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SYMPOSIUM REVIEW

Myths and realities of the cardiac vagus

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Abstract There is continuing belief that cardiac parasympathetic postganglionic fibres are sparse or absent from the ventricles. This review of the literature shows that the supposition is a myth. Early studies considered that fine silver-stained fibres coursing amongst ventricle myocardial cells were most likely cardiac parasympathetic postganglionic fibres. The conclusions were later supported by acetyl cholinesterase staining using a method that appeared not to be associated with noradrenaline nerve fibres. The conclusion is critically examined in the light of several recent histological studies using the acetyl cholinesterase method and also a more definitive technique (CHAT), that suggest a widespread location of parasympathetic ganglia and a relatively dense parasympathetic innervation of ventricular muscle in a range of mammals including man. The many studies demonstrating acetylcholine release in the ventricle on vagal nerve stimulation and a high density of acetylcholine M_2 receptors is in accord with this as are tests of ventricular performance from many physiological studies. Selective control of cardiac functions by anatomically segregated parasympathetic ganglia is discussed. It is argued that the influence of vagal stimulation on ventricular myocardial action potential refractory period, duration, force and rhythm is evidence that vagal fibres have close apposition to myocardial fibres. This is supported by clear evidence of accentuated antagonism between sympathetic activity and vagal activity in the ventricle and also by direct effects of vagal activity independent of sympathetic activity. The idea of differential control of atrial and ventricular physiology by vagal C and vagal B preganglionic fibres is examined as well as differences in chemical phenotypes and their function. The latter is reflected in medullary and supramedullary control. Reference is made to the importance of this knowledge to understanding the normal physiology of cardiac autonomic control and significance to pathology.

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Introduction

There is a consensus repeated in most medical text books that cardiac sympathetic nerves innervate the sino-atrial (SA) and atrioventricular (AV) nodes, the atria, the ventricles and conducting tissue, whereas the idea that parasympathetic nerve supply is mainly limited to the atria and nodal tissues is perpetuated. This has occurred in spite of many anatomical and physiological studies showing the presence of cardiac vagus nerves coursing throughout

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the ventricles as well as having a profound influence on ventricular rate and rhythm, and affecting contractility. Recent research accounts have drawn attention to the misconception (Crick *et al.* 1999; Dhein *et al.* 2001; Harvey & Belevych, 2003; Kawano *et al.* 2003; Hoover *et al.* 2004; Ulphani *et al.* 2010). Additionally, recent research of cellular and molecular events in ventricle muscle provide substantial knowledge of vagal cholinergic muscarinic regulation of cardiac ventricular function (Casadei, 2001) that further emphasises the importance of vagal innervation of the heart. Despite these contributions, the understanding of fundamental mechanisms of parasympathetic regulation of the heart lags behind our knowledge of sympathetic control. The present review attempts to refocus our sight onto vagal parasympathetic influence on the heart and particularly the ventricles.

Anatomical evidence

Do sympathetic efferents in the cervical vagus nerve project to the heart?

It is first important for me to deal with an oft-repeated supposition regarding the efferent fibre composition of the cervical vagus nerves. In contrast to common belief there are no cardiac sympathetic efferent nerves projecting to the heart via the nodose ganglion in these nerve bundles, at least in the rabbit, cat and dog (see Evans & Murray, 1954). This was clearly shown by Heinbecker & O'Leary (1933*a*,*b*) who stimulated the cervical vagus before and after allowing 10–20 days for vagal preganglionic efferent fibres to degenerate following extracranial supranodose ganglion vagotomy. This procedure left sympathetic postganglionic efferents undamaged. Significantly it abolished all cardiac effects on stimulating the cervical vagus. Early studies also showed that those sympathetic postganglionic fibres reported to pass from the cervical sympathetic nerve via the nodose ganglion were destined for the bronchial circulation and pulmonary blood vessels (McSwiney & Spurrel, 1933; Richardson & Hinsey, 1933). Confirmation came in two later studies using similar procedures (Daly & Hebb, 1952; Daly & Evans, 1953). The conjecture of a cardiac vago-sympathetic fibre composition in the cervical nerve trunk may have arisen because stimulation of the cervical vagus lower in the neck may excite cardiac sympathetic branches from the stellate ganglion (Randall & Armour, 1977). This may be the explanation for the recent report on two dogs that displayed a sympathetic tachycardia during stimulation of cervical vagus nerve via an implanted electrode (Onkka *et al.* 2013).

Therefore, providing stimulating electrodes are positioned on the rostral part of the vagus nerve nearer to the nodose ganglion one can be confident that cardiac sympathetic postganglionic nerves are not activated together with vagal preganglionic fibres.

Vagal nerve supply to the chambers of the heart

It is universally accepted that parasympathetic nerves densely innervate the atria, SA and AV nodes and conducting tissue. This has been extensively described in several reviews (Randall, 1984; Levy & Martin, 1996). Accordingly it is agreed that activity in vagal fibres to the heart can cause cardiac slowing via decreased excitability at the SA and AV nodes, and a decrease in force of atrial contraction and in rate of atrioventricular conduction. However, there is now substantial evidence for a vagal nerve influence on ventricular rhythm and contractility. These atrial and ventricular effects are dependent upon excitation of ganglion cells located in discrete clusters on the epicardium of the atria and in atrial and ventricular septum (Singh *et al.* 1996; Gatti *et al.* 1997; Dickerson *et al.* 1998). There is evidence that each cluster of postganglionic neurones targets different regions of the heart and selectively controls cardiac muscle action not only in adjacent regions but also in all four chambers of the heart (Yuan *et al.* 1994; Armour, 2008). This is described as a topographical functional organisation of the vagal cardiac ganglia (Randall *et al.* 1987; Billman *et al.* 1989; Sampaio *et al.* 2003). However, it had been generally thought that parasympathetic postganglionic fibres in the ventricles are sparse and effects on the ventricular muscle are minimal and highly dependent on interaction with sympathetic activity. This idea is critically examined in the following sections.

Evidence for vagal innervation of the cardiac ventricles

Historically, anatomical studies (Cullis & Tribe, 1913; Nonidez, 1939, 1943) have generally been considered to indicate an absence of direct parasympathetic innervation of the ventricles. A more careful examination shows that even Nonidez (1939), in his observations on autonomically decentralised hearts, interpreted the fine heavily silver-impregnated fibres coursing amongst ventricle muscle fibres to be postganglionic fibres of the vagus nerve. Using similar methods a more substantial parasympathetic innervation was demonstrated by Tcheng (1951) and Davis *et al.* (1952). Confirmation was forthcoming from subsequent studies in dog, cat and human hearts (Cooper, 1965; Jacobowitz *et al.* 1967) in which the thiocholine method developed by Karnovsky & Roots (1964) was used to identify acetylcholinesterase (AChE) the enzyme responsible for hydrolysing acetylcholine (ACh). These data were interpreted as showing a relatively rich parasympathetic postganglionic

innervation distributed throughout the ventricles albeit less than in the atria. Not surprisingly, identification of cholinergic nerve fibres by AChE staining was questioned since other types of nerves have been observed to express the cholinergic enzyme. To get round this problem, Kent *et al.* (1974) compared the increase in ventricular fibrillation threshold caused by cervical vagus stimulation with effects of directly stimulating the cardiac ganglia. This was done before and after selectively destroying the postganglionic neurones in the heart ganglia with the neurotoxin vinblastine. Post-lesion there was an absence of AChE in the ventricles, and vagus nerve stimulation no longer increased fibrillation threshold. In a further experiment adrenergic denervation with 6-hydroxydopamine (6OHDA) did not affect the presence of AChE or the action of vagus nerve stimulation. Therefore, Kent *et al.* (1974) concluded that AChE was abundant in cholinergic neurones but sparse or absent in adrenergic or sensory neurones in the heart. A similar conclusion was reached by Bermani *et al.* (1982) who also used the thiocholine method to examine innervation of bronchi in rabbits. They showed that AChE-positive axons lacked catecholamine fluorescence. More recently several studies have refined the thiocholine method and interpreted the AChE staining as showing the cardiac cholinergic innervation to be widespread across all chambers of the heart. It is similar in mouse (Rysevaite *et al.* 2011), guinea pig (Batulevicius *et al.* 2005), rat (Zang *et al.* 2005), cat (Johnson *et al.* 2004), dog (Blomquist *et al.* 1987; Pauza *et al.* 2002), pig (Crick *et al.* 1999; Ulphani *et al.* 2010), sheep (Saburkina *et al.* 2010) and human (Kent *et al.* 1974; Pauza *et al.* 2000; Kawano *et al.* 2003) and in bats (O'Shea & Evans, 1985). These authors concluded that cholinergic nerves richly innervate the epicardial and endocardial surfaces of the ventricles as well as of the atria.

Although the evidence strongly favoured the interpretation that histochemical identification of AChE in the heart is a marker of cholinergic fibres the interpretation needed to be confirmed with a more reliable marker. This has now been achieved in studies in rat and mouse that have identified cholinergic fibres with choline acetyltransferase (CHAT) immunohistochemistry (Yasahura *et al.* 2007; Rysevaite *et al.* 2011; Pauza *et al.* 2013). This method convincingly identifies only ACh fibres, since CHAT is the enzyme that catalyses the final step in the synthesis of ACh. In general the pattern of cholinergic parasympathetic postganglionic innervation of the heart conducting system and heart chambers, including the ventricles, was confirmed in these studies.

In summary, present knowledge is that the cardiac vagal preganglionic nerves pass close to the cardiopulmonary vessels and superior vena cava (Kaye *et al.* 1970) to synapse on ganglion cells located in several epicardial fat pads on the dorsum of the atria close to sites of entry of the major veins and surface of the ventricles as well as the interventricular groove and the interatrial and interventricular septa (Ardell & Randall, 1986; Randall *et al.* 1986*a*,*b*; Gatti *et al.* 1995, 1997). These are now considered the principal sites of preganglionic termination. The number of these principal parasympathetic ganglia varies across species, four identified in the rat, eight in the cat and ten in human hearts (Pardini *et al.* 1987; Singh *et al.* 1996). Parasympathetic postganglionic axons project from these ganglia to selective regions of the atria and cardiac conduction system and ventricles. With regard to both right and left ventricle innervation there are several supraventricular ganglia from which many parasympathetic postganglionic axons project across the atrioventricular groove (Priola *et al.* 1977; Blomquist *et al.* 1987). Presumably these are the AChE-stained fibres observed on the epicardium in recent studies (Ulphani *et al.* 2010; Taggart *et al.* 2011). There is also an important parasympathetic postganglionic innervation, shown in the dog, that projects from the interventricular septum to supply the right ventricle to decrease its contractility and another that projects from a ganglion near the cranial margin of the left ventricle that participates in the decrease in left ventricle contractility produced by cervical vagus nerve stimulation (Gatti *et al.* 1997; Dickerson *et al.* 1998). The anatomical data are summarised schematically in Fig. 1.

These data describing a significant parasympathetic postganglionic innervation of the ventricles are supported by numerous observations of the distribution of muscarinic receptors in both right and left ventricles (Fields *et al.* 1978; Yamada *et al.* 1980; Syrota *et al.* 1985; Hancock *et al.* 1987; Deighton *et al.* 1990; Yang *et al.* 1992; Dunlap *et al.* 2002; Mittmann *et al.* 2003) as well as a high density of chemically identified AChE (Crick *et al.* 1999; Dunlap *et al.* 2002; Gill *et al.* 2003; Zang *et al.* 2005).

There is also convincing evidence that ACh, detected by microdialysis, is released from various postganglionic sites in the left ventricular myocardium of mice and cats following vagal stimulation (Akiyama *et al.* 1994; Akiyama & Yamazaki, 2001; Shimizu *et al.* 2009). These studies showed that the concentration of released ACh was positively correlated with the frequency of stimulation of the cervical vagus nerve and with decreases in heart rate. Furthermore, ganglion blockade with hexamethonium prevented the effect of vagal stimulation on ACh release (Akiyama *et al.* 1994; Akiyama & Yamazaki, 2001; Shimizu *et al.* 2009). It is worth noting that the findings of a higher density of cholinergic fibres and of acetycholine in the atria may in part be due to the location of both pre- and postganglionic neurones in this region.

Functional evidence of vagal control of the cardiac ventricles

Supporting the anatomical and pharmacological data are several lines of physiological evidence showing that parasympathetic postganglionic nerve fibres must come into close apposition to ventricular myocardial fibres.

Eliakim *et al.* (1961) showed, in dogs with complete experimentally induced bundle branch block, that vagal stimulation caused a small but significant decrease in the rate of ventricular contraction that was blocked by atropine and mimicked by ACh. In subsequent studies by others, measurements of the monophasic action potential from surface electrodes on the left ventricle myocardium have shown that the duration and effective refractory period is lengthened during vagus nerve stimulation (Martins & Zipes, 1980*a*; Ng *et al.* 2001; Brack *et al.* 2007, 2011). A significant message that also follows from these and numerous other studies is that the parasympathetic postganglionic nerve fibres must innervate the cardiac ventricles (Hirsch, 1971; Martins & Zipes, 1980*b*, 1983; Itto & Zipes, 1994).

There are three ways in which the terminal fibres of the vagus can influence the myocardium: one is dependent on interaction with the sympathetic nerves, another is an independent direct action on the myocardium, and a third is via interaction with neurones of the intrinsic cardiac plexuses.

Dependent vagal effects

Sympathetic-dependent vagal effects on the myocardium. It is clear that in intact anaesthetised animals increased vagal activity has a significant negative inotropic effect on the ventricle of rat (Nalivaiko *et al.* 2009), rabbit (Brack *et al.* 2006), cat (Gatti *et al.* 1997), dog (De Geest *et al.* 1964), pig and human (Lewis *et al.* 2001). This is most marked in the presence of sympathetic tone where a muscarinic receptor activation by parasympathetic nerve-released ACh suppresses the action of sympathetic nerves on the myocardium (Henning & Levy, 1991; Levy & Schwartz, 2004; Brack *et al.* 2006). Much evidence suggests that this action is partly a pre-junctional effect whereby acetylcholine released from parasympathetic postganglionic terminal varicosities reduces the amount of catecholamine released from sympathetic nerves (Levy, 1977), an action which has been studied in detail in atria (Paterson, 2001; Dawson *et al.* 2008).

The sympatho-vagal interaction may also be post-junctional and due to the cholinergic intracellular elevation of cGMP that then inhibits cAMP so reducing the sympathetic adrenergic-activated hyperpolarization activated slow depolarizing (I_f current) and the L-type Ca^{2+} current in the myocyte. The interaction results in a decrease in contractility. The experimental observations of these phenomena, particularly by Levy & Zieske (1969) led to the formulation of the concept of accentuated

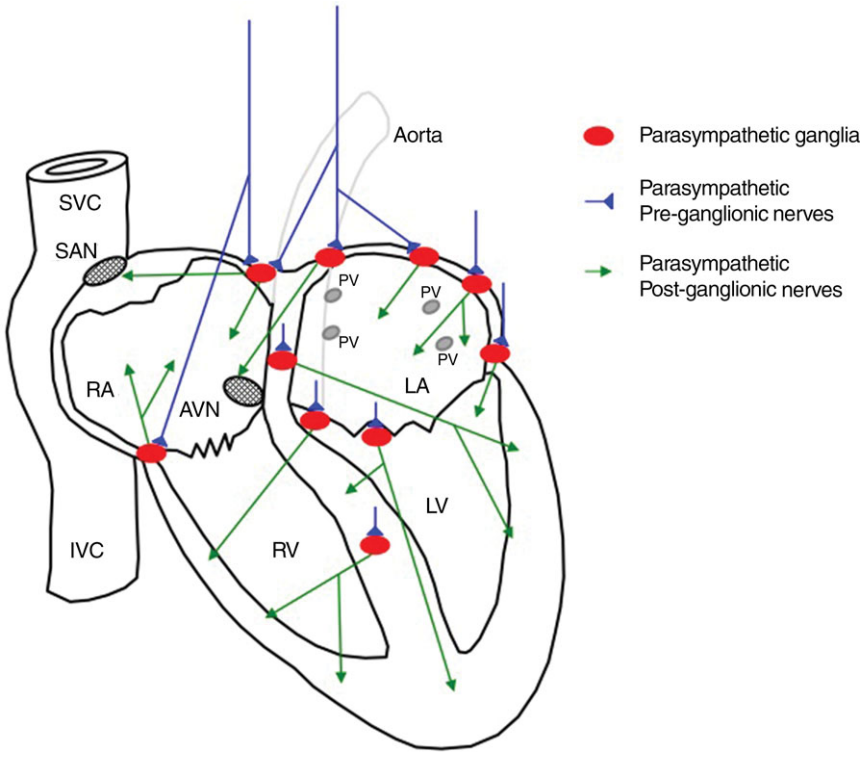

Figure 1. Summary of the parasympathetic innervation of the heart that is discussed in the text
Schematic diagram of an anterior view of the human heart with the two vagal cardiac preganglionic nerves in blue that project to synapse in parasympathetic ganglia (red) that are distributed at several sites on the epicardium and within the atrial and ventricular septa. The diagram does not indicate the extent of the distribution of the right and left vagal nerves to either side of the heart. The parasympathetic postganglionic neurones send axons (green) to different regions of the heart including the sinoatrial node (SAN) and atrioventricular node (AVN) and all cardiac chambers including the ventricles and conducting tissue. The anatomical location is diagrammatic. The anterior portion of the heart has been excluded in the cut-away diagram. The major blood vessels, including the superior vena cava (SVC), inferior vena cava (IVC), the entry of the pulmonary veins (PV) and aorta are indicated (diagram by Kieran Brack based on original drawing by John Coote).

antagonism (Levy, 1977; Levy & Schwartz, 2004). The postsynaptic mechanism for adrenergic–cholinergic interaction also has a firm molecular basis. Paterson's group using a guinea pig atrial preparation has shown that vago-sympathetic nerve interaction depends on the following events. Depolarisation at the parasympathetic nerve endings leads to the formation of nitric oxide (NO) derived from L-arginine by the activation of nitric oxide synthase. The NO facilitates parasympathetic nerve release of acetylcholine (Herring *et al.* 2000; Herring & Paterson, 2001; Paterson, 2001; Mohan *et al.* 2002) and inhibits sympathetic transmission (Paton *et al.* 2002; Li *et al.* 2007) via cGMP- and cAMP-dependent modulation of neuronal calcium levels, and this in turn regulates exocytosis (Wang *et al.* 2007).

It is likely that similar molecular events are also involved in the vago-sympathetic interaction in the ventricles (Levy & Schwartz, 2004).

The description now needs to be further extended in the light of the discovery of numerous local factors such as peptides released from myocardial cells, vasculature and intrinsic neurones, that can alter the release of chemical transmitters of parasympathetic postganglionic or sympathetic nerves (Herring & Paterson, 2009).

In conclusion functional studies of cardiac sympatho-vagal balance provide clear robust and abundant evidence for a parasympathetic postganglionic nerve presence in the ventricles that interacts with sympathetic nerve terminals in all species so far studied.

Independent vagal effects

Sympathetic-independent vagal effects on the myocardium. Many studies have shown that stimulation of the vagus nerves to the heart can decrease heart rate and decrease atrio-ventricular conduction independent of sympathetic nerve activity (e.g. Conlon *et al.* 1996). This action is facilitated by neuronal nitric oxide (Conlon & Kidd, 1999).

There is also robust evidence that parasympathetic postganglionic nerve fibres have direct effects on the ventricular myocardial cells acting independently of sympathetic activity. Vagal stimulation is capable of significantly lengthening the effective refractory period and the duration of the monophasic action potential recorded from the surface of the ventricle, in the isolated innervated paced rabbit heart where there is an absence of sympathetic tone (Ng *et al.* 2001; Brack *et al.* 2007, 2011). Vagal stimulation has also been shown to directly depress the force of ventricular contraction in the absence of sympathetic tone. This is in contrast to several early studies that reported very small or no inotropic effects of vagus nerve stimulation on the ventricles (Sarnoff *et al.* 1960; Harman & Reeves, 1968; Higgins *et al.* 1973). On the contrary, Gatti *et al.* (1997) have demonstrated that cervical vagus nerve stimulation can reduce left ventricle contractility by around 20% in anaesthetised cats in which the heart is paced and the sympathetic influence prevented by β adrenoreceptor blockade. Similarly, Nakano *et al.* (1998) measured changes in the right heart and showed that cervical vagus stimulation decreased dP/dt of right atrium and right ventricle in autonomically decentralised paced hearts of anaesthetised dogs. This seemingly direct action was given a more convincing basis by a study also in dogs (Xenopoulos & Applegate, 1994). The authors measured the changes in the end-systolic pressure–volume relationship in the left ventricle obtained from pressure–volume loops to give a load-insensitive measure of ventricular contractile performance. Vagal stimulation caused a negative inotropic effect that was still present, although reduced, after sympathetic denervation and β adrenoreceptor blockade. Similar results have been described in pig and humans (Lewis *et al.* 2001) and in the working heart–brainstem preparation of rat (Nalivaiko *et al.* 2009). There are other studies reporting small but significant vagally induced negative inotropy of the ventricles in rabbit when sympathetic tone has been pharmacologically blocked (Garcia-Perez & Jordan, 2001). However, Brack *et al.* (2006) could not confirm this in their isolated innervated rabbit heart preparation. The different results from the two laboratories could be due to incomplete β adrenergic blockade in the study by Garcia-Perez & Jordan (2001). However, it may be worth noting that there is an interesting and possibly important difference in the two studies. In the Garcia-Perez & Jordan (2001) experiments the effect was a characteristic of unmyelinated vagal preganglionic efferents that were stimulated in the absence of larger vagal fibres, these larger fibres having been blocked by anodal current. Different actions of large and small vagal efferents have also been described in the rabbit (Ford & McWilliam, 1986; Woolley *et al.* 1987).

An action via intrinsic neural plexuses. Cardiac vagal preganglionic termination and location of ganglia containing parasympathetic postganglionic neurones is complicated by the presence of intrinsic neurones that form plexuses with numerous connections throughout all cardiac chambers (Randall *et al.* 1996; Pauza *et al.* 2002; Armour, 2008). These systems may interact within the network and also with the parasympathetic pre- and postganglionic nerves, but it is important to understand that the neurones forming the network of connections that are referred to as the intrinsic nerve plexuses are not the same as the parasympathetic postganglionic neurones (Randall *et al.* 2003) that I have depicted in Fig. 1.

How information is processed by cardiac parasympathetic postganglionic neurones is partly explained by data from intracellular studies of cardiac

ganglion cells recorded in isolated atria *in vitro* (e.g. Edwards *et al.* 1995) and in a ground-breaking *in vitro* preparation in which the heart is functionally connected to the brainstem (McAllen *et al.* 2011) allowing definitive identification of postganglionic vagal neurones in the atrium. These show that the efficiency of synaptic transmission is high but not necessarily 1:1 and the cells are capable of gating high frequency inputs. Thus the cardiac vagal ganglia can determine the level of postganglionic parasympathetic activity transmitted to the heart and do not act as a simple relay. The question of how much influence the intrinsic nerve plexuses have on the efficacy of transmission at the parasympathetic ganglia and on extrinsic autonomic postganglionic terminals is still unclear. However, there is some evidence that the effect of parasympathetic action can be enhanced or depressed by intrinsic nerves (Armour, 2008). A more challenging concept is the extent to which the intrinsic plexuses act independently as a 'Little Brain' as proposed by Randall *et al.* (1996). This remains an important area for further investigation. This is particularly so in view of the scope for both physiological and pathophysiological factors to alter cardiac vagal parasympathetic transmission.

Vagal modulation of ventricular rhythm

Perhaps the most striking and well-documented action of the cardiac vagus nerves is their protective effect on the vulnerability for ventricular fibrillation (Verrier & Lown, 1984; Schwartz, 1996; Brack *et al.* 2012). This is in contrast to the effects of the parasympathetic nerves on the atrium where they reduce the action potential duration and effective refractory period of atrial muscle cells (Liu & Nattel, 1997) hence lowering the threshold for fibrillation. Part of the protective influence on the ventricle appears to be contingent on the level of cardiac sympathetic tone (Kolman *et al.* 1975). Thus in dogs, whilst the protective action was shown to be attenuated by atropine, it was prevented by β adrenergic blockade (Kolman *et al.* 1975; Rabinowitz *et al.* 1976; Verrier & Lown, 1984). Similarly in dogs and cats vagal stimulation prevented ventricular arrhythmias induced by raised cardiac sympathetic activity following coronary artery occlusion (Kent *et al.* 1973; Myers *et al.* 1974; Corr & Gillis, 1974; Zuanetti *et al.* 1987). These conclusions were then tested in a demanding series of studies on conscious animals (reviewed by Schwartz, 1996). In dogs with a healed myocardial infarction, cardiac vulnerability was tested by subjecting them to exhaustive exercise. It was shown that those animals with a higher resting vagal tone as measured by baroreflex heart rate sensitivity (BRS) were less likely to experience ventricular fibrillation and sudden death compared to dogs with low BRS. The data are consistent with the presence of parasympathetic postganglionic nerve fibres in the ventricles but having an *indirect* action. That is, where sympathetic tone is high the protective effect of vagal activity is due, at least in part, to accentuated antagonism involving cholinergic-induced formation of nitric oxide (Paterson, 2001).

There is also convincing evidence supporting a direct and non-cholinergic protective action of vagal nerve fibres on pathological alterations in ventricular rhythm (Brack *et al.* 2012). The most compelling evidence comes from a series of studies over the last 12 years by André Ng and colleagues. These experiments depended on the development of a novel isolated Langendorff rabbit heart preparation in which the parasympathetic and sympathetic nerves are patent but inactive and can be stimulated independently (Ng *et al.* 2001; Brack *et al.* 2004). The studies revealed that the parasympathetic nerves have two separate independent actions in the cardiac ventricle. One is a cholinergic–muscarinic effect on contraction rate and ventricular effective refractory period (Brack *et al.* 2009). The other concerns the protection from fibrillation by increased vagal activity. The latter turned out to be an independent effect of NO on action potential duration restitution. This action was selectively blocked by an neuronal nitric oxide synthase (nNOS) antagonist and unaffected by atropine. Thus it appeared to be mediated via NO release independently of ACh action at M_2 post-junctional receptors (Brack *et al.* 2007, 2009, 2011). This highly novel discovery depended on a series of technically demanding studies, one of which used the selective NO fluorescent indicator technique developed by the group for use in the beating heart (Patel *et al.* 2008). In a parallel study it was confirmed that stimulation of the vagus nerve increased nNOS-dependent NO fluorescence (Brack *et al.* 2009). There are several ways in which this may occur. One is that NO released from cholinergic fibres acts at a different post-junctional site to ACh. Another suggested by the latter authors is that this anti-arrhythmic action of the vagus in the rabbit is exerted by a select population of parasympathetic postganglionic nitrergic nerves. There is anatomical data in support of this speculation (Klimaschewski *et al.* 1992; Tanaka *et al.* 1993; Hoover *et al.* 2009). The possibility of specific vagal nitrergic neuro-junctional transmission in the heart was also alluded to by Rubino *et al.* (1996). Otherwise it has always been assumed that the parasympathetic postganglionic nerve fibres are cholinergic and phenotypically homogeneous.

A further complexity is that diverse chemical phenotypes and receptors are expressed within the intrinsic cardiac plexuses and this expands the possible chemical messengers that might participate in cardiac performance if they interact with postganglionic fibres (Singh *et al.* 1999).

Conceptually, the idea of vagal pathways that are separate to the classical cholinergic ones is not new since it

is established in parasympathetic control of the gastrointestinal tract. Medulla vagal preganglionic neurones innervating the gut have been shown to form two parallel projections: an excitatory one synapsing with cholinergic postganglionic neurones and another inhibitory one synapsing with non-cholinergic postganglionic neurones containing NO (Chang *et al.* 2003). A similar arrangement appears in the sacral parasympathetic innervation. Part of the parasympathetic innervation of the lower urinary tract and vas deferens in the rat has been identified as nitrergic (Persson *et al.* 1997; Ventura *et al.* 1998; Fry *et al.* 2010).

The central regulation of cardiac vagal neurones

The evidence reviewed above supports the notion of specialised functional organisation within the terminal parasympathetic innervation of the heart. This is reflected in the central nervous organisation of the vagal motor nuclei in the medulla and parallel functional pathways of the preganglionic neurones projecting to specific cardiac loci including the ventricles (Randall *et al.* 1986*a*,*b*; Pardini *et al.* 1987; Billman *et al.* 1989; Nakano *et al.* 1998; Dickerson *et al.* 1998; Sampaio *et al.* 2003; Cheng *et al.* 2004).

Preganglionic cardiac vagal neurones

Electrophysiological studies were the first to provide robust evidence for the location within the medulla of vagal cardiac preganglionic neurones (McAllen & Spyer, 1976; Jordan *et al.* 1982). Histological studies with tracer dyes injected either into the heart ganglia (retrograde; Izzo *et al.* 1993; Standish *et al.* 1995; Massari *et al.* 1995, 1998; Ter Horst *et al.* 1996; Hsieh *et al.* 1998; Corbett *et al.* 1999; Jones, 2001; Jordan, 2011) or into the medullary nuclei (anterograde; Cheng *et al.* 1999; Cheng & Powley, 2000; Cheng *et al.* 2004) show that preganglionic cardiac neurones mainly arise from two nuclei in the caudal medulla oblongata. A larger group of about 80% are located in the posterior ventrolateral nucleus ambiguus (NA). These have small myelinated axons (B fibres) that can powerfully slow heart rate, conduction and force of contraction. A second group comprising a significant population (20%) of cardiac vagal neurones originates from the dorsal motor nucleus (DMNV) and a scattering of neurones in an intermediate zone. These have more slowly conducting unmyelinated axons. Selective stimulation of the unmyelinated dorsal motor nucleus axons in the vagus nerve affects atrioventricular conduction and force of ventricular contraction and also reduces heart rate, but it has a slower onset and different pharmacology to that seen following stimulation of the B fibres in the vagus nerve (Jones *et al.* 1995; Garcia-Perez & Jordan, 2001; Jones, 2001). Apart from conduction speed the two groups of neurones have quite different discharge patterns. The NA cardiac neurones display a respiratory rhythm and receive baroreceptor input and chemoreceptor input. The DMNV neurones have a more irregular non-respiratory-dependent discharge (Jones *et al.* 1998) and appear to be unaffected by baroreceptors and chemoreceptors (Ford *et al.* 1990; Jones *et al.* 1998). Cardiac vagal neurones in the intermediate zone have similar characteristics to those in the DMNV (Kong *et al.* 2007). This arrangement has led to speculation that it reflects a differential function of the two groups (Jones *et al.* 1998; Wang *et al.* 2000). The idea is supported by a study showing that DMNV and NA terminals, each anterogradely labelled with different fluorescent tracers, target separate populations of principal neurones in an intrinsic cardiac ganglion, and furthermore DMNV neurones also innervated small intensely fluorescent (SIF) cells in ganglia (Cheng & Powley, 2000; Cheng *et al.* 2004). In contrast Jones (2001) has proposed that the rhythmic respiratory input conveyed by the faster vagal B fibres may interact at the same cardiac ganglia with the tonic input conveyed by the slow C fibres. This would suggest that B and C fibres converge on common postganglionic neurones but at present there is no direct evidence to support this. Another possibility is that some of the C fibres act independently and have a different action on the myocardium. In the rabbit the bradycardic effect of stimulating all the C fibres in the vagus nerve is not blocked by hexamethonium, the cholinergic nicotinic receptor antagonist, unlike its action on the effect of stimulating the B fibre component (Woolley *et al.* 1987; McWilliam & Woolley, 1988; Garcia-Perez & Jordan, 2001). This suggests that the action of C fibre stimulation on heart rate is mediated by a non-cholinergic mechanism. These studies on rabbits also showed that the effects of unmyelinated vagal preganglionic fibres on slowing atrioventicular conduction and decreasing ventricular contactility were also resistant to hexamethonium. The observation of differential actions of cardiac vagal fibres is supported by immunohistochemical characterisation showing that there are at least four chemically distinct types of cardiac vagal preganglionic neurones in the rat (Takanaga *et al.* 2003). Therefore, it appears that different cardiac functions may be regulated by different phenotypes of vagal preganglionic neurones. This has also been alluded to in studies in dog (Dickerson *et al.* 1998), cat (Wang *et al.* 2000) and rabbit (Woolley *et al.* 1987). Consistent with these data are results of experiments with microinjection of glutamate into different regions of the nucleus ambiguus in dogs that show a longitudinal cardiotopic organisation of negative chronotropic and dromotropic vagal preganglionic neurones (Gatti *et al.* 1996; Massari *et al.* 1998). In accord with this, Sampaio *et al.* (2003) showed different cardiac effects on the

stimulation of two quite separate ganglionic plexuses in the heart: one at the junction of the superior vena cava and right atrium and the other at the junction of the pulmonary veins and left atrium. The first plexus elicits a bradycardia whilst stimulation of the second slows atrio-ventricular impulse conduction.

Adding further weight to this idea are experiments in both cat and dog showing two populations of cardiac vagal preganglionic neurones in the medulla, one that was inhibited by lung inflation and one that was unaffected (Daly & Kirkman, 1989).

Supramedullary control

Emotional events or clinically life-threatening brain lesions are associated with adverse electrocardiographic changes (Oppenheimer & Lima, 1998; Oppenheimer, 2006; Cheshire & Saper, 2006; Samuels, 2007). It is thus unsurprising that cardiac ventricular rhythm disturbances have long been known to accompany experimental manipulation of several brain regions above the level of the medulla. In many cases the effects are shown to be due to heightened sympathetic activity induced by regions such as the hypothalamic and midbrain 'defence areas' (Coote, 2004). Of relevance to the present discussion are those regions that have selective cardiac vagal effects.

For example, in the paraventricular nucleus of the hypothalamus oxytocin-type neurones have been shown to project to the dorsal motor nucleus of the vagus and the nucleus ambiguus of the medulla (Sawchenko & Swanson, 1982; Luiten *et al.* 1985) and some of their connections are on vagal cardiac preganglionic neurones. Rogers & Hermann (1986) showed that stimulation of these oxytocin neurones caused a bradycardia and later Darlington *et al.* (1989) demonstrated that this was a cholinergic vagal effect on heart rate, it being blocked by intravenous atropine and hexamethonium. The explicit functional role of this system is unclear.

Of more functionally explicit importance is an island of cerebral cortex lying deep within the fold of the lateral fissure (Sylvian fissure) on each side of the brain and known as the insula. Its posterior region has profound effects on autonomic control of the heart (Cechetto & Saper, 1987). In an ingenious series of experiments, it was shown that stimulation in human and monkey of this part of the right insula increases cardiac sympathetic tone whereas stimulation of the left insula increases cardiac vagal tone (in the rat it was opposite) (Oppenheimer & Cechetto, 1990; Oppenheimer *et al.* 1992; Zhang *et al.* 1998). The influence of the insula cortices is of considerable clinical importance since cerebrovascular events such as stroke and others like epilepsy are often accompanied by cardiac arrhythmias. The arrhythmias appear to occur more frequently after left insula infarction suggesting sympatho-vagal balance has been impaired. Also the long-term cardiac stability for stroke patients is improved if the vascular event mainly involves the right insula (Cheshire & Saper, 2006; Oppenheimer, 2006).

Conclusion

In conclusion the myth that vagal fibres are sparse in the cardiac ventricles and only have weak actions on their physiology is incorrect. This account highlights the importance of cardiac vagal innervation to the normal functioning of the ventricles as well as to the atria (Fig. 1). There are many gaps still to be filled. There is no full description of the terminal innervation and neurochemistry of the parasympathetic postganglionic nerves in the atrial and ventricular myocardium. It is also unclear to what extent activity of the intrinsic plexuses influences the efficacy of extrinsic autonomic neurones. The normal functioning of a cardiotopic arrangement of parasympathetic pre- and postganglionic neurones is also a puzzle. Also much has still to be learned regarding the non-cholinergic mechanisms and molecular pathways targeted by multiple co-transmitters. Meanwhile present knowledge needs to be more explicitly presented in textbooks and lectures for the benefit of physicians and scientists alike. A sound and full knowledge of the anatomy and physiology of the parasympathetic nerves supplying the heart is essential if we are to fully understand heart dysfunction and in particular how brain damage or disease can have serious adverse cardiac effects (Taggart *et al.* 2011).

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Additional information

Competing interests

None declared.