Decreased Contractility of the Left Ventricle is Induced by the Neurotransmitter Acetylcholine, but not by Vagal Stimulation in Rats

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SUMMARY

There is still controversy with respect to how an increase in vagal tone changes left ventricular (LV) contractility. It is possible that a difference in LV vagal innervation density may affect the inotropic effect. To test this, we examined the effects of vagal stimulation and acetylcholine (ACh) infusion on the rat ventricle, in which LV vagal innervation density is sparse and a negative force-frequency relationship is uniquely observed. To evaluate LV contractility, we developed an in situ Langendorff preparation, in which the effects of changes in afterload, preload, and coronary flow during an intervention were minimized. Both vagal stimulation and ACh infusion significantly increased LV systolic pressure (34 ± 16%; 36 ± 22%, respectively) and its maximum positive first derivative with slowing of heart rate (51 ± 17%; 46 ± 18%). These effects of vagal stimulation were abolished by pretreatment with atropine. During a fixed heart rate, LV systolic pressure was not changed by vagal stimulation, however, it was decreased slightly but significantly (11 ± 8%) by ACh infusion. In conclusion, LV contractility changes due to ACh release during vagal stimulation were negligibly small, presumably due to a sparse vagal innervation density in rats, and therefore, a bradycardia-dependent indirect positive inotropic effect may be dominant compared to a direct negative inotropic action during vagal stimulation. Thus, the integrated effect of vagal nerve stimulation on LV contractility is different among species, because it is determined by a direct negative inotropic effect, which depends on the vagal innervation density in the left ventricle, as well as by bradycardia-dependent indirect inotropic changes. (Jpn Heart J 2003; 44: 257-270)

Key words: Force-frequency relationship, Vagal innervation density, In situ Langendorff preparation

The effects of vagal stimulation on left ventricular (LV) contractility have been controversial for a long time. Previous reports1,2) claim to have shown a direct negative inotropic action on the left ventricle. However, indirect negative inotropic effects mediated by a positive force-frequency relationship2,3) or vago-
thetic interaction\textsuperscript{4-6} are also known to occur during vagal stimulation. The controversies in previous reports seem to be partly due to species differences in parasympathetic innervation density and muscarinic receptor density in the ventricle.\textsuperscript{7,8} If LV vagal innervation density is low as observed in rabbit or rat heart,\textsuperscript{3,7,8} the direct effect of parasympathetic stimulation on LV contractility, if any, would be small, and vice versa. Therefore, administration of acetylcholine (ACh), a neurotransmitter in the parasympathetic nervous system, is also needed to detect how species differences in vagal nerve innervation affect the contractile response during parasympathetic nerve activation. In addition, a reduction in heart rate (HR) via parasympathetic stimulation\textsuperscript{9,10} affects ventricular contraction differently in different species, e. g., reduced contraction with decreased HR (a positive force-frequency relationship) in rabbits, or enhanced contraction with decreased HR (a negative force-frequency relationship) in rats. Thus, to settle the controversy regarding the effects of parasympathetic stimulation on ventricular contractility, parasympathetic nerve stimulation must be conducted with and without a fixed HR in the same heart. Such experiments should resolve the question as to how vagal nerve activation \textit{in situ} affects ventricular contraction directly or indirectly, regardless of species differences.

Previous controversial results may be due to the methods or preparations used. In this study, we attempted to clarify the effects of parasympathetic nerve activation on LV performance. Vagal stimulation and ACh infusion were carried out with and without a fixed HR, minimizing the influences of loading conditions and of changes in coronary flow in an \textit{in situ} rat Langendorff preparation with isovolumic LV contraction. With this preparation, we could estimate LV contractility while the central and cardiac autonomic nervous systems were kept intact.

\textbf{METHODS}

The study conformed to the Guidelines for Animal Experiments of Fukushima Medical University, the Japanese Government Animal Protection and Management Law (No. 115), and the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

\textbf{Experimental preparations:} Twenty-nine Wistar rats weighing 180-220 g were anesthetized by intraperitoneal administration of sodium pentobarbital (50 mg/kg) following diethylether inhalation. Each rat was placed in a supine position on a thermostated table which was kept at 37\textdegree C during the experiment. Electrocardiograms (ECG) were obtained from a bipolar CC5 lead using needle electrodes on the chest wall, and continuously monitored to discriminate sinus bradycardia from atrioventricular block during bradycardia. After making a midline incision in the neck, the right and left vagal nerves were carefully isolated and transected,
achieving as much cranial proximity as possible. Each vagal nerve was placed on a platinum bipolar electrode for electrical stimulation. They were transiently stimulated with monophasic square pulses of 3 msec in width, 10 V, and 10 Hz to confirm a bradycardiac response.\textsuperscript{10)} If adequate bradycardiac responses for both vagal nerves (i.e., more than a 30\% reduction in HR) were not obtained, the animal was not included in the experiment ($n = 5$). Five of the remaining 24 rats were used for confirmation of the reproducibility of vagal stimulation. The other 19 rats were randomly divided for use with the following 3 experimental protocols: vagal stimulation and biochemical analyses with or without ventricular pacing ($n = 7$), an atropine study ($n = 5$), and an ACh study ($n = 7$) as described below.

After a laparotomy, heparin sodium (200 IU) was injected into the inferior vena cava to prevent blood coagulation and the diaphragm was incised, and thoracotomized at a midsternal line. The thorax was opened and a polyethylene cannula (PE 205, Clay Adams, Parsippany, NJ, USA) was inserted retrogradely into the ascending aorta through a small incision and tied up for coronary artery perfusion of the heart with oxygenated modified Krebs-Henseleit solution. It took less than three minutes to begin the perfusion of the heart after thoracotomy. Another 6 gage polyethylene cannula (Venula, Top, Japan) was inserted retrogradely into the abdominal aorta distal to the renal arteries for retrograde perfusion with the same solution to nourish the central nervous system and peripheral autonomic nerves in the thorax and neck, as in our previous study.\textsuperscript{11)}

The Krebs-Henseleit solution consisted of, in mmol/L, NaCl 118, KCl 4.7, CaCl\textsubscript{2} 2.5, KH\textsubscript{2}PO\textsubscript{4} 1.2, MgSO\textsubscript{4} 1.2, NaHCO\textsubscript{3} 25 and glucose 11. Coronary perfusion was performed at a constant flow of 10 mL/min with a peristaltic pump (Micro Tube Pump MB-3BB, Tokyo Rikakikai, Tokyo). Coronary flow and coronary perfusion pressure were monitored continuously with an electromagnetic flowmeter (Model EF-010T, Nihon Kohden, Tokyo) and a pressure transducer (AP 641G, Nihon Kohden), respectively. The Krebs-Henseleit solution was continuously bubbled with a mixture of 95\% O\textsubscript{2} and 5\% CO\textsubscript{2}. The oxygenated Krebs-Henseleit solution was kept temporarily in a double-lumen column with an internal capacity of 20 mL for trapping air bubbles and warming it to 37$^\circ$C. A 16 gage double-lumen catheter (Argyle, Nippon Sherwood Medical Industries Ltd., Tokyo) with a latex balloon on the tip was inserted into the LV cavity through the incision in the left atrial free wall. The balloon and catheter lumen were filled with physiological saline and air was carefully removed before insertion. The catheter was connected to a pressure transducer (Model EF-010T, Nihon Kohden) for monitoring HR, LV pressure, and the first derivative of LV pressure (LVdP/dt) of isovolumetric contraction. The LV end-diastolic pressure in the balloon was adjusted to 5 mmHg, and the LV volume was kept constant during the experiment. A polyethylene needle, 8-10 mm long, (SR-OT 2051C, Terumo, Tokyo)
was inserted into the left ventricle through the apex for drainage of effluent from LV Tebesian veins. Another polyethylene cannula (PE 205) was inserted into the right atrium through the inferior vena cava to drain coronary sinus effluent. To prevent contamination of the coronary sinus effluent by perfusate from the systemic veins, the bilateral superior venae cavae were ligated. Bilateral external jugular veins were cut to allow free drainage of perfusate from the systemic veins.

**Electrical stimulation of the vagal nerves:** The vagal nerves were stimulated as described above for 90 seconds using an electrical stimulator (SEN-2201, Nihon Kohden). The electrical stimulation of right, left, or bilateral vagal nerves was performed in a random order after the hemodynamic variables became stable in the prestimulation stage or recovered to the baseline values following stimulation. The degree of bradycardia has been reported to be similar from right and left vagal stimulation, and there were also no significant differences in HR, coronary perfusion pressure, or LV pressure between right and left vagal stimulation or between right, left, and bilateral vagal stimulations in this study (data not shown). We used bilateral vagal stimulations to stimulate the whole heart. Subsequently, bilateral vagal nerves were stimulated at frequencies of 5, 10, and 20 Hz in a random order to examine the effects of increasing stimulation frequencies on cardiac responses. As previously reported, changes in HR and the other hemodynamic variables were significantly larger with 10 Hz stimulation compared to 5 Hz, but not significantly smaller compared to 20 Hz (data not shown). It has been reported that the effects of intense vagal nerve stimulation, such as with 15 and 20 Hz, but not with 10 Hz, on the cardiovascular system, especially on coronary circulation, are evident and vagal stimulation at 20 Hz increases coronary flow by 62% via vasoactive intestinal peptide released endogenously from the cardiac vagal nerve. Therefore, we chose bilateral electrical vagal stimulation at 10 Hz for 90 seconds. In addition, the reproducibility of cardiac responses within the range of 10% changes was confirmed up to 5 times with stimulation intervals of 10 minutes (n = 5).

**Experimental protocols:**

**Protocol 1: Vagal stimulation with and without ventricular pacing.** To eliminate the effects of HR changes on myocardial contractility, we performed vagal stimulation under a fixed HR induced by right ventricular pacing with a cardiac stimulator (Demand Pacemaker, Type E-2991, Devices Implants Ltd., UK). After baseline vagal stimulation was performed, the pacing rate was set at approximately 10 beats/min higher than the spontaneous rate before pacing in each experiment. Two minutes after hemodynamic variables were stabilized during pacing, vagal stimulation was repeated, and compared to the changes without pacing (n = 7).

**Protocol 2: Effects of atropine pretreatment on cardiac responses to vagal
stimulation. Vagal stimulation without right ventricular pacing was performed in the absence of atropine and then repeated 3 minutes after a bolus administration of 0.1 mg of atropine sulfate to block muscarinic receptors (n = 5).

**Protocol 3: ACh infusion with and without ventricular pacing.** To examine whether the cardiac effects of ACh infusion and vagal stimulation are different, a 10^{-3} mol/L ACh (Daiichi Seiyaku, Tokyo) solution was infused at 0.1 mL/min for 4 minutes from a sidearm of the coronary perfusion line using an infusion pump (Syringe pump PSK-51, Nikkiso, Japan), resulting in 10^{-6} mol/L ACh in the coronary perfusate. This dose was selected to produce an HR reduction similar to that resulting from vagal nerve stimulation which we observed in a preliminary study, and this dose was also used in previous studies. Duplicate ACh infusion studies were done without and with right ventricular pacing (n = 7). ACh infusion was started after 2 minutes of pacing and was continued for 4 minutes.

**Biochemical analyses:** The effluent from the right atrium was collected before and from 60 to 90 seconds of vagal stimulation to measure oxygen tension and norepinephrine concentration (Protocol 1, n = 7). PO2, PCO2, and pH in the perfusion fluid were also measured with a gas analyzer (JBA-150K, Blood Gas Analyzer, Jookoo, Tokyo) as previously reported. Myocardial oxygen (O2) consumption (mL O2/mL) was calculated using the following formula: 0.0237 × (PA - PV) / 760, where PA is the partial O2 pressure of the oxygenated Krebs-Henseleit solution in the aortic inflow line, PV is the partial O2 pressure of the coronary venous effluent, and 0.0237 is the solubility coefficient of O2 in the Krebs-Henseleit solution (mL O2/mL per atmosphere at 37°C). The difference in myocardial O2 consumption before and after vagal stimulation was defined as ΔO2 consumption (mL O2 /mL). The remaining effluent from the right atrium was collected into a tube containing 0.6 mol/L perchloric acid and stored at -80°C until norepinephrine was measured by high-performance liquid chromatography (HPLC-725, Tosoh, Tokyo).

**Data acquisition:** During an experiment, ECG, LV pressure, LV dP/dt, coronary perfusion pressure and flow were continuously monitored (Polygraph system, Nihon Kohden), and recorded (Thermal array recorder, Nihon Kohden) and stored on magnetic tape (PC208A, SONY, Japan).

Hemodynamic variables were averaged for 10 seconds at the baseline state before, and at 15, 30, 60 and 90 seconds during vagal stimulation and 30 sec after the cessation of stimulation. In the ACh infusion studies, HR gradually decreased, presumably due to mixing of the perfusate and ACh solution in the dead space at the connection of the sidearm. Therefore, we measured the hemodynamic variables before, at the time of minimal HR during infusion, and 5 minutes after infusion. For ACh infusion with ventricular pacing, the hemodynamic variables were measured at the time of maximal change of LV pressure (at about
2 to 3 minutes following ACh infusion). Data were also averaged for 10 seconds at each period.

**Statistical analysis:** Data are presented as the mean ± SD. ANOVA was used to determine the statistical difference among groups, followed by Fischer's post hoc test. A level of $P < 0.05$ was considered statistically significant.

**RESULTS**

**Cardiac responses to vagal stimulation:** The HR, LV pressure, LV dP/dt, and coronary perfusion pressure and flow responses during bilateral vagal stimulation at 10 Hz without right ventricular pacing are shown in Figures 1A, and 2A, and the

![Figure 1](image_url)

**Figure 1.** Changes in hemodynamic variables caused by vagal stimulation without (Figure A) and with (Figure B) right ventricular pacing. Arrows indicate the start of vagal stimulation. Coronary flow was kept constant at 10 mL/min. Pulse interval, left ventricular peak systolic pressure, and maximal positive dP/dt increased almost instantaneously after electrical vagal nerve stimulation without ventricular pacing (Figure A). In contrast, these variables were not changed by vagal stimulation with ventricular pacing (Figure B). LVP = left ventricular pressure; LVdP/dt = first derivative of LVP; CPP = coronary perfusion pressure; Flow = coronary flow; Rec = recovery period after vagal stimulation.
### Table. Changes in Hemodynamic Variables during Vagal Stimulation or ACh Infusion

<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>HR (beats/min)</th>
<th>CPP (mmHg)</th>
<th>LVP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>+dP/dt (mmHg/sec)</th>
<th>−dP/dt (mmHg/sec)</th>
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<td><strong>Vagal stimulation (n=7)</strong></td>
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<tr>
<td>Pre</td>
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<td>107±26</td>
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<td>3725±547 bcdef</td>
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<td>118±14</td>
<td>5.5±1.6</td>
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<td>60</td>
<td>148±43 af</td>
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<td>116±19</td>
<td>5.8±1.2</td>
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<td>114±21</td>
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<td>3283±256</td>
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<td>111±25</td>
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<td>5.5±0.7</td>
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HR=heart rate; CPP=coronary perfusion pressure; LVP=left ventricular peak systolic pressure; LVEDP=left ventricular end-diastolic pressure; +/−dP/dt, maximal positive/negative first derivative of LVP; pre, prestimulation or pre-ACh infusion; ACh, at maximum change of HR or LVP during ACh infusion, post: 30 sec after vagal stimulation or 5 minutes after ACh infusion. Values are means±SD. a, P<0.01 vs Pre; b, P<0.05 vs Pre; c, P<0.05 vs 30 sec; d, P<0.05 vs 60 sec; e, P<0.05 vs 90 sec; f, P<0.01 vs Post, g, P<0.05 vs Post.
Table. Vagal stimulation instantaneously decreased HR and increased LV peak systolic pressure and maximal positive LV dP/dt in all rats. HR decreased maximally (51±17%) from 215±34 to 105±33 beats/min (P<0.01 vs prestimulation) during the first 15 seconds of stimulation, gradually increased despite vagal stimulation, and then recovered to the prestimulation value at 30 seconds after vagal stimulation. LV peak systolic pressure and maximal positive LV dP/dt increased by 34±16% from 103±21 to 138±13 mmHg (P<0.05 vs prestimulation), and by 24±20% from 3000±447 to 3725±547 mmHg/sec (P<0.05 vs prestimulation) at 15 seconds following stimulation, respectively. Increases in LV peak systolic pressure as well as positive LV dP/dt were maximum at 15 seconds and then gradually returned to the prestimulation level, similar to the observed changes in HR. Myocardial O₂ consumption decreased significantly from 5.4±0.4 to 3.9±0.3×10⁻² mL O₂/mL (n=7, P<0.01) by vagal stimulation, and norepinephrine in the venous effluent did not change significantly from 0.3±0.3 to 0.1±0.2 pmol/L (n=7, NS).

Cardiac responses to vagal stimulation with right ventricular pacing: Figure 1B shows representative recordings of HR, LV pressure, LV dP/dt, and coronary perfusion pressure and flow during vagal stimulation with constant HR induced by ventricular pacing. LV peak systolic and maximal positive LV dP/dt were not changed by vagal stimulation (Figure 2B and Table). Myocardial O₂ consumption (n=7, 5.1±0.9 to 5.0±0.8×10⁻² mL O₂/mL, NS), and norepinephrine concentration in the venous effluent (n=7, 0.4±0.4 to 0.2±0.2 pmol/L, NS) were not changed by vagal stimulation during pacing.

Vagal stimulation with atropine sulfate pretreatment: None of the hemodynamic variables were changed by vagal stimulation after pretreatment with atropine sulfate without ventricular pacing (Table).

Cardiac responses to ACh infusion: Figure 3A shows representative recordings of HR, LV pressure, LV dP/dt, and coronary perfusion pressure and flow during ACh infusion without right ventricular pacing. HR and other hemodynamic variables reached their maximal changes at approximately 2 to 3 minutes of ACh infusion, after which they recovered slightly. As shown in the Table, HR significantly decreased by 46±18% from 205±22 to 111±38 beats/min (n=7, P<0.01 vs pre-stimulation). LV peak systolic pressure and maximal positive LV dP/dt increased significantly by 36±22% from 102±19 to 139±32 mmHg (P<0.05 vs prestimulation), and by 27±25% from 3100±474 to 3933±664 mmHg/sec (P<0.05 vs prestimulation), respectively. These changes in HR, LV peak systolic pressure, and maximal positive LV dP/dt were similar to those at 15 seconds of vagal stimulation without ventricular pacing.

Cardiac responses to ACh infusion with right ventricular pacing: Representative recordings of ACh infusion with right ventricular pacing are shown in Figure 3B,
Figure 2. Changes in hemodynamic variables caused by vagal stimulation without (Figure A) and with (Figure B) right ventricular pacing. Pooled data are shown as means ± SD. Vagal stimulation was performed for 90 seconds (indicated by black bar). Abbreviations are the same as in Figure 1. a; P<0.01 vs prestimulation value (pre); b; P<0.05 vs pre; c; P<0.05 vs 30 sec; d; P<0.05 vs 60 sec; e; P<0.05 vs 90 sec; f; P<0.01 vs poststimulation value (post); g; P<0.05 vs post.
while the Table shows the mean values from ACh infusion during a fixed HR induced by ventricular pacing. LV peak systolic pressure slightly but significantly decreased by 11±8% from 96±15 to 85±14 mmHg (n=7, P<0.05 vs prestimulation) and maximal positive LV dP/dt decreased by 13±9% from 3033±315 to

Figure 3. Changes in hemodynamic variables caused by ACh infusion without (Figure A) and with (Figure B) right ventricular pacing. The recordings were before (Control), during (ACh infusion), and 5 minutes after ACh infusion (Post). Coronary flow was kept constant at 10 mL/min. As shown in Figure 3A, LV peak systolic pressure (95 to 167 mmHg) and maximal positive dP/dt (3000 to 4450 mmHg/sec) increased remarkably in accordance with bradycardia from 180 to 42 beats/min following ACh infusion. CPP also increased, presumably due to decreased metabolic demand and the direct vasoconstrictive action of ACh. In contrast, LV peak systolic pressure and maximal positive dP/dt were decreased by ACh infusion with ventricular pacing, as shown in Figure B. Abbreviations are the same as in Figure 1.
2650 ± 380 mmHg/sec ($P < 0.05$ vs prestimulation).

**DISCUSSION**

The main results from this study are that LV contractility increases with a slowing of HR by vagal stimulation, and that the increase in LV contractility is mostly due to a negative force-frequency relationship because LV contractility is not changed by vagal stimulation with a fixed HR induced by ventricular pacing. On the other hand, a small but significant negative inotropic action for ACh was observed with a fixed HR induced by ventricular pacing. These results indicate that the direct effect of vagal stimulation on LV contractility is basically negative inotropic, probably depending on the density of parasympathetic innervation and the density of muscarinic receptors in the ventricle in individual species. In contrast, the modulation of LV contractility due to HR reduction is negative or positive inotropic, also depending on the species tested. In general, since the direct negative inotropic effect (reduction by $11 \pm 8\%$) observed under ACh infusion with pacing is not overly strong without sympathetic nerve stimulation, the effect of parasympathetic stimulation will be largely affected by inotropic change due to HR reduction, or an intrinsic positive or negative force-frequency relationship.

**Methodological considerations:** In our experimental model, the presence of norepinephrine in the coronary perfusate indicated a certain degree of sympathetic tone. However, the following problems should be discussed. First, HR decreased maximally within 15 seconds after vagal nerve stimulation, but then increased gradually toward its prestimulation value. The mechanism of this is unclear, but it may have been induced by the inactivation of ACh by a high concentration of cholinesterase and the desensitization of cholinergic receptors in the sinus node.\(^{17}\) Second, in contrast to other species,\(^{1-5,18}\) enhanced LV contraction was observed in rat hearts in response to vagal nerve stimulation, indicating a negative force-frequency relationship.\(^{19}\) However, the mechanism of this relationship is unclear from this experiment.

**Effect of vagal stimulation on LV contractility:** Although early investigations failed to show any significant influence of vagal stimulation on LV contractility,\(^{20,21}\) later studies showed that vagal stimulation elicits a negative LV contractile response in the intact animal.\(^{1-5,18}\) However, these studies have been criticized because vagal stimulation not only alters HR but also LV loading and coronary hemodynamic conditions that also affect the strength of LV contraction in *in vivo* experiments. Moreover, it is likely that species differences also contribute to the differences in the results, since the force-frequency relationship is positive in rabbits\(^3\) and dogs\(^2,22\) and negative in rats.\(^{19}\) In addition, muscarinic ACh receptor density in the ventricles is lower in rabbits and rats than in dogs, and lower by one
sixth to one tenth than in the atria in rabbits and rats but not in dogs, \(^7,8\) while ACh content, which indicates vagal innervation density in the ventricle, is one tenth that of the sinus node in rabbits and rats. \(^23,24\)

In this study, LV contractility was not changed by vagal stimulation during ventricular pacing, which confirms previous results with rabbit heart. \(^3\) In contrast, a small negative inotropic effect of ACh was found in this study during ventricular pacing. This different contractile response to ACh administration and vagal nerve stimulation with constant HR might be due to the sparse distribution of vagal nerves in the rat ventricle. The degree of bradycardia was not different with the two interventions, vagal stimulation, and ACh infusion, probably because of similar concentrations of ACh in the sinus node. However, it would be expected that the concentration of ACh in the ventricle might be higher with ACh infusion than with vagal stimulation. \(^24\) McMorn, \textit{et al} reported that \(10^{-6}\) mol/L ACh decreased the contraction of rat ventricular myocytes by approximately 24\%. \(^14\) Thus, it is also possible that vagal stimulation directly induces a negative inotropic action if vagal nerve distribution is abundant, and therefore, ACh release during vagal stimulation is large in the left ventricle, as in dogs. This may explain previous contradictory reports. The negative inotropic effect of vagal stimulation in rabbit heart using the slope of the end-systolic pressure-volume relationship was attributed primarily to a negative chronotropic effect, \(^3\) whereas vagal stimulation exerted a significant direct negative inotropic action that was independent of its bradycardic effect or the level of sympathetic tone, using the same index in dogs. \(^2\) Accordingly, although there are significant controversies on the effects of vagal stimulation on the contractile function of the heart, these are considered to be mainly brought about from methodological problems or differences in the species used. On the other hand, the present experimental design enabled us to clearly resolve this issue because the chronotropic and inotropic effects due to vagal stimulation were independently determined under controlled experimental conditions. In addition, the effects of ACh infusion were compared to those of vagal stimulation, in which the principle of vagal stimulation of cardiac function was shown irrespective of the different vagal innervation densities in different species.

Vasoactive intestinal peptide, as well as ACh, is released from vagal nerve endings and exerts a positive inotropic effect\(^25,26\) and vasodilating action on coronary arteries.\(^13\) The fact that LV contractility was not changed by vagal stimulation after atropine treatment may suggest that the positive inotropic effect of vasoactive intestinal peptide is quite small or absent in rats, probably due to the negligible release of vasoactive intestinal peptide.\(^27\)

Finally, parasympathetic nerve stimulation did not affect LV contractility at a fixed HR, and even in rat ventricle showing augmented contraction with a
decreasing HR induced by vagal nerve stimulation, LV contractility itself decreased if ACh was injected at a fixed heart rate, as found in dogs. An indirect effect caused by inhibiting adenyl cyclase via Gi protein, and direct effects mainly caused by activation of the ACh-regulated potassium channel current have been suggested as the mechanisms for ACh induced negative inotropism. Moreover, the ACh induced negative inotropic effect may be augmented by an “accentuated antagonism” action via sympathetic nerve stimulation. Whatever the reason, this study strongly suggests that the controversy about this issue in the whole heart may be partly explained by the different densities of vagal innervation and muscarinic receptors in the left ventricle. Further study is needed to clarify how the present results may be extended to the contractile response to vagal stimulation in normal and diseased human hearts.

REFERENCES