Progress in the study of vagal control of cardiac ventricles

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Abstract: Autonomic nervous system plays an important role in the regulation of mammalian heart, and it is divided into the sympathetic and parasympathetic (vagal) subsystems. The parasympathetic (vagal) control of the atria involves modulation of chronotropic, dromotropic and inotropic activities, but the role of the parasympathetic innervation of the ventricles is still unclear. There is a common misconception that the sympathetic nerves in nerate all over the heart; while the parasympathetic nerves only innervate the superventricular part of the heart, but not the ventricles. Recent evidence indicates that the cholinergic innervation of the left ventricle is functionally very important in some mammalian species. The present article reviews the evidence of vagal control in the ventricles from the anatomy and histochemistry, molecular biology, and function areas. Additionally we overview the vagal (muscarinic) regulation of cardiac contractile function and its signal transduction.

Key words: vagus nerves; muscarinic receptor; ventricle; inotropism; signal transduction

Introduction
Autonomic nervous system plays an important role in the regulation of mammalian heart, and it is divided into the sympathetic and parasympathetic (vagal) subsystems[1,2]. The sympathetic and partial parasympathetic efferent signals follow descending pathways in the lateral funiculus of the spinal cord that terminate on cell bodies placed in the intermediolateral and intermediomedial columns. The sympathetic innervation of the heart arises from the preganglionic neurons in the intermediolateral column of the spinal cord, extending from roughly the first through fifth thoracic segments. The primary visceral motor neurons are in the adjacent thoracic paravertebral and prevertebral ganglia of the cardiac plexus. The parasympathetic preganglionic...
are in the dorsal motor nucleus of the vagus nerve and the nucleus ambiguus. The parasympathetic preganglionic fibres extend through the right and left vagus nerves, projecting to parasympathetic ganglia in and around the heart and great vessels\[1,3\].

Sympathetic efferent nerves are present throughout the atria, especially in the sinoatrial (SA) node, and ventricles, including the conduction system of the heart. The right vagus nerve primarily innervates the SA node, whereas the left vagus innervates the atria-ventricular (AV) node; however, there can be significant overlap in the anatomical distribution. Atrial muscle is also innervated by vagal efferents, whereas the ventricular myocardium is only sparsely innervated by vagal efferents\[1,4\]. The major neurotransmitters of the sympathetic and parasympathetic systems are norepinephrine and acetylcholine, respectively, which define the stimulatory and inhibitory physiologic effects. Sympathetic stimulation increases heart rate (positive chronotropy), inotropy and conduction velocity (positive dromotropy), whereas parasympathetic stimulation of the heart has opposite effects\[5\].

The parasympathetic (vagal) control of the atria involves modulation of chronotropic, dromotropic and inotropic activities, but the role of the innervation of the ventricles is still unclear. In fact, there is a common misconception that the sympathetic nerves innervate all over the heart, while the parasympathetic nerves only innervate the supraventricular part of the heart, but not the ventricles, since the vagal fibres are hardly found in the ventricles, and muscarinic agonists have little or no effect on ventricular function under basal conditions\[6-8\]. Yet, this is clearly not the case. Some papers have reviewed the muscarinic regulation of the heart\[9-12\], but most of them focus on the chronotropic and the dromotropic activity, and mainly in the atria and the conduct system of the ventricles. The present article reviews the evidence of vagal innervation in the ventricles, vagal (muscarinic) regulation of the cardiac contractility, and its signal transduction. Special attention is paid to the distribution of vagus nerves in the ventricle and the direct inotropic response to vagal (muscarinic) activation.

**Evidence for vagal innervation of the ventricles**

**Histochemical evidence of vagal innervation in the mammalian ventricles**

Since 1960s, the innervation of the human heart has been studied microscopically by the use of methylene blue method and the silver impregnation technique. However, these methods could not distinguish adrenergic from cholinergic nerves. Thereafter, the innervation of the human heart was further studied by histochemical and immunohistochemical methods\[16,17\].

Acetylcholine (ACh), which is released from the parasympathetic nerves and is distributed in different regions of the heart, may correlate with the level of cholinergic innervation. Acetylcholine is synthesized by transport of choline into the cytosol of the nerve terminal through the high-affinity choline transporter (CHT) and acetylation by choline acetyltransferase (ChAT). Vesicular ACh transporter (VACHT) transfers ACh to storage vesicles in nerve terminals or varicosities. The transmitter is then stored in vesicles. ACh is released on vagus nerve stimulation, subsequently activates muscarinic receptors. The effects of ACh are terminated by rapid degradation by acetylcholinesterase (AChE). So the four main ACh-related proteins become the targets of histochemical and immunohistochemical studies about the cholinergic innervation\[16-28\].

With histochemical and immunohistochemical methods, cholinergic nerves were found in the ventricle of dog\[100\], pig\[10\], guinea-pig\[101\], rat and human\[102\]. Electron microscopy of the ventricular muscle of the dog has revealed the presence of cholinergic nerve endings\[100\] in the left ventricle. Kawano et al\[102\] investigated the distribution of autonomic nerves in the human heart by using histochemical methods. Their results showed that there were more AChE-positive nerves and tyrosine hydroxylase (TH)-positive nerves in the atrium than in the ventricle, and more at the base than at the apex of the ventricle. There were more AChE-positive nerves in the subendocardial area than in the subepicardial area of the myocardium. There were more TH-positive nerves than AChE-positive nerves in the ventricle. Crick et al\[103\] compared the distribution of innervation between the endocardial, myocardial and epicardial tissues of the four chambers of the pig heart, by using quantitative immunohistochemical and histochemical techniques. Their results showed that the myocardium of the right and left atria possessed higher percentage of AChE-positive nerves than the ventricular myocardium, and the epicardial tissues of the left ventricle possessed a significantly higher percentage of AChE-positive nerves than the other three chambers, which suggested that AChE-positive nerves of the endocardial plexus displayed a right to left gradient in density, which was absent from both the myocardial and epicardial tissues. Gill et al\[104\] separated G1 and G4 AChE molecular forms in different regions of guinea-pig heart and found that the activities of both were highest in the left side of the left ventricle [LV(L)] among different regions...
of the heart. The functional importance of these differences is unclear, but they may relate to the involvement of the left side in the sequential regulation of contractility in different regions of the heart during the heart cycle. Although early studies in the rat showed that the cholinergic innervation of the ventricle was sparse\cite{6}, the AChE-positive nerves were found in the Sprague-Dawley rats ventricle with Karnovsky-Roots method in our laboratory, whereas, M\textsubscript{2}-muscarinic receptors were also found in the rat ventricles by using immunohistochemical method. Additionally, we also compared the distribution of cholinergic nerves and M\textsubscript{2}-muscarinic receptors in the three layers of the rat left ventricular free wall, the results showed that the counts of cholinergic nerves distribution in the subendocardial and subepicardial tissues were higher obviously than those in the midcardial tissue, however, the area and grey degree of M\textsubscript{2}-muscarinic receptors were not different in the three layers statistically \cite{Xu XL, Zang WJ, et al., 2005; unpublished data}.

ChAT antibodies had limited utility for identifying cholinergic nerves in peripheral tissues until recently \cite{20}. ChAT-immunoreactive nerve fibers were abundant in guinea-pig cardiac ganglia, and were also observed in large vagosympathetic fiber bundles, in interganglionic fiber bundles, and passing individually within the myocardium. Immunoreactivity for ChAT was also observed in a large proportion of the small tyrosine hydroxylase-immunoreactive neurons that exist in guinea pig cardiac ganglia \cite{21}. VChAT antibodies have been used to identify cholinergic neurons and nerve fibers in brain and peripheral tissues, including rat and human heart \cite{22-25}. The antibodies to the CHT have been used for immunohistochemical detection in multiple species and tissues \cite{26-29}. However, comparison of labeling patterns for CHT and AChE suggests that AChE histochemistry overestimates the density of cholinergic innervation in the heart \cite{17}, because the expression of AChE is also found in some noncholinergic neurons \cite{104}. They found that AChE-positive nerve fibers were more abundant than CHT-immunoreactive (CHT-IR) nerves in working atrial and ventricular myocardium. The highest density of CHT-IR nerves in ventricular myocardium was localized in the conducting system, but the CHT-IR nerves were rarely observed in left ventricular free wall whereas the AChE-positive nerves were observed in left ventricle \cite{17}.

Although both divisions of the autonomic nervous system are crucial for the regulation of the heart, the anatomic distribution of cholinergic nerves is less precisely known than that of sympathetic innervation. These findings mentioned above also imply that cholinergic innervation of ventricular myocardium may vary significantly between species. The distribution and abundance of cholinergic nerves in ventricular muscle warrant further investigation in different species by using more specific cholinergic probes or methods.

**Molecular biological evidence in vagal (muscarinic) regulation of the ventricles**

It is also evident that muscarinic receptors are expressed throughout all areas of the heart, including the ventricles. In receptor-binding studies by using \cite{18} QNB, the atrial regions of rabbit, rat \cite{105}, chick \cite{106}, and human \cite{29,30} hearts were shown to be more densely endowed with muscarinic receptors than the ventricular myocardium. The density of receptor binding in these species was similar in right and left atria and, likewise, for right and left ventricles. Furthermore, radioligand-binding studies have revealed the presence of muscarinic receptors in guinea-pig \cite{31} and canine ventricles \cite{32}. Regional differences exist for the distribution of M\textsubscript{2}-receptors in the human heart: the number of M\textsubscript{2}-receptors is significantly higher in the atria (up to 2.5-fold) than in the ventricular myocardium \cite{13,29}. It has been reported that in guinea-pig, dog and chick atria and ventricles comparable densities (atrium vs ventricle) of muscarinic receptor were found \cite{106,107}, while in rabbit, rat and frog the density of muscarinic receptor was five times higher in atrium as compared to that in the ventricle \cite{105,107}.

In our laboratory, the species difference in M-receptors distribution in the heart was also observed. The results showed that the density of M-receptors is significantly higher in the atria (up to 3-fold in rats; up to 1.5-fold in guinea-pigs; up to 3.5-fold in rabbits) than that in the ventricles \cite{33}.

Receptor cloning studies have demonstrated the existence of five different muscarinic receptor subtypes (M\textsubscript{1}~M\textsubscript{5}) \cite{34,35,36}. Because their functional properties correspond to those previously defined by pharmacological criteria, it has been recommended that the M\textsubscript{1}, M\textsubscript{2}, M\textsubscript{3}, M\textsubscript{4}, and M\textsubscript{5} nomenclature be used to describe both the pharmacological and the molecular subtypes \cite{34}. The chromosomal localization of the human M\textsubscript{1}~M\textsubscript{5} receptor genes is 11q12-13, 7q35-36, 1q43-44, 1p12-11.2, and 15q26, respectively \cite{34}. There is a general agreement that the predominant form of the muscarinic receptors in the human heart is the M\textsubscript{2}-receptor. Using RT-PCR or ISH, the mRNA of muscarinic receptor subtypes has been detected in the ventricles in various species, such as human, rat, and guinea-pig, etc., although it is significantly lower than that in the
atria. The summary of the references is seen in table 1. Our Western blotting results also showed that the M2-receptors expressed evidently in the rat left and right ventricles, although the M2-receptors in the atria were higher (up to 2–2.5-fold) than that in the ventricles (Fig. 1) (Chen LN, Zang WJ, et al., 2005; unpublished data).

<table>
<thead>
<tr>
<th>Species</th>
<th>Preparation</th>
<th>Methods</th>
<th>Muscarinic receptor subtypes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Atria and ventricles</td>
<td>RT-PCR</td>
<td>M₁ + M₂ + M₃ + M₄ + M₅</td>
<td>Hellgren et al., 2000[35]</td>
</tr>
<tr>
<td>Human</td>
<td>Atria and ventricles</td>
<td>RT-PCR</td>
<td>+ + + + +</td>
<td>Wang et al., 2001[36]</td>
</tr>
<tr>
<td>Rat</td>
<td>Dispersed ventricular myocytes</td>
<td>RT-PCR</td>
<td>+ + + + +</td>
<td>Sharma et al., 1996[37]</td>
</tr>
<tr>
<td>Rat</td>
<td>New born ventricular myocytes in culture</td>
<td>RT-PCR</td>
<td>+ + + + +</td>
<td>Colecraft et al., 1998[38]</td>
</tr>
<tr>
<td>Rat</td>
<td>Atria and ventricles</td>
<td>RT-PCR</td>
<td>&lt;1% &gt;90% &lt;3% &lt;1% &lt;5%</td>
<td>Krejci and Tucek, 2002[39]</td>
</tr>
<tr>
<td>Guinea-pig</td>
<td>Ventricles</td>
<td>RT-PCR</td>
<td>+ ND ND ND ND</td>
<td>Gallo et al., 1993[40]</td>
</tr>
</tbody>
</table>

ND, not determined; RT-PCR, reverse transcription-PCR.

Table 1. References on the occurrence of mRNAs for multiple muscarinic receptor subtypes in mammalian heart

![Fig. 1. Expression of the M2-receptors in the four chambers of normal adult rat heart. A: Western blots of membrane obtained from rat atria and ventricles. B: The bar plot showing the expression of the M2-receptors in the four chambers of normal adult rat heart. LA, left atria; RA, right atria; LV, left ventricles; RV, right ventricles. The ratio of OD (M₂ receptor)/OD (GAPDH) of RV was used as 100%. The values shown are means ± SEM, n = 7. *P<0.05. (From Chen LN, Zang WJ, et al., 2005; unpublished data).](image)

Functional evidence in vagal (muscarinic) control of the ventricles

Recent evidence indicates that the cholinergic innervation of the left ventricle is functionally very important in some mammalian species. Electrical stimulation of the vagus nerves of the left ventricular myocardium in the cat caused increased release of acetylcholine[50], and stimulation of the vagus nerve reduced the contractility of the left ventricle in dog[6,51], pig and human[52]. Vagal stimulation and ACh infusion both decreased left ventricular systolic pressure (LVSP) and its maximum positive first derivative with slowing of heart rate[53]. Vagal stimulation shortened the effective refractory period (ERP) in dog anterior and posterior left ventricular free wall and the septum[108]. The data from Yang et al.[54] showed that ACh increased cardiac contractility in isolated rat heart. Ates et al.[55] also found that ACh (1×10⁻⁷, 5×10⁻⁷, and 1×10⁻⁶ mol/L) perfusion increased significantly the cardiac contractility in the Langendorff rat heart. It was found that there were differences in the electrophysiological response between canine ventricular subendocardium and subepicardium to acetylcholine. In canine ventricular epicardium, ACh produced an accentuation of phase 1 magnitude and slowing of the second action potential upstroke, resulting in prolongation of APD at ACh concentrations of 1×10⁻⁷ and 1×10⁻⁶ mol/L; ACh in a concentration of 1×10⁻⁴ mol/L suppressed the plateau, thus...
ZANG Wei-Jin et al: Progress in the Study of Vagal Control of Cardiac Ventricles

causing a marked abbreviation of the action potential[56]. Casadei’s review[2] provided further evidence for a vagal (muscarinic) regulation of left ventricular function in human.

The direct inotropic effect was found in other species, such as ferret[57-59], rat[60], dog[61], guinea-pig[62, 63] and human[52]. We did a series of functional experiments to improve the direct effect of acetylcholine on the ventricles. It was found that acetylcholine could inhibit the contractile force of the guinea-pig ventricular tissues directly[62]. Moreover, acetylcholine also exerted a direct negative inotropic effect on the isolated ventricular myocytes of guinea-pig (Fig. 2)[63].

Additionally, recent experimental and clinical studies have shown that vagus nerves activity suppresses some ventricular arrhythmias and has a protective effect against sudden cardiac death[64-66]. Vagal stimulation or acetylcholine application can mimic ischemic preconditioning to protect the heart during cardiac ischemia[67,68]. Moreover, the acetylcholine-sensitive potassium current (I_K,ACh) has also been observed in rat[60], ferret[42], feline[69] and human[70] ventricular myocytes, although the current is much smaller than that in the atria. However, there is still controversy with respect to how an increase in vagal tone changes left ventricular (LV) contractility. Recent work has demonstrated that decreased contractility of the left ventricle was induced by the neurotransmitter acetylcholine, but not by vagal stimulation in rats[53]. So the vagal regulation of the ventricular function still needs to be investigated further.

### Inotropic effects of vagal (muscarinic) stimulation in the ventricle

There are four inotropic effects of vagal (muscarinic) stimulation in the ventricle: a direct negative inotropic effect of vagal (muscarinic) stimulation on ventricular tissue; an indirect negative inotropic effect of vagal (muscarinic) stimulation on ventricular tissue; a positive inotropic response to vagal (muscarinic) activation prolonged or after washout; a positive inotropic response to high concentration of muscarinic agonists[5,58]. This part is focused on the direct inotropic effect of muscarinic activation in the ventricle.

**Direct negative inotropic effect of vagal (muscarinic) stimulation on ventricular tissue**

It is generally accepted that an indirect negative inotropic effect of muscarinic agonists exists in the mammalian ventricle, whereas a direct negative inotropic effect occurs in the atrium but not in the ventricle[5,58], with the

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**Table 2. References on the occurrence of mRNAs for subunits of K_ACh in mammalian heart**

<table>
<thead>
<tr>
<th>Species</th>
<th>Methods</th>
<th>GIRK1 Atria</th>
<th>GIRK1 Ventricles</th>
<th>GIRK4 Atria</th>
<th>GIRK4 Ventricles</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>Rat</td>
<td>Northern blot</td>
<td>++</td>
<td>±</td>
<td></td>
<td></td>
<td>Kubo et al., 1993[44]</td>
</tr>
<tr>
<td>Rat</td>
<td>Northern blot</td>
<td>++</td>
<td>±</td>
<td></td>
<td></td>
<td>Dascal et al., 1993[45]</td>
</tr>
<tr>
<td>Rat</td>
<td>Northern blot</td>
<td>++</td>
<td>±</td>
<td></td>
<td></td>
<td>Krapivinski et al., 1995[46]</td>
</tr>
<tr>
<td>Rat</td>
<td>ISH</td>
<td>++</td>
<td>±</td>
<td></td>
<td></td>
<td>Spauschus et al., 1996[47]</td>
</tr>
<tr>
<td>Rat</td>
<td>ISH</td>
<td>++</td>
<td>±</td>
<td></td>
<td></td>
<td>Karschin et al., 1994[48]</td>
</tr>
<tr>
<td>Pig</td>
<td>Northern blot</td>
<td></td>
<td>±</td>
<td>++</td>
<td>++</td>
<td>Iizuka et al., 1995[49]</td>
</tr>
</tbody>
</table>

++, strong expression; ±, weak expression; ISH, in situ hybridization.
exception of the ferret\textsuperscript{[57-59]} and rat\textsuperscript{[60]}. However, the data\textsuperscript{[55,61]} from other species show that direct inotropy is also present. The results of Yang et al.\textsuperscript{[61]} showed that ACh exerted a direct negative inotropic effect on cell shortening of the ventricular myocytes in dogs. In guinea-pig ventricles, we did a series of experiments in muscle strips and isolated cardiac myocytes. We found that ACh exerted direct concentration-dependent effects not only on the atria but also on the ventricles, although the inhibitory effects of ACh on the atria were stronger than that on the ventricles\textsuperscript{[55]}. It was also found that the direct negative inotropic effect of ACh existed in the rat ventricular myocardium and isolated myocytes, but no direct inotropic response to ACh was detected in rabbit ventricular myocytes (Fig. 3) (Chen LN, Zang WJ, et al., 2005; unpublished data).

The decrease in basal contractile force by muscarinic receptor agonists is probably attributable to at least one of the following mechanisms\textsuperscript{[60]}: (1) the shortening of the APD by activation of potassium channels via direct effects of the G-protein \( \beta \gamma \)-subunits\textsuperscript{[71,72]} reducing the time window for \( I_{\text{Ca,L}} \), (2) a decrease in \( I_{\text{Na,K}} \)\textsuperscript{[59]}, (3) a direct decrease in \( I_{\text{Ca}^2^+} \) current\textsuperscript{[58]}, (4) the inhibition of mobilization of intracellular \( I_{\text{Ca}^2^+} \) ions, thereby causing accumulative depletion of intracellular stores of \( I_{\text{Ca}^2^+} \)\textsuperscript{[5,58,73]}

### Indirect negative inotropic effect of vagal (muscarinic) stimulation on ventricular tissue

Stimulation of muscarinic receptors causes direct negative chronotropic effects on atria and inotropic effects on isolated tissues. In ventricles, however, the negative inotropic effect can be demonstrated when basal force of contraction has been enhanced in advance by cAMP-elevating agents, such as \( \beta \)-adrenergic receptor agonists, forskolin, or phosphodiesterase inhibitors. This inhibitory action is termed an ‘indirect inhibitory action’ (secondary to inhibition of facilitated cAMP-mediated processes), an ‘anti-adrenergic effect’, or an ‘accentuated antagonism’ because the effect has been observed most often as an antagonistic effect on the \( \beta \)-adrenoceptor-mediated positive inotropic effect\textsuperscript{[5,58]}.

The indirect negative inotropic effect occurs in ventricular myocardium that has been prestimulated with \( \beta \)-adrenoceptor agonists so that cAMP levels are elevated due to activation of adenyl cyclase. The main mechanism involved is inhibition of the stimulated adenyllylcyclase. In parallel with this indirect negative inotropic response a reduction in intracellular \( I_{\text{Ca}^2^+} \) concentrations (which previously have been elevated via \( \beta \)-adrenergic stimulation) can be detected\textsuperscript{[90,74]}.

The cardiac L-type \( I_{\text{Ca}^2^+} \) current \((I_{\text{Ca,L}})\) is inhibited by muscarinic stimulation in both atrium and ventricle\textsuperscript{[74]}, while in the atrium no prior elevation of intracellular cAMP concentration is required, in the ventricle the inhibitory effect on \( I_{\text{Ca,L}} \) is typically seen only if cAMP has been elevated\textsuperscript{[75,76]}.

This effect is thought to account for the negative inotropic regulation by cholinergic stimulation.

#### Positive inotropic response to vagal (muscarinic) activation prolonged or after washout

Positive inotropic responses have also been observed if exposure to muscarinic agonists is prolonged or after washout. In guinea-pig ventricular myocardium and single myocyte, we found that during ACh perfusion, there was a desensitization in the effects of decreasing contraction and cell shortening, whereas, a rebound phenomenon occurred in both tissue and myocytes after washout (Fig. 2)\textsuperscript{[62,63]}. Moreover, the desensitization and rebound phenomena of the inotropic effect of ACh were in parallel with the changing APD effect (Fig. 4)\textsuperscript{[62,63]}. Additionally, desensitization of acetylcholine on the inhibition effects of blood pressure was found in anesthetized canine\textsuperscript{[77]}. The desensitization is attenuation of a negative inotropic response and, thus, is not equivalent to classic positive inotropic responses. The desensitization is possibly attributable to a mechanism such as muscarinic receptor endocytosis, G protein phosphorylation, G protein-coupled receptor kinase phosphorylation, or potassium channel down regulation\textsuperscript{[78,83]}. Figure 5 shows that the desensitization of \( I_{\text{Ca,AC}} \) was in most part due to muscarinic receptor and the modification of G protein\textsuperscript{[490]}. The rebound of the contraction may involve an increase in the intracellular Na\textsuperscript{+} concentration with subsequent activation of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger and a rise
in the intracellular Ca\(^{2+}\) transient\(^{[5, 58, 84, 85]}\). In addition, the increase in the Ca\(^{2+}\) sensitivity of the myofibrils may also attribute to the positive inotropic response\(^{[58]}\). However, the exact underlying mechanisms are still unclear.

**Positive inotropic response to high concentration of muscarinic agonists**

High concentrations of carbachol (1×10\(^{-5}\)--1×10\(^{-4}\) mol/L) have been shown to exert a small positive inotropic effect in guinea-pig papillary muscles\(^{[56]}\) that could be antagonized by atropine (1×10\(^{-5}\) mol/L). Positive inotropic effects of high concentrations of muscarinic agonists have also been described in the rat atrium\(^{[109]}\), in rat ventricular cardiomyocytes\(^{[109]}\), guinea-pig ventricle\(^{[89]}\) and rabbit atrial muscle\(^{[58]}\). This positive inotropic effect was preceded by an increase in IP\(_3\)\(^{[86]}\), suggesting a role for phospholipase C. In addition, phospholipase D can also be activated by high concentrations of muscarinic agonists\(^{[110]}\), leading to the formation of choline and phosphatidic acid. Moreover, the positive inotropy of high concentrations of muscarinic agonists appear to occur in concert with increases in intracellular Na\(^{+}\) activity\(^{[84]}\), and an increase in free intracellular Ca\(^{2+}\) concentration as described in rat ventricular myocytes\(^{[110]}\). This action may involve the activation of reversed mode Na\(^{+}/Ca^{2+}\) exchange\(^{[87]}\), subsequent to the rise in intracellular Na\(^{+}\) activity. High concentrations of muscarinic agonists can also induce the release of norepinephrine from sympathetic nerve terminals, thereby exerting an indirect positive inotropic effect\(^{[111]}\).

**Mechanisms of the different effects of ACh on the atrial and ventricular myocardium**

The effects of ACh on atria and ventricles are quite different. ACh produces direct effects in the atrium that are unrelated to changes in intracellular cyclic adenosine monophosphate (cAMP), mainly via muscarinic-sensitive potassium channels\(^{[5, 58]}\). In contrast, ACh exerts indirect effects on the ventricle with the presence of β-adrenoceptor agonists, so that cAMP levels are elevated due to activation of adenylyl cyclase\(^{[5, 58, 88]}\).

About the direct intropic effect, the atria are more sensitive to ACh than the ventricles, which might be explained\(^{[85]}\) by (1) there being more muscarinic receptors in the atria than in the ventricles\(^{[5, 10, 39]}\), (2) \(I_{K,ACh}\) is greater in the atria than in the ventricles\(^{[89]}\), (3) the vagal innervation density is lower in the ventricles than in the atria\(^{[89]}\), or (4) ACh has a higher affinity to the atrium than to the ventricle according to the result that there are comparable muscarinic receptor densities (atrium vs ventricle) in guinea-pig\(^{[107]}\).

However, the major reason for these differences between atrial and ventricular myocardium is a fundamental difference in the effector mechanisms activated by acetylcholine in atria and ventricles. In both atria and ventricles, activation of M\(_2\)-receptors, coupling to a PTX-sensitive G-protein (G\(_i/G_o\)), leads to an inhibition of adenylyl cyclase and hence inhibits the increase in intracellular cAMP; thus leads to a reduction in the L-type Ca\(^{2+}\) current (previously enhanced by cAMP). This appears to be the predominant mechanism of inhibitory force of contraction enhanced by cAMP-elevating agents (indirect inhibitory action)\(^{[4, 5]}\). In human atrial myocytes, however, acetylcholine additionally opens an inwardly rectifying potassium channel (\(I_{K,ACh}\)) through direct effects of the G protein β\(_7\)γ subunits\(^{[71, 72]}\).
This results in hyperpolarization, slowing of heart rate, shortening of the action potential duration, abbreviation of the L-type Ca2+ current and reduction of force of contraction (direct inhibitory action). In human ventricular myocardium, on the other hand, stimulation of M2 receptors has no direct negative inotropic effect, and it is still a matter of debate whether acetylcholine might activate the $I_{K, ACh}$.

**Signal transduction of vagal (muscarinic) regulation in the heart**

Muscarinic cholinergic receptors are members of the large superfamily of G protein-coupled receptors (GPCRs). GPCRs share a common overall structure characterized by seven transmembrane domains with three extracellular and three intracellular loop domains, an extracellular N-terminal and an intracellular C-terminal tail. The transmembrane domains are more highly conserved than the loops or the N- and C-terminal tails. GPCRs couple to various effectors via heterotrimeric (αβγ) G proteins that elicit responses via actions of both α and βγ subunits.

Among muscarinic receptors, M1, M3, and M5 preferentially couple to $G_{i/o}$, and, in turn, lead to inhibition of adenyl cyclase (AC), activation of inwardly rectifying K⁺ channels, and inhibition of voltage-dependent Ca²⁺ channels. M1, M3, and M5 preferentially couple to $G_{q/11}$, which leads to activation of phospholipase C and the generation of diacylglycerol, which activates intracellular calcium. Additional signaling systems involved are effects on K⁺ and Ca²⁺ channels and activation of phospholipase A₂, phospholipase D, and protein tyrosine kinases.

Muscarinic cholinergic receptors play a role in the regulation of the ventricular contractile function in balance with adrenergic receptors. The main mechanism of action seems to be inhibition of GTP-activated adenylate cyclase activity mediated by a GTP-binding α-regulatory protein ($G_i$). $G_i$ inactivates the catalytic subunit of adenylate cyclase, thereby reducing intracellular cAMP levels and exerting an anti-adrenergic effect. Therefore, the parasympathetic system acts through muscarinic cholinergic inhibition of β-adrenergic cardiac responsiveness. In this way, adrenaline is enhanced and inotropic stimulation is induced by noradrenaline after parasympathetic blockade with atropine. Because the predominant muscarinic receptor is M1 cholinergic receptor and the function of other muscarinic receptor subtypes is still unclear, in this part, we focus on the signal pathway of the inotropic response induced by muscarinic activation.

Muscarinic activation induces the inotropic effects via cAMP-dependent pathway. Muscarinic agonists inhibit the contratile force possibly through decreasing cAMP formation and increasing cAMP breakdown. Decreased cAMP formation is caused by muscarinic receptor stimulation. This effect is most apparent when adenylyl cyclase is stimulated, for example, by activation of adrenergic receptors with catecholamines or forskolin. Simultaneous addition of cholinergic agonists decreases the amount of cAMP formed in response to the catecholamine, in some tissues almost completely. The result is diminished activation of cAMP-dependent protein kinase A (PKA) and decreased substrate phosphorylation catalyzed by this kinase. The mechanism by which the muscarinic receptor inhibits adenyl cyclase (5/6) is through activation of an inhibitory GTP-binding protein, $G_i$. The α subunit of $G_i$ competes with the α subunit of the G protein activated by stimulatory agonists ($G_s$) for regulation of adenyl cyclase.

Muscarinic activation increases cAMP breakdown through NO-cGMP-PDE₂ pathway. Early studies demonstrated that exposure to ACh was associated with the production of cGMP in cardiac tissue. However, there was never a consistent correlation between ACh effects and cGMP levels. Nevertheless, it is clear that at least in some cardiac myocyte preparations, exogenous cGMP or cGMP analogs can antagonize cAMP-mediated ion channel responses. Such effects have been attributed to exogenous cGMP stimulating the activity of type 2 phosphodiesterase (PDE₂), resulting in an increase in cAMP breakdown. Therefore, evidence linking either of these potential effects of cGMP to muscarinic receptor activation has been inconsistent.

In contrast, muscarinic agonists facilitate the contratile force may through increasing cAMP formation and decreasing cAMP breakdown. Although muscarinic receptors do not interact with $G_s$, increases in cAMP formation are seen under some circumstances. One clear manifestation of such an effect is the rebound increase in heart rate as well as contractility that can be observed immediately following termination of vagal stimulation or cessation of exposure to ACh. Moreover, rebound responses to ACh can be observed in isolated cardiac myocytes. These may result from stimulatory effects of βγ subunits released from G proteins or effects of elevated intracellular Ca²⁺ on specific isoforms of adenylyl cyclase (4/7). Muscarinic stimula-
tory responses may be mediated by direct activation of AC via the \( \beta_\gamma \) subunits of a PTX-sensitive G protein \( G_i \) or \( G_o \) or by inhibition of PDE\(_5\) via production of nitric oxide (NO) and cGMP\([4]\). Direct stimulation of AC by \( \beta_\gamma \) subunits is only observed under conditions, such as the presence of a \( \beta_1\)-adrenergic receptor agonist, that can activate AC via direct interaction of the a subunit (\( \alpha_s \)) of the stimulatory G protein \( G_s \).

It is conceivable for muscarinic receptor activation to produce both facilitation as well as inhibition of cAMP-dependent ion channel responses via a NO/cGMP-dependent mechanism. Whether one would expect to see an inhibitory and/or stimulatory response would then depend on the relative level of expression of the different PDE isoforms, and whether or not they are coupled to muscarinic receptor activation\([94]\). The illustration of the detailed mechanism is shown in Fig. 6.

Stimulation of phosphoinositide hydrolysis may be involved in the inotropic response to muscarinic stimulation. Activation of phosphoinositide-specific phospholipase C by muscarinic agonists stimulates phosphoinositide hydrolysis. Activation of the \( \beta_1 \) isofrm of phosphoinositide-specific phospholipase C (PI-PLC) is mediated through the \( \alpha \) subunit of a GTP-binding protein, \( G_{q/11} \). This is the primary mechanism by which muscarinic receptors regulate this enzyme. However, some PLC isoforms, most clearly \( \beta_2 \), also are activated by \( \beta_\gamma \) subunits. This probably accounts for the pertussis toxin-sensitive, \( G_i/G_o \)-mediated activation of PI-PLC seen when high levels of cloned \( M_2 \) receptors are expressed stably in some cell lines. The hydrolysis of phosphatidylinositol 4,5-bisphosphate yields two potential second messengers, IP\(_3\) and diacylglycerol (DAG).

Muscarinic agonists cause rapid activation of G protein-
coupled, inwardly rectifying potassium channels (GIRKs). This muscarinic effect can be mimicked by GTP analogs in whole-cell clamp experiments[101], and the response is sensitive to pertussis toxin, which ribosylates and inactivates G\textsubscript{i} and a related protein, G\textsubscript{o}. Stimulation of a specific PTX-sensitive G-protein-coupled receptor (GPCR) leads to the activation of K\textsubscript{ACs} channels in atrial myocytes via a membrane delimited pathway. It is now commonly accepted that G\textsubscript{b\gamma} is the activator of K\textsubscript{ACs} channels, whereas G-protein \textalpha subunit may determine the specificity and duration of the G-protein action[71,72]. This is a primary mechanism by which muscarinic agonists cause hyperpolarization of cardiac atrial cells, as well as of neurons. However, whether the activation of K\textsubscript{ACs} channel plays a role in the ventricular contraction is still unclear, and needs further investigations.

Concluding remarks
The paper reviews the evidence in the innervation of vagus nerves in the ventricles, distribution and expression of muscarinic receptors and acetylcholine-sensitive potassium channel, and the mechanisms in vagal (muscarinic) regulation of cardiac contractile function, which have contributed immensely to our current understanding of vagal (muscarinic) control of the ventricular function, especially in the working ventricular myocardium. However, there are some neuropeptides co-localized with ACh or noradrenaline in cardiac autonomic nerves, such as neuropeptide Y, substance P, calcitonin gene-related peptide, etc.[15]. Vagal stimulation induces the release of substances other than ACh from vagal postganglionic axons, e.g. somatostatin[112]. Additionally, there is an interaction between sympathetic nerves and parasympathetic nerves in vivo[3]. Additionally, there exists electromechanical heterogeneity[56,102] and species difference of vagal innervation in the ventricles. Therefore, the result of vagal stimulation could be more complex than muscarinic activation, although ACh is the main neurotransmitter of the vagus. Despite M\textsubscript{2} receptor is the predominant muscarinic receptor in the heart, the non-M\textsubscript{2} receptor may play an indispensable role in heart regulation. Further research in the vagal control of the heart may provide a better understanding of the geneses and development of the cardiovascular diseases, and benefit for the therapeutic intervention.

REFERENCES
et al.: Progress in the Study of Vagal Control of Cardiac Ventricles


ZANG Wei-Jin et al: Progress in the Study of Vagal Control of Cardiac Ventricles


98 Dedkova EN, Wang YG, Blatter LA, Lipsius SL. Nitric oxide signalling by selective beta2-adrenoceptor stimulation prevents...

Abi-Gerges N, Szabo G, Otero AS, Fischmeister R, Mery PF. NO donors potentiate the beta-adrenergic stimulation of \( I_{\text{Ca,L}} \) and the muscarinic activation of \( I_{\text{K,ACh}} \) in rat cardiac myocytes. J Physiol 2002; 540: 411-424.


