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Cardiac innervation in acute myocardial ischaemia/reperfusion injury and cardioprotection

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Abstract

Acute myocardial infarction (AMI) and the heart failure (HF) that often complicates this condition, are among the leading causes of death and disability worldwide. To reduce myocardial infarct (MI) size and prevent heart failure, novel therapies are required to protect the heart against the detrimental effects of acute ischaemia/reperfusion injury (IRI). In this regard, targeting cardiac innervation may provide a novel therapeutic strategy for cardioprotection. A number of cardiac neural pathways mediate the beneficial effects of cardioprotective strategies such as ischaemic preconditioning and remote ischaemic conditioning, and nerve stimulation may therefore provide a novel therapeutic strategy for cardioprotection. In this article, we provide an overview of cardiac innervation and its impact on acute myocardial IRI, the role of extrinsic and intrinsic cardiac neural pathways in cardioprotection, and highlight peripheral and central nerve stimulation as a cardioprotective strategy with therapeutic potential for reducing MI size and preventing HF following AMI. This article is part of a Cardiovascular Research Spotlight Issue entitled ‘Cardioprotection Beyond the Cardiomyocyte’, and emerged as part of the discussions of the European Union (EU)-CARDIOPROTECTION Cooperation in Science and Technology (COST) Action, CA16225.

1. Introduction

Acute myocardial infarction (AMI) and the heart failure that often complicates this condition, are among the leading causes of death and disability worldwide. To reduce myocardial infarct (MI) size and prevent heart failure, novel therapies are required to protect the heart against acute ischaemia/reperfusion injury (IRI). In this regard, the dense cardiac network of parasympathetic and sympathetic nerves and their interactions with the intrinsic cardiac nerve system (ICNS), may provide novel targets for cardioprotection. This cardiac neural network influences myocardial rhythm and contractile function, and the susceptibility to acute IRI. It also contributes to cardioprotective strategies such as ischaemic preconditioning (IPC) and remote ischaemic conditioning (RIC). In this article, we provide an overview of cardiac innervation with a focus on acute myocardial IRI, the role of extrinsic and intrinsic cardiac innervation in cardioprotection, and highlight peripheral and central nerve stimulation as a cardioprotective strategy with therapeutic potential for improving clinical outcomes in AMI patients.

2. An overview of the cardiac neural network

The heart is innervated by a complex interacting hierarchal network of neural pathways within the central nervous system (CNS), intrathoracic extracardiac ganglia, and intrinsic cardiac ganglia of the ICNS (see **Fig.1**).[1] The heart is supplied and controlled by sympathetic and parasympathetic nerves, which receive sensory inputs from heart, blood vessels and other organs. The parasympathetic nerves interface with ganglionic neurons of the ICNS, whereas the sympathetic nerves traverse ganglia without synapsing on ganglionic neurons, and together they provide beat-to-beat regulation of heart rhythm and contractile function. Sympathetic stimulation increases heart rate and cardiac contractile function through activation of beta adrenoceptors, and vagal activation reduces heart rate and in some species, cardiac contractile function, through activation of muscarinic receptors (reviewed in [2, 3]).

Pre-ganglionic fibres of the parasympathetic nervous system arise from the medulla oblongata, and via the vagal nerves, secrete acetylcholine (Ach) which binds to the nicotinic Ach receptors on the plasma membrane of postganglionic fibres. These in turn secrete Ach, which binds to the type 2 muscarinic Ach receptors present on the plasma membrane of cardiac cells in the sinoatrial (SA) node, atrioventricular (AV) node, left ventricle, and to some extent also other parts of the heart, resulting in a reduction in contraction rate of cardiac muscle by shortening its action potential duration and conduction velocity, by hyperpolarising SA nodal cells that reduce heart rate.

Within the myocardium there exists an ICNS, comprising cardiac ganglia and interconnecting neurons (known as ganglionic plexuses), which process sensory information and modulate efferent post-ganglionic parasympathetic and sympathetic activity within the heart, in the absence of any central modulation (reviewed in [3]). The extrinsic parasympathetic and sympathetic nerves access the ICNS arterially, around the roots of the pulmonary artery and aortic root, and interface with the venous portion of the heart around the roots of the pulmonary veins and superior vena cava. The number of cardiac ganglia varies between species from 19 in mice to over 800 in humans, and they are mainly located on the dorsal atrial surface, around the base of the aorta and pulmonary artery, dorsal and ventral to the pulmonary veins, and on the anterior ventricular surface. From these cardiac ganglia, intrinsic cardiac nerves extend epicardially from ganglionic plexuses to innervate the atria, interatrial septum and the ventricles. A number of neurochemicals have been found within the ICNS, the presence of which highlight the existence of both parasympathetic and sympathetic nervous components within the atria and ventricles. The majority of the cardiac ganglia are cholinergic (containing choline acetyltransferase, responsible for the synthesis of acetylcholine) which innervate supraventricular myocardium in and around the sinoatrial and atrioventricular nodes as well as the left ventricle, and these co-exist with both neuronal nitric oxide synthase (responsible for producing nitric oxide), and vasoactive intestinal peptide. The cardiac ganglia also include adrenergic nerve fibres (containing tyrosine hydroxylase, for the production of noradrenaline) within the left and right coronary subplexuses that

innervate the ventricles, with which neuropeptide Y is co-released. Within the ICNS there are also neuronal subpopulations that are non-adrenergic and non-cholinergic.[4] Activation of the ICNS can result in local and/or remote cardiac changes with effects on cardiac function and rhythm that are dependent on location. The ganglionic plexuses can modulate postganglionic parasympathetic nerve activity and selectively modulate vagal control of heart rate, atrio-ventricular conduction and left ventricular inotropy.[5-7] The ganglionic plexuses may also help mediate the differential effects of sympathetic nerves stimulation of the heart, with nerves arising from the left sympathetic chain influencing LV contractile function and electrical conduction via the AVN to a greater degree than the right, whilst the nerves arising from the right sympathetic chain have a more significant modulator effect on sinus rate via the sinoatrial node.[8] The ICNS can respond to a variety of stimuli including acute IRI and influence cardiac function on a beat-to-beat basis and have been implicated in both acute IRI and cardioprotection.[9]

3. Cardiac innervation and acute myocardial ischaemia/reperfusion injury

Sensory nerve endings may detect consequences of acute ischaemia, such as hypoxia, lactate, K^+ and low pH, which stimulate cardiac sensory nerves to release their neuropeptide transmitters.[3, 10] Local afferent function of these sensory nerves may have a strong influence on cardiac function through cardio-cardiac reflexes and initiate adaptive responses due to their nitric oxide (NO) and vasoactive neuropeptides, such as calcitonin gene-related peptide (CGRP), substance P, somatostatin.[10-12] Indeed, in selective sensory desensitisation by capsaicin, a ligand for TRPV channels, cardiac sensory nerves were demonstrated to strongly influence gene expression patterns in rat hearts,[13] regulation of the cardiac NO-cGMP system,[11] SERCA function,[14] with potential effects on cardiac function. Moreover, cardiac sensory nerves play a role in acute myocardial injury and adaptation to ischaemic stress,[12, 15] and in the mechanism of doxorubicin-induced heart failure.[16] Intact cardiac sensory nerves have been shown to protect against acute IRI-induced cell death via the local release of NO and cytoprotective neuropeptides.[17] Sympathetic efferent nerve

terminals release norepinephrine and exacerbate IRI-induced cardiac cell death directly and indirectly by deterioration of oxygen supply and by increasing oxygen demand.[2]

Cardiac sympathetic afferent denervation attenuates cardiac remodelling and improves cardiovascular function in rats with heart failure.[18] Modulation of neural networks outside the heart can also impact on post-AMI remodelling with renal nerve denervation preventing adverse post-AMI LV remodelling and preserving vascular function in both spontaneously hypertensive rats and normotensive rats, effects which were mediated by reduced neprilysin activity and preservation of circulating natriuretic peptide levels.[19] Interestingly, blockade of beta-adrenoceptors directly in the brain via chronic intracerebroventricular administration of metoprolol attenuated post-AMI LV remodelling in a rat model of myocardial infarction-induced heart failure, suggesting that the action of certain beta-blockers in the brain could contribute to the beneficial effect of beta-blockers in the failing heart.[20]

Cardiac sympathetic neurons in the stellate ganglia co-express neuropeptide Y (NPY) and the neurotransmitter norepinephrine (NE). Following acute myocardial IRI, axonal damage and the inflammatory response to injury, result in suppression of NPY and NE expression, and enhanced expression of neuropeptides such as vasoactive intestinal peptide, substance P, and galanin. Habecker et al [21] observed extensive axon damage after AMI, and this was associated with a significant increase in galanin (a peptide which promotes regeneration of sensory neurons[22]) in cardiac sympathetic neurons in the left ventricle, suggesting the existence of an endogenous protective strategy based on neuropeptides in cardiac sympathetic neurons. The susceptibility to acute myocardial IRI differs between cardiomyocytes and neurons, and found that cardiac sympathetic neurons are more susceptible to acute myocardial IRI than cardiomyocytes [23].

Most cardiac neurons of the ICNS are perivascular, making them susceptible to acute myocardial IRI, thereby setting an environment for neuronal remodelling following AMI. In response to acute myocardial IRI, pathological and degenerative changes to the cardiac ganglia occur with the appearance of cytoplasmic inclusions, a feature in common with

neuronal degeneration disorders. Acute myocardial IRI induces reorganisation and remodelling within ganglionic plexuses of the ICNS in the first 7 days post-AMI, resulting in increased adrenergic sensitivity and enhanced neuronal nitric oxide synthase (nNOS) expression within parasympathetic postganglionic neurons within the ICNS.[24] Pathological features of damaged cardiac nerves include enlargement, and degenerative changes to dendrites and axons and the appearance of cytoplasmic inclusions.[25, 26] Neuronal remodelling also occurs within regions of non-infarcted myocardium, presumably enabling a compensatory response in remote myocardium and impacting on post-AMI cardiac remodelling. In this regard, it has been proposed that enhanced nNOS expression plays a protective role, attenuating the initial increase in centrally derived sympathetic activity and facilitating parasympathetic neuronal inputs.[24] However, the actual interplay between the ICNS and extrinsic vagal or sympathetic nerves in the setting of AMI needs to be further elucidated.

3.1. Protection of cardiac neurons against acute myocardial IRI

The majority of studies investigating cardioprotective strategies for protecting the heart against the detrimental effects of acute IRI have focused on preventing cell death of cardiomyocytes and only few studies have explored the beneficial effects on cardiac neurons. During acute myocardial ischaemia, damage to cardiac sympathetic neurons results in the release of norepinephrine (NE) into the myocardial interstitial space. IPC can reduce myocardial NE levels following acute myocardial IRI in rat and rabbit hearts.[27-29] Miura et al[29] demonstrated that the detrimental effects of acute myocardial IRI on cardiac sympathetic nerves were reduced by IPC, and the mechanism underlying this neuroprotective effect was attributed to K_{ATP} channel opening. The neurotrophin, nerve growth factor (NGF) is known to support survival and differentiation of sympathetic neurons, and is elevated following AMI in a spinal nerve-dependent manner (thoracic epidural anaesthesia prevented the increase in NGF following AMI).[30] Strande et al[31] have shown

that NGF administered prior to ischaemia reduced MI size in an *in vivo* rat AMI model, and this effect was mediated through PI3K and NOS. A recent clinical study has shown that limb RIC can reduce the muscle sympathetic nerve activity in the forearm induced by acute ischemia, and this was associated with decreased production of an erythrocyte marker of oxidative stress and the reduction of NO availability, and ameliorated ischaemic reactive hyperaemia.[32] Further studies are required to investigate whether cardioprotective strategies can protect cardiac parasympathetic neurons and the ICNS against the detrimental effects of acute myocardial IRI.

3.2. Cardiac innervation and ventricular arrhythmias following acute myocardial ischaemia/reperfusion injury

Ischaemia can directly provoke cardiomyocyte electrical instability, action potential duration (APD) heterogeneity and arrhythmias as a result of ATP depletion, lactate production, reactive oxygen species, K^+ accumulation, and other substances e.g endothelin, which enhances the response of perivascular afferent nerves to autonomic reflexes. Some myocardial ischaemic events can be triggered and enhanced by abnormal central autonomic activity such as emotional stress leading to an imbalance in cardiac sympathovagal tone, reflexly increasing cardiac sympathetic activity leading to further coronary vasoconstriction.[33, 34] This is often accompanied by vagal withdrawal perpetuating the clinical scenario. Since the ICNS has an integrative role and can exert considerable influence on cardiodynamics, it is possible that significant interaction occurs between the heart's 'little brain'[3] locally and with peripheral nerves that mediate important mechanisms underlying arrhythmogenesis during acute myocardial IRI. Vagal control of heart rate and release of Ach in non-ischaemic ventricular regions are both blunted following a developed myocardial infarct,[35] showing that regional ischaemia can affect non-ischaemic sites both proximal and distal to the insult site.

Preclinical studies support the notion that sympathetic nervous system (SNS) activity is pro-arrhythmic,[36, 37] whilst vagal nerve stimulation is anti-arrhythmic.[38] High levels of vagal activity exert powerful anti-arrhythmic effects, which can counter the effects of acute

ischaemia and sympathetic activation. Mechanisms are complex and include indirect effects of accentuated antagonism against sympathetic activity and direct protective effects on electrophysiological parameters, Ca^{2+}-handling and other important factors such as inflammation and gap junctions.[39] Increased dispersion of repolarisation is an important mechanism in ventricular arrhythmogenesis, and sympathetic stimulation increases dispersion of repolarisation *in vivo*,[40] especially in the ischaemic border which increases propensity to arrhythmias.[41]

In an innervated isolated heart preparation,[42] the kinetics of the APD restitution relationship appear to be a key mechanism by which sympathetic stimulation precipitates ventricular fibrillation (VF), resulting in a steepening of APD-restitution slope- this facilitates alternans and hence wave-breaks to generate VF.[43] Vagal nerve stimulation, on the other hand, protects against VF initiation by flattening the slope, an effect which is mediated via nitric oxide from neuronal NO synthase,[44] a property which appears to be independent of muscarinic activation.[45] This NO-mediated protection has been shown to be important with intrapericardial perfusion of L-arginine increasing NO synthase activity, and protecting against VF in open chest dogs during acute coronary artery occlusion.[46] Recent proof-of-concept evidence suggests that vagal stimulation via low level tragus stimulation can reduce arrhythmias related to acute myocardial IRI in patients with STEMI, a finding which needs to be confirmed in larger studies.[47]

Cardiac remodeling as a result of AMI exaggerates influences on dispersion and APD kinetics which results in increased arrhythmogeneity coupled with cardiac fibrosis, regional denervation,[48] and adaptive nerve sprouting and heterogeneous hyper-innervation.[49] Neural remodelling also occurs in the stellate ganglion,[50] and ICNS which further promotes instability in the already arrhythmogenic environment.[26] Preclinical studies have shown a beneficial effect of reducing sympathetic tone through renal artery denervation on ventricular arrhythmias associated to post-AMI LV remodeling.[51, 52]

Clinically, left cardiac sympathetic denervation is effective in reducing arrhythmia burden in otherwise refractory ventricular arrhythmias,[53] but with accompanying side effects.

Recent evidence supports a cardiotopic arrangement whereby functionally distinct neurons arise from discrete regions of the sympathetic chain,[54] which should be targeted for more focused therapy. On the other hand, vagal protection against VF initiation appears to be mediated through a specific population of anti-fibrillatory nitrergic neurons,[55] although other indirect and non-arrhythmic mechanisms may also be at work including anti-inflammatory actions and effects on gap junctions.[39] Clinical studies using implanted vagal nerve stimulators in patients with heart failure have not produced positive outcomes to date.[56] Much work is needed to understand the mechanisms underlying the autonomic modulation of lethal arrhythmias especially following AMI, in order to develop effective therapeutic options.

3.3. Coronary vascular effects of cardiac sympathetic and vagal innervation in myocardial IRI

Both, cardiac sympathetic and vagal nerve activation impact on coronary blood flow through changes in heart rate with secondary effects on MI size.[57] Their direct effect on the coronary circulation is more immediate and short-lasting such that it is of greater importance in acute episodes of reversible ischaemic injury than in AMI. Sympathetic activation during exercise, excitement or pain not only increases heart rate and cardiac contractile function through activation of β-adrenoceptors, but also coronary vasoconstriction of epicardial and resistive vessels through activation of α-adrenoceptors.[58] In the presence of coronary stenosis, α-adrenergic coronary vasoconstriction is powerful enough to induce lactate production and ischaemic contractile dysfunction.[59, 60] Acute myocardial ischaemia then elicits a further positive-feed back activation of cardiac sympathetic nerves which then results in progressive α-adrenergic coronary vasoconstriction but can be eliminated by spinal anaesthesia.[34] Coronary collateral vessels in dogs have no functional α-adrenoceptors. Hence, the blood flow into collateral-dependent myocardium is not reduced by sympathetic activation.[61] Accordingly, chronic sympathetic denervation does not increase collateral blood flow or reduce infarct size after 3 h coronary occlusion in conscious dogs,[62] and the same is true in anaesthetised rabbits.[63, 64] However, chronic sympathetic denervation in mice attenuates

post-infarct inflammation and adverse remodeling.[65] In anaesthetised pigs, carvedilol but not propranolol improved coronary blood flow after 3 h coronary occlusion/reperfusion and reduced coronary no-reflow, suggesting an action through α- rather than ß-adrenoceptor blockade.[66] α-Adrenergic coronary vasoconstriction contributes to acute myocardial ischaemia also in humans.[67] In particular, the cardiac sympatho-excitatory reflex elicits α-adrenergic coronary vasoconstriction during stenting in patients with stable angina and with AMI, and α-blockade may therefore improve blood flow during reperfusion following AMI.[68]

Activation of cardiac vagal nerves reduces MI size, not only through HR reduction, but through a number of mechanisms, including improved mitochondrial function, attenuated formation of reactive oxygen species, and inflammation.[69] There is no evidence that cardiac vagal nerve activation improves collateral blood flow during coronary occlusion. However, cardiac vagal nerve activation just prior to reperfusion not only reduces MI size[70, 71], but also decreases areas of no-reflow after reperfusion.[71] Vagal activation by electrical stimulation of the auricular tragus also reduced MI size in patients with AMI.[47]

3.4. Cardiac innervation and inflammation

The sympathetic control of the immune cell system has been investigated in conditions such as rheumatoid arthritis, asthma, sepsis, and colitis. Peripheral effects of SNS activation have been linked to the release of monocytes from the bone marrow,[72] macrophage programming,[73] cytokine expression of various immune cells,[74] and B cell antibody production.[75] More recently, the SNS has been suggested to play a role in the immune response to cardiovascular disease.[76]

Following AMI, the inflammatory response to acute myocardial IRI plays a critical role in determining MI size and subsequent LV remodelling (reviewed in [77, 78]). Recent studies have investigated the role of the cardiac SNS in the regulation of the inflammatory response to AMI. In a murine model of AMI, Ziegler et al[65] surgically removed the right and the left superior cervical ganglia, which resulted in near complete loss of myocardial sympathetic innervation in the LV anterior wall. Although this method of cardiac sympathetic denervation

did not affect acute myocardial injury and MI size, it did attenuate myocardial inflammation (with less infiltration of macrophages, neutrophils and T cells), and prevent subsequent adverse LV remodelling in terms of less cardiomyocyte hypertrophy, and preserved cardiac function. These findings confirm the importance of chronic SNS activation in post-AMI heart failure as a contributor to adverse LV remodelling. However, the mechanisms through which the cardiac SNS modulates the inflammatory response post-AMI is not known and requires further study. Interestingly, the interaction between the SNS and the immune cell system appears to be mutual, with a study showing less sympathetic hyper-innervation of remote myocardium post-AMI following chemical depleting macrophages with systemic clodronate.[79]

4. Cardiac innervation and cardioprotection

A number of experimental studies have investigated the role of cardiac innervation, and more recently the ICNS in endogenous cardioprotective strategies.[80] Pacing-induced preconditioning requires intact cardiac capsaicin-sensitive sensory innervation, and the release of NO and CGRP from capsaicin-sensitive nerves may be involved in the mechanism of pacing-induced preconditioning.

Cardiomyocytes per se are capable of synthesising and releasing ACh, an intrinsic cholinergic system which is known as the non-neuronal cholinergic system within the heart.[81] Ach is also produced in the myocardium during acute myocardial ischaemia, and exogenous acetylcholine can be a trigger of IPC cardioprotection.[82] Bilateral vagotomy did not inhibit ischaemia-induced Ach release in the myocardium.[83, 84] The role of Ach in the ICNS as a mediator of IPC has recently demonstrated the involvement of intrinsic cardiac ganglia. In an isolated perfused rat heart subjected to acute IRI, the ganglion blocker, hexamethonium, and the muscarinic receptor antagonist, atropine, abrogated IPC cardioprotection. Interestingly, IPC increased acetylcholine in the perfusate, and the cardioprotection induced by this perfusate in a naïve rat heart was also blocked by hexamethonium.[85] However, the mechanism through which IPC stimulates the intrinsic cardiac ganglion is not clear and whether this pathway is operative *in vivo* is not known. In contrast to these findings, an

earlier study by Kudej et al[86] had found that intact cardiac nerves were not required for classical IPC in a porcine acute myocardial IRI model, but were required for the second window of protection through the activation of α1-adrenergic receptor and increased expression of iNOS and COX-2. Atropine and bilateral vagotomy did not abolish the infarct-limiting effects of classical IPC in rats.[87, 88]

In the field of cardioprotection, most studies have focused on the role of peripheral and cardiac innervation in RIC, the phenomenon by which brief cycles of non-lethal ischaemia and reperfusion to an organ or tissue away from the heart are able to protect the heart against AMI.[89-95]

4.1. Cardiac innervation and cardioprotection by remote ischaemic conditioning

The actual mechanisms underlying cardioprotection induced by RIC remain unclear, although a neuro-hormonal pathway has been implicated **(Fig. 2)**.[96] With respect to the neural component of RIC cardioprotection, an intact neural pathway is required for application of RIC to the remote organ or tissue. The neural element to the stimulus was demonstrated in some of the earliest experimental studies of RIC, which showed that the cardioprotection induced for example by transient mesenteric ischaemia was completely abrogated when animals were pre-treated with the ganglion blocker hexamethonium.[97] Resection of the neural pathway to the lower limb abolished RIC induced cardioprotection by transient limb ischaemia,[98 88, 99] showing the dependency of the RIC stimulus upon neural connections between the remote organ and the heart.

However, these observations predated the finding that a key component of RIC is the release of cardioprotective substances into the blood, the plasma and plasma dialysate from animals and humans subjected to transient limb ischaemia being highly cardioprotective when used to perfuse naïve hearts subjected to prolonged ischaemia, or when used to pre-treat isolated cardiomyocytes subjected to simulated IRI.[100, 101] While the identification of the substance (or substances) released by RIC remain to be determined completely, there is

little doubt that their release is dependent upon intact neural pathways to the triggering organ. For example, the aforementioned abrogation of RIC by femoral nerve transection prior to limb RIC was associated with failure to release humoral factor(s), the plasma dialysate from such animals having no cardioprotective activity when tested in Langendorff preparation.[102] The testing of 'cardioprotectivity' of plasma in this way has proven to be a useful biomarker for dissecting the neurohumoral pathways potentially involved in other conditioning stimuli.[103] It is perhaps unsurprising, given the earlier discussion, that direct stimulation of the femoral nerve leads to release of humoral factor(s) and recapitulates the cardioprotectivity associated with transient limb ischaemia.[104] However other, less direct, neural stimuli appear also to invoke this neurohumoral response. For example, it has long been known that local IPC of the heart and other organs involves the stimulation of capsaicin-sensitive sensory nerves (C-sensory fibres),[105] and more recently both surgical incision (presumably via stimulation of sensory fibres) and direct stimulation of sensory nerves in the skin (using topical capsaicin) was shown to induce potent 'remote' cardioprotection.[106] In subsequent studies, topical capsaicin[88, 104] and stimulation of sensory nerves via transcutaneous nerve stimulation[107] were both shown to release cardioprotective humoral factor(s) into the blood. Interestingly, this humoral response was abolished by pre-treatment with topical DMSO (a sensory nerve blocker) and intra-arterial injection of the nitric oxide (NO) donor SNAP, presumably via the neuro-inhibitory effects of NO on unmyelinated sensory nerves. Similarly, although conceivably working via other signalling pathways, the 'preconditioning' effect of targeted electro-acupuncture was associated with release of humoral factor(s) and can provide equally potent cardioprotection to that of RIC induced by transient limb ischaemia in experimental animals.[108] Interestingly, electro-acupuncture has been shown to be cardioprotective in the clinical setting, where it reduces peri-operative myocardial injury in patients undergoing cardiac surgery.[109, 110]

Activation of the somatosensory system, the spinal cord, and the autonomic nervous system have been shown to mediate the release of yet unidentified humoral factor(s) that elicit the response in the target organ in the setting of RIC. The sensory afferent nerve

appears to be the pivotal communication from the conditioned limb or organ as release of the humoral mediator following RIC depends on an intact sensory pathway.[102, 111, 112] The stimulus may not only originate from local IRI in the conditioned organ or limb, but may also be initiated by local surgical trauma, which appears to recruit similar signaling pathways within the heart as RIC.[106, 112, 113]

Spinal cord involvement in RIC has been supported by loss of RIC cardioprotection with spinal cord transection at T7-T10, or intrathecal spinal opioid receptor blockade with naloxone, and MI size reduction can be recapitulated via spinal cord stimulation at C8-T2.[114, 115] It appears that cardiac sympathetic nerves are involved in the observed MI size reduction upon spinal cord stimulation, and this cardioprotective effect is attenuated by the α1-blocker, prazosin, and the ß-blocker, timolol.[115] It is well-established that systemic administration of morphine is cardioprotective but it has recently shown that lower doses of morphine can be administered intrathecally into the cerebrospinal fluid to induce cardioprotection.[116-118] This protective effect appears to be mediated by spinal μ-opioid receptors and signals through the spinal NOS-NO-cGMP pathway.[117, 118]

The efferent cardioprotective efficacy of the humoral mediator on the myocardium is dependent on functioning intrinsic neural loops and recruitment of intrinsic cardiac ganglia, which regulate cardiac neural activity in the absence of any extra-cardiac neural input. Transmission via intrinsic cardiac ganglia is dependent on acetylcholine release to activate nicotinic acetylcholine receptors (nAchR) on the post-ganglionic nerve and initiate a nerve impulse. The ganglionic blocker, hexamethonium, which prevents transmission of information at the ganglia by antagonising nAchR, abrogates protection by local bradykinin administration or RIC in most,[97, 106, 119] but not all studies[120]. Another ganglionic blocker, trimetaphan, also abrogates the protection by RIC from ischaemia-reperfusion induced endothelial dysfunction in humans.[121]

Further studies are clearly required, but the potential role of direct or indirect neural stimulation as a cardioprotective strategy is compelling, and further understanding of the

neural component of the neuro-humoral pathways of RIC may be important in understanding the vagaries of response when RIC is used clinically.

4.2. Vagal nerve stimulation and cardioprotection

The role of vagal stimulation, either as part of a remote stimulus or via direct stimulation is an emerging area of interest in the field of cardioprotection. Electrical stimulation of the vagal nerve is cardioprotective. Vagal stimulation reduced MI size in an *in vivo* rat AMI model when performed either prior to ischemia or a the onset of reperfusion, with preconditioning vagal stimulation activating the Akt/GSK-3β muscarinic pathway, whereas postconditioning vagal stimulation activated α7-nicotinic acetylcholine receptors and JAK2, independently of the cholinergic anti-inflammatory pathway[122]. In a comprehensive study Donato and colleagues[123] demonstrated the involvement of these neural pathways in RIC induced cardioprotection via limb ischaemia. The need for afferent innervation to the limb was confirmed, since cardioprotection was abrogated in animals undergoing femoral and sciatic nerve transection. More importantly, prior transection of the spinal cord, or the left and right vagus nerves at the midcervical level, or pretreatment with atropine, also abolished the cardioprotective effect of remote preconditioning by transient limb ischaemia. Mastitskaya and colleagues further dissected the role of vagal innervation in experiments using highly selective sectioning of different branches of the vagus nerve.[124, 125] The authors concluded that the posterior gastric branch of the vagus alone was pivotal in signal transduction of the preconditioning stimulus from limb to heart. Although they did not prepare plasma dialysate for confirmation of a coincident humoral signal, Mastitskaya concluded that their results suggest “that the circulating factor (or factors) of RPc are produced and released into the systemic circulation by the visceral organ(s) innervated by the posterior gastric branch of the vagus nerve”. In a different study that vagal stimulation induced the release of glucagon like peptide 1 (GLP1),[126] and GLP-1 signaling has been shown to limit MI size in isolated hearts and intact pigs[127], as well as in proof-of-concept clinical trials.[128] Although the signal

transduction involved is not clear, there is increasing evidence that GLP-1 signaling induces a metabolic shift towards glycolysis in cardiomyocytes which is independent of insulin.[129, 130]

Interestingly, it has recently been shown in pigs and rats that the vago-splenic axis is required for RIC cardioprotection.[131] Splenic denervation or splenectomy abolished protection and muscarinergic stimulation of an isolated perfused spleen released a substance which reduced infarct size in an isolated perfused heart, indicating that the integrity of the vago-splenic axis is essential for RIC cardioprotection. However, the nature of the spleen-derived cardioprotective substance was not identified. Also, the role of the vago-splenic axis in the more clinically relevant remote ischaemic perconditioning or postconditioning, was not investigated, but this limitation applies also to all other of the above studies. Although the underlying mechanisms are not known, it is proposed that the spleen acts as a source of neuroprotective,[132] and cardioprotective substances.[131] Most recently, acute cardioprotection via vagal nerve stimulation has been tested in the clinical setting of AMI with the demonstration that transcutaneous vagal activation by low-level electrical stimulation at the right tragus reducing MI size.[47, 133]

Chronic neuropathic pain impacts on the susceptibility to acute myocardial IRI,[134] and MI size was reduced in a murine model of chronic neuropathic pain. This cardioprotective effect could be recapitulated via activation of anterior nucleus of paraventricular thalamus (PVA)-dependent parasympathetic pathway, as evidenced by the fact that pharmacological inhibition of Erk activation in the PVA abolished neuropathic pain-induced cardioprotection, whereas activation of PVA neurons pharmacologically, or by optogenetic stimulation, induced cardioprotection.

4.3 Anesthesia and cardioprotection by neural stimulation

Any anesthesia impacts on the autonomic nervous system and its balance. Of particular concern with respect to cardioprotection is the use of pentobarbital anaesthesia in experimental studies since pentobarbital augments sympathetic activity and its impact on ischaemic/reperfused myocardium. Accordingly, sympathetic denervation augments

ischaemic myocardial blood flow and reduces MI size in pentobarbital-anaesthetised dogs,[135, 136] and this effect is not seen in conscious, chronically instrumented dogs.[62] Of even greater concern is the use of propofol in exerimental and clinical studies on cardioprotection.[137] Propofol interferes with *γ*-aminobutyrate-mediated central nervous control of cardiac vagal nerves.[138, 139] Propofol, in contrast to volatile anesthesia, interferes with the cardioprotection by RIC in rats[140] and in patients undergoing cardiovascular surgery,[141-143] and this interference may have accounted for the apparent lack of cardioprotection in two large randomised clinical trials.[144-146]

4.4. Diabetic neuropathy as modulator of cardioprotection by RIC

The efficacy of IPC is decreased in animal and human models of diabetes mellitus, [147-153] while the responses to RIC in humans with diabetes have been varying.[111, 154, 155] Depending on the presence of peripheral neuropathy, dialysed plasma from diabetic patients subjected to RIC has revealed differential responses. Plasma from diabetic patients without neuropathy was cardioprotective in naïve recipient rabbit hearts, while plasma from patients with peripheral neuropathy failed to provide cardioprotective plasma.[111] The findings confirm the interaction between the neural and humoral components of RIC and that release of the humoral mediator following RIC is dependent on an intact sensory innervation in the conditioned limb.[99]

As described above, the vagal nerve is an essential neural mediator for limb RIC cardioprotection and facilitates the release of the blood-borne mediator.[101, 123, 124] However, studies exploring the impact of autonomic neuropathy upon the efficacy of RIC in a human context have not been identified. Despite deprivation of extra-cardiac innervation, experimental studies using isolated hearts have demonstrated consistent attenuation of the efficacy of RIC by diabetes. Acute myocardial IRI appears to be dependent on diabetes duration, but the efficacy of RIC is not.[156-158] Moreover, the majority of studies have been conducted in young experimental animals with a low likelihood of diabetic complications. Although extra-cardiac autonomic neuropathy may be involved, it does not seem to be a

leading mechanism behind the impaired response to RIC in diabetic individuals.

Degenerative changes and reduced numbers of nerve fibers and intracardiac ganglia have been demonstrated in patients and animal models of type 1 and 2 diabetes,[159-161] and the density of cholinergic nerves may be changed in diabetic rats.[162] The functional impact of disarrays in intrinsic neural cardiac loops and cardiac ganglia, which regulate cardiac neural activity and intracellular signaling pathways involved in cytoprotection, and interference with RIC is currently unknown.

5. Clinical implications and future perspectives

Limb RIC appears to the most promising strategy for limiting MI size in patients with AMI.[163] There is compelling evidence that neural stimulation is a key element in triggering and coordinating RIC cardioprotection, but the contribution of the neural network is complex and depends on the type of RIC intervention (pre, per, postconditioning), the animal species and other factors, and that may be additive or redundant. Elucidating the exact role of the different neuronal pathways involved in each situation appears as an essential step to bring the maximal benefit of RIC strategies to patients. It should help optimise RIC protocols for different clinical contexts, as the type of ischaemic insult to the myocardium, age, sex, comorbidities and co-medications, as well as to identify situations of resistance to RIC strategies and opportunities for combination therapies. It should also help to develop new treatments that could reproduce the cardioprotection afforded by remote ischaemia with pharmacological or physical methods or combinations of both. Among these new treatments, different modalities of direct nerve stimulation and neuromodulation appear as a promising, safe and effective strategy. In this regard transcutaneous vagal nerve stimulation has been shown to reduce MI size in AMI patients,[47] and electro-acupuncture has been reported to reduce peri-operative myocardial injury patients undergoing cardiac surgery.[109, 110] Finally, limb RIC appears to be the most promising strategy for limiting MI size in patients with AMI,[163] and whether it can improve clinical outcomes is being tested in the CONDI2/ERIC-PPCI trial,[164] which reports its result in Summer 2019.

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Figure 1: Hierarchy of cardiac innervation to the heart

This figure shows the complex and hierarchal interactions between the different components of the neural pathways of the central nervous system (CNS), intrathoracic extracardiac ganglia, and intrinsic cardiac ganglia of the intrinsic cardiac nervous system.
This figure has been modified from [1] with permission.

Figure 2: Cardiac innervation and cardioprotection

The heart is innervated by the cardiac sympathetic and parasympathetic afferent and efferent neural pathways which interact with intrinsic cardiac nerves within the heart to modulate myocardial function, susceptibility to acute IRI, and cardiac arrhythmias. Cardioprotection induced by endogenous strategies such as ischaemic preconditioning (IPC) and remote ischaemic conditioning (RIC) can modulate the intrinsic cardiac nerves and peripheral sensory afferent nerves in the limb and the vagus nerve, respectively. IPC cardioprotection in the isolated perfused has shown to be dependent on the function of intrinsic cardiac nerves within the heart. RIC which comprises brief non-lethal cycles of ischaemia and reperfusion to the limb, via cuff inflation/deflation, causes local autacoid release. This in turn activates sensory afferent neurons which relay, via the spinal cord, to the dorsal nucleus of vagal nerve (DMVN) in the CNS. Activation of nuclei within the DMVN results in increased vagal nerve firing to the heart which, via release of acetylcholine (Ach) and subsequent activation of muscarinic Ach receptors induces the cardioprotective phenotype. In addition, following activation of afferent sensory neurons in the conditioned limb, there is release of a dialysable cardioprotective factor into the systemic circulation. The source of this factor remains unknown, although possibilities include i) from the conditioned limb itself ii) from the central nervous system iii) from pre-/post-ganglionic parasympathetic nerve endings within the heart iv) from a non-conditioned remote organ/tissue such as the gut or spleen. Neural stimulation of sensory afferent nerves (by RIC, transcutaneous nerve stimulation [TENS], trauma, electro-acupuncture [EA] or topical capsaicin) or of the vagus nerve can induce cardioprotection. This figure has been modified from [92].