptors and cholinesterase-positive nerves in the myocardium of hyperlipidaemic rats (Wang *et al.*, 2010). Exercise training by swimming improves cardiovascular autonomic activity in rats (Sant'Ana *et al.*, 2011; Barbosa Neto *et al.*, 2013). In a human study, increased vagal activity, induced by exercise training, was a predictor of favourable prognosis, post-myocardial infarction (La Rovere *et al.*, 2002). Other clinical studies have found that physical exercise improved cardiac sympathovagal balance, i.e., enhancing vagal tone while suppressing sympathetic tone, and increased heart rate variability (Tsai *et al.*, 2006; Soares-Miranda *et al.*, 2009; Guiraud *et al.*, 2013).

More studies are clearly needed to (i) assess the potential influence of different modalities (constant repeated exercise

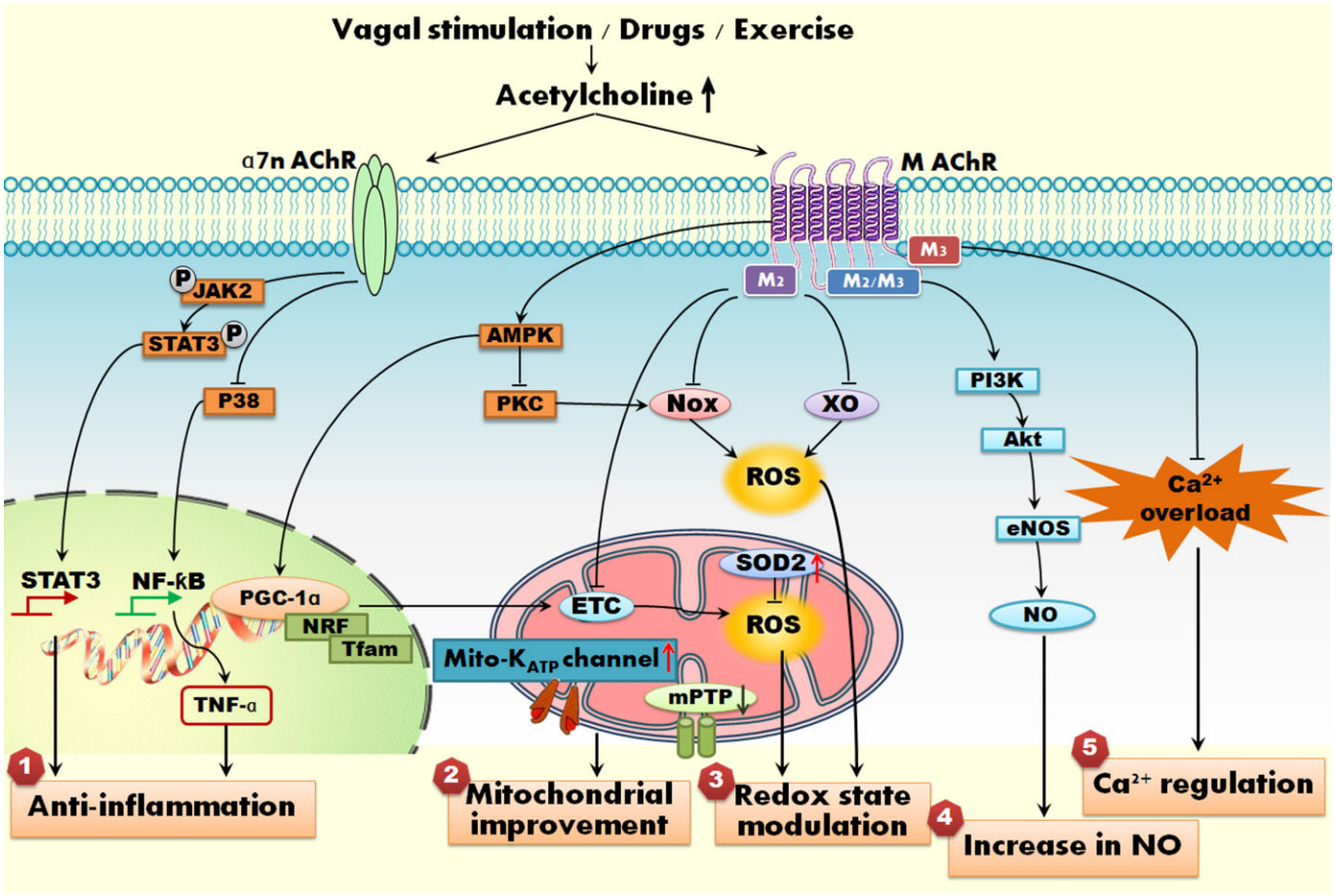

Figure 1

Schematic representation of signalling pathways and mechanisms regulated by vagal activation. The mechanisms contributing to the beneficial effect of improved vagal activity are multifactorial, including (1) its anti-inflammatory effects by activating JAK2/STAT pathway and inhibiting NF-κB activation which is dependent upon α7nACh receptor; (2) mitochondrial improvement (increasing mitochondrial biogenesis and function, mitochondrial K_{ATP} activation and inhibiting mitochondrial permeability transition pore (mPTP) opening); (3) redox state regulation (suppressing ROS generation and promoting ROS elimination); (4) increase in NO through PI3K/Akt/eNOS pathway; (5) down-regulation of calcium overload. SOD2, Mn superoxide dismutase.

or aerobic interval training) and parameters (intensity, frequency and duration) of exercise training on vagal tone; (ii) perform medical supervision from the start of exercise training in clinical settings, and establish a personalized protocol of training consisting of appropriate duration, frequency and intensity of exercise training according to the systemic physical evaluation; and (iii) provide a more comprehensive and thorough understanding of the mechanisms underlying the enhancement of vagal tone induced by exercise.

Underlying protective mechanisms of improved vagal activation

We have reviewed the beneficial and therapeutic effects of restoration or even augmentation of vagal tone in cardiovascular diseases and explored the new therapeutic options targeting modulation of vagal activity. Here, we will summarize the mechanisms contributing to the salutary effect of improved vagal activity. In addition to the fundamental action of the vagal nerve such as heart rate reduction and antagonism of sympathetic actions, the mechanisms also include its anti-inflammatory effects, increase in NO, regulation of redox state, improvement of mitochondrial biogenesis and function, and potential calcium regulation (Figure 1).

Role of anti-inflammatory pathways activated by the vagal nerve

It is well known that the activation of efferent vagal nerve fibres can modulate local and systemic inflammatory responses, now known as the 'cholinergic anti-inflammatory pathway' (Tracey, 2007; Johnston and Webster, 2009; Huston and Tracey, 2011) and there is an essential role for the α7nACh receptors in this anti-inflammatory pathway (Huston and Tracey, 2011; Liu and Su, 2012). Two major anti-inflammatory signalling pathways are activated by the vagal nerve: the JAK/STAT pathway and the pathway involving the transcription factor NF-κB (de Jonge and Ulloa, 2007).

Activated α7nACh receptors can recruit and phosphorylate JAK2 and, subsequently, trigger STAT3 activation. JAK2/STAT3 signal transduction is critical for the anti-inflammatory potential of vagal nerve stimulation. Another crucial part of the cholinergic anti-inflammatory pathway is mediated through the suppression of NF-κB activation (de Jonge and Ulloa, 2007). A landmark study suggested that vagal stimulation ameliorated LPS-induced injury in a murine model through reducing the release of a series of inflammatory cytokines (Borovikova *et al.*, 2000). Substantial evidence has accumulated that vagal stimulation plays a pronounced anti-inflammatory effect and exerts protective effects in a wide range of inflammatory conditions. Vagal stimulation reduced the number of neutrophils in myocardial tissue following I/R and the plasma levels of inflammatory markers (LPS-inducible CXC chemokine and CCL2) (Calvillo *et al.*, 2011). We showed that vagal stimulation decreased TNF-α expression and TNF receptor 1/TNF receptor 2 ratio, thus favourably attenuating ischaemia-induced cardiac dysfunction in a rat model (Kong *et al.*, 2011). More recently, we demonstrated that vagal stimulation promoted STAT3 phosphorylation and depressed NF-κB activation in mesenteric arteries, triggering peripheral vascular protection following myocardial I/R, and these effects were prevented by treatment with α7nACh receptor shRNA, indicating the key role of α7nACh receptor in this anti-inflammatory effect of vagal stimulation (Zhao *et al.*, 2013a). The underlying mechanism might include (i) a direct effect of vagal stimulation on the vasculature including enhancing local ACh concentration, and activating α7nACh receptor-dependent cholinergic pathway to improve endothelial structure and function, and (ii) indirect effects where reduced infarct size and improved cardiac function induced by vagal stimulation could lead to ameliorated inflammatory response, resulting in remote vascular protection. The anti-inflammatory effects of cholinergic stimulation are increasingly being appreciated, and these findings may be of great significance in elucidating vagal nerve-mediated signalling cascade and its potential role in modulating the vasculature. Our *in vitro* study also showed that ACh suppressed an elevation of TNF-α expression induced by hypoxia via regulation of p38 MAPK phosphorylation in cardiomyocytes (Li *et al.*, 2011).

Effects of improved vagal activity on NOS regulation and NO signalling

NO is generated by three NOS isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). NOS activity is expressed in neuronal cells, myocytes and endothelial cells, and the consequent generation of NO modulates a wide range of biological functions depending on the different NOS isoforms. The effects of NO can be beneficial, neutral or even detrimental, according to the specific isoform. Thus, NO generated from eNOS exerts a critical role in the modulation of cell growth, vasodilation and inhibition of platelet aggregation, whereas NO derived from nNOS modulates myocardial function and calcium-handling proteins (Zhang *et al.*, 2014). iNOS is not expressed under basal conditions and appears to be up-regulated in various pathological conditions, such as heart failure and ageing (Hamann *et al.*, 2013; Gong *et al.*, 2014). eNOS-derived NO has been shown to play many vasculoprotective roles in the CVS (Lei *et al.*, 2013). However, if eNOS is uncoupled, the consequent generation of superoxide anion, along with reduced NO bioavailability, predisposes to cardiovascular damage (Lorin *et al.*, 2014). The large amounts of NO generated by iNOS are regarded as detrimental, contributing to pro-inflammatory reactions and tissue damage (Lorin *et al.*, 2014). In cardiovascular diseases such as I/R injury, hypertension and heart failure, the expression of the different NOS isoforms are adversely changed and dysfunctional NO signalling may be causally related to various pathological processes associated with cardiovascular disorders (He *et al.*, 2011; 2013; Hamann *et al.*, 2013). Thus, it is conceivable that reversing NOS and NO signalling dysfunction could favourably affect the outcome in cardiovascular disease. Notably, in a recent study conducted in dogs with heart failure, protein expression of eNOS in left ventricular tissues was markedly decreased, and expressions of iNOS and nNOS were elevated, compared with levels in normal dogs. Chronic vagal stimulation therapy significantly enhanced the expression of eNOS and suppressed that of iNOS and nNOS (Hamann *et al.*, 2013). eNOS activation by vagal stimulation may be associated with up-regulation of the PI3K/Akt signalling cascade, mediated via muscarinic M_2 or M_3 receptors (Lu *et al.*, 2013; Miao *et al.*, 2014). These data are in agreement with the concept that PI3K/Akt is a critical upstream signalling component in the activation of eNOS (Morello *et al.*, 2009; He *et al.*, 2013). Further studies of the specific mechanisms by which vagal stimulation improves iNOS and nNOS are clearly required.

Redox state regulated by vagal activation

The redox status is a critical factor in the pathogenesis of a variety of diseases. Tsutsumi *et al.* (2008) demonstrated that vagal stimulation suppressed myocardial reactive oxygen species (ROS) production via muscarinic receptors, in a murine model of chronic heart failure. Recently, we reported that vagal stimulation up-regulated the antioxidant enzyme Cu/Zn-superoxide dismutase and Mn-superoxide dismutase activities and suppressed oxidative stress as measured by reduced myocardial superoxide production, activity of NADPH oxidase (Nox) and expressions of nitrotyrosine, Rac1and p67phox in a rat model of myocardial ischaemia (Kong *et al.*, 2012a). Moreover, the significant anti-oxidative effects of vagal stimulation involved adenosine monophosphate-activated protein kinase (AMPK)-PKC-Nox pathway (Kong *et al.*, 2012a). Kim *et al.*, (2008) showed that ACh treatment inhibited long-term hypoxia-induced increase in ROS and also suppressed the ROS-mediated apoptosis. Studies in our laboratory showed that ACh reduced angiotensin II- and hypoxia-induced ROS in cardiomyocytes (Li *et al.*, 2011; Liu *et al.*, 2011). More recently, we demonstrated that ACh inhibited mitochondria-, xanthine oxidase- and Nox-derived ROS production, thus protecting rat cardiomyocytes against hypoxia/reoxygenation (H/R)-induced oxidative stress, and these beneficial effects were mainly mediated by M_2 receptors (Miao *et al.*, 2013). Therefore, there is good evidence that, at least a part of, the positive effects of vagal activation might be due to a promotion of anti-oxidative actions and a decrease of pro-oxidant effects.

Mitochondria as a new therapeutic target

Mitochondrial dysfunction is now recognised as a critical factor contributing to the pathogenesis of a wide variety of cardiovascular diseases, including ischaemic heart disease, I/R injury, cardiomyopathy and vascular diseases. The mitochondria are, consequently, regarded as new targets for therapies of these diseases (Groschner *et al.*, 2012; Walters *et al.*, 2012; Dromparis and Michelakis, 2013; Kluge *et al.*, 2013; Tang *et al.*, 2014). Importantly, the cardioprotective effects of vagal stimulation against I/R injury were demonstrated by a reduction in infarct size, attenuation of left ventricular dysfunction, restoration of myocardial ATP content, up-regulation of cell-survival transduction and inhibition of pro-apoptotic signals, and all these salutary effects were abolished by a permeability transition pore opener, illustrating that vagal stimulation protected against I/R injury by inhibiting mitochondrial permeability transition pore opening (Katare *et al.*, 2009). Several investigations, including our own, reported that the protective role of ACh appeared to be mediated by activation of mitochondrial K_{ATP} channels (Oldenburg *et al.*, 2003; Lu *et al.*, 2006). Recently, we have shown that ACh inhibited the mitochondrial morphological abnormalities, promoted mitochondrial biogenesis (elevated mitochondrial DNA copy, mass and density) and improved mitochondrial function (increased activities of mitochondrial complexes, membrane potentials and ATP synthesis) in cardiomyocytes subjected to H/R, putting forward the exciting notion of ACh as a new mitochondrial nutrient. These beneficial effects on mitochondrial biogenesis and function were mediated by the AMPK/PPAR-γ co-activator-1α signalling pathway (Sun *et al.*, 2013). The mitochondria now appear to be a vital component of the cardioprotective programme induced by vagal activity.

Potential calcium regulation by vagal activation

Growing evidence indicates that intracellular Ca^{2+} overload with a defect in Ca^{2+} transport system is a major contributor to the pathogenesis of cardiovascular disorders (Talukder *et al.*, 2009; Marks, 2013). Thus, attenuation of Ca^{2+} overload and modulation of Ca^{2+} handling pathways could be protective. Previous studies demonstrated that choline, an agonist of muscarinic M_3 receptors, decreased ischaemia-induced intracellular Ca^{2+} overload, thus exerting protective effects against ischaemia-induced arrhythmias (Wang *et al.*, 2012a). Pharmacological activation of M_3 receptors also prevented the increase in intracellular Ca^{2+} concentration in the model of angiotensin II-induced cardiac hypertrophy (Wang *et al.*, 2012b). Also, the protective effects of choline against ischaemic myocardial injuries were partly mediated by amelioration of intracellular Ca^{2+} overload (Yang *et al.*, 2005). Wang *et al.* (2012a) demonstrated that choline resulted in an attenuation of Ca^{2+} overload via down-regulation of the reverse mode of Na^+/Ca^{2+} exchanger and L-type Ca^{2+} channels. Further studies are required to confirm the association between improved vagal modulation and attenuation of Ca^{2+} overload. The importance of endoplasmic reticulum-mitochondria Ca^{2+} crosstalk in cardiovascular diseases has been increasingly appreciated (Paillard *et al.*, 2013), and it may be a new molecular target for vagal modulation.

Present challenges and future prospects

Despite the well-established benefits of vagal stimulation in various cardiovascular diseases, the characteristics of the stimulation applied are vary markedly between different experimental settings (Uemura *et al.*, 2010; Calvillo *et al.*, 2011; Kong *et al.*, 2011). In order to find the optimal set of vagal stimulation parameters, we applied uniform design methods and set up six stimulation modes with different voltage, frequency, pulse width and duration of vagal stimulation in a rat model of acute myocardial infarction (Kong *et al.*, 2012b). The results have suggested that (i) the stimulation duration of vagal stimulation is a decisive factor in determining its cardioprotective function and (ii) the parameter set (3 V, 2 Hz, 1 ms, 240 min) was evaluated as the best set of variables to obtain the maximum of beneficial outcomes and to provide a moderate heart rate reduction. These findings may help to provide guidance for selection of appropriate vagal stimulation parameters in humans in a clinical setting.

It should be noted that slow rates of breathing enhanced baroreflex sensitivity and increased vagal activity, exerting a beneficial effect in heart failure patients or in hypertensive patients (Bernardi *et al.*, 2002; Joseph *et al.*, 2005). Yoga, a breathing practice, has also been reported to lead to improved vagal functions (Mason *et al.*, 2013). The data suggest that regulation of the respiration can favourably affect vagal activity, thereby inducing cardioprotective effects. On the other hand , in terms of respiratory tract tissues innervated by the vagal nerve, stimulation of the vagus can induce bronchoconstriction especially in asthmatic subjects (Li *et al.*, 2005), indicating an important limitation of vagal stimulation therapy. Similarly, β-blockers are usually contraindicated in patients with bronchial asthma. These findings might be relevant for exploring the strategies that selectively and effectively target the CVS.

In addition, the combination of electrical vagal stimulation and β-blockade produced better-preserved myocardial function compared with treatment with β-blockers alone, in dogs with experimental heart failure (Sabbah *et al.*, 2007). The benefit of this therapy may be associated with (i) antagonism of the action of the sympathetic nervous system; (ii) a direct action on enhancing vagal tone; and (iii) a combination of these factors. Li *et al.* (2014) demonstrated that pharmacological normalization of vagal activity by an AChE inhibitor, donepezil, together with inhibition of the renin–angiotensin system by losartan, could improve long-term survival in a rat model of chronic heart failure, suggesting that this combination could be a new pharmacotherapy for chronic heart failure patients. In view of the beneficial effects of restoration or even augmentation of vagal tone that can be achieved by various therapeutic interventions (direct electrical stimulation of vagal nerve, pharmacological, and exercise strategies) as described earlier, the combination of these therapeutic strategies is clearly a feasible and promising possibility deserving further study.

When the clinical application of feasible, safe and efficient stimulation of the vagal nerve in patients with cardiovascular disease, such as heart failure, has gained wide

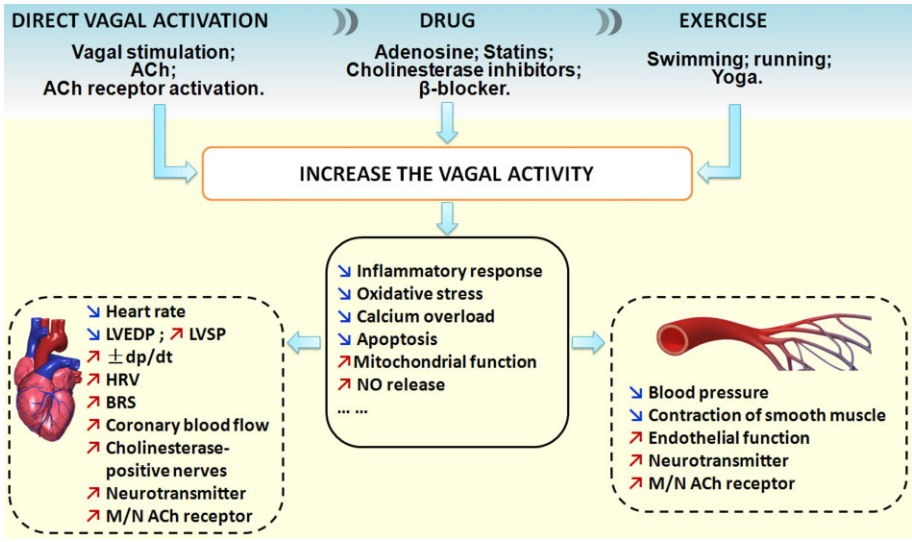

Figure 2

Beneficial effects on cardiac and vascular function are provided by the modulation of vagal activity, including direct vagal activation (vagal stimulation, ACh administration and ACh receptor activation), pharmacological modulation (adenosine, cholinesterase inhibitors, statins) and exercise training.

acceptance, it should be possible to consider how non-cardiological conditions may benefit from this strategy. There is evidence for a potentially regulatory role of vagal activity in tumours. For instance, vagus nerve activation decreased breast cancer metastasis (Erin *et al.*, 2012) and vagal activity, reflected by heart rate variability, could also predict clinical prognosis in patients with non-small-cell lung cancer or prostate cancer, in a historical prospective design (De Couck *et al.*, 2013). Gidron *et al.* (2014) suggested that higher vagal activity might exert protective effects against the adverse effects of advanced cancer stage in patients with colorectal cancer or prostate cancer. Further studies are clearly required to elucidate the specific mechanism(s) underlying the anti-tumour effects of vagal activation. The validity of the protective effects of vagal activation awaits further investigation in distinct types of cancer.

Conclusion

In this review, we have tried to provide an overview of recent studies for the role of vagal nerve modulation in a wide range of cardiovascular diseases. Although not exhaustive, this review shows that there is a surge of data to suggest that decreased vagal function is closely related to disease development and poor clinical outcomes in patients with cardiovascular disorders. From the perspective of 'autonomic neural regulation', we have attempted to explore a variety of ways to normalize or enhance vagal activity (i.e. direct vagal activation, pharmacological and exercise strategies) and provide valuable information for combination therapy, contributing to establishment of a comprehensive system on vagal modulation (Figure 2). This may help to combine clinical therapy and improvement of lifestyle in an attempt to provide important benefits and promise for prevention and treatment of cardiovascular diseases. In addition, we summarize and discuss the multifactorial mechanisms involved in the beneficial effect of improved vagal activity, including its anti-inflammatory effects, increase in NO, regulation of redox state, improvement of mitochondrial biogenesis and function, and potential calcium regulation, in order to sketch a clear and whole picture of the underlying mechanisms. The insights into the mechanisms may be of significance in exploration and development of promising and novel therapeutic approaches for enhancing vagal activity and normalizing autonomic balance.

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Conflicts of interest

None.

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