Sodium channel enhancer restores baroreflex sensitivity in conscious dogs with heart failure

Weiqun Shen, Robert M. Gill, Jian-Ping Zhang, Bonita D. Jones, Angela K. Corbly, and Mitchell I. Steinberg
Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana
Submitted 12 April 2004; accepted in final form 18 November 2004

BAROREFLEX IMPAIRMENT is a prominent characteristic of the heart failure syndrome (5, 12, 23, 38). Blunted baroreflex sensitivity and reduced heart rate variability directly contribute to the morbidity of chronic heart failure as well as to acute coronary events (1, 35); thus preserving baroreflex sensitivity in heart failure should be clinically relevant. Impaired Na+ and Ca2+ regulation has been suggested to mediate alterations of baroreflex function (18), and a voltage-dependent Ca2+ channel promoter was reported recently to restore baroreflex sensitivity in heart failure (36).

Synthetic Na+ channel enhancers have been proposed as a novel class of cardiac positive inotropic agents (8, 9, 32). These agents prolong the open state of cardiac Na+ channels and secondarily enhance reverse-mode Na+/Ca2+ exchange, resulting in an increase of intracellular free Ca2+ (25–27). Their cardiac positive inotropic effects have been demonstrated repeatedly in vitro (6, 10, 21, 25) as well as in vivo (3, 11, 30, 34). Our recent study (30) in conscious dogs with pacing-induced heart failure demonstrated that the Na+ channel enhancer LY-341311 not only increased cardiac contractile performance but also produced a prominent neurally mediated bradycardia in dogs with heart failure, indicating an improved balance between sympathetic and parasympathetic regulation. Thus we hypothesized that the enhancement of Na+ channel opening and the secondary increase of cellular Ca2+ could positively improve impaired baroreflex function in conscious dogs with pacing-induced heart failure.

The primary goal in the present study was to assess specifically the impact of augmented Na+ channel opening on baroreflex sensitivity in heart failure by assessing the baroreflex response during reflex tachycardia in response to temporary pharmacological and mechanical alterations of arterial pressure. We selected the Na+ channel enhancer R-4-[3-{1-(diphenylmethyl)-3-azetidinyl]oxy}-2-hydroxypropylamino)-1H-indole-2-carbonitrile-5-mandelate (LY-368052) for study because it is a close structural analog of LY-341311, which was previously studied, but is ~10 times more potent (19, 32). The secondary goal in the study was to reveal the possible efferent neural mechanisms during the cardiac chronotropic responses to the Na+ channel enhancer by examining the bradycardic effect of LY-368052 in the presence and absence of ganglionic blockade with hexamethonium or β-adrenergic receptor blockade with propranolol. In addition, we compared the cardiac inotropic and lusitropic responses to the Na+ channel enhancer before and after the development of heart failure to ascertain the extent to which this agent retains its hemodynamic effects in the presence of congestive heart failure (CHF).

METHODS

Animal and Surgical Preparation

Male adult mongrel dogs (20–30 kg) were anesthetized with isoflurane in oxygen and ventilated with a respirator after induction with acepromazine (0.03 mg/kg im) and propofol (5.5 mg/kg iv). A left thoracotomy was performed through the fifth intercostal space under sterile technique. Tygon catheters were implanted in the descending thoracic aorta, left atrial appendage, and left ventricle (LV) for measuring pressures. A solid-state miniature pressure transducer (model P6; Konigsberg, Pasadena, CA) was placed into the LV chamber via an apical stab incision for recording LV pressure (LVP).

Address for reprint requests and other correspondence: W. Shen, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285 (E-mail: shen_weiqun@lilly.com).
A pair of piezoelectric ultrasonic crystals was placed on opposing anterior and posterior endocardial surfaces of the LV for measuring LV internal diameter (LVID). A screw-in pacing lead was attached to the right ventricular free wall, and stainless steel pacing wires were placed on the left atrium. An occluder was placed on the inferior vena cava (IVC). All instruments were secured with sutures. The catheters and lead wires were externalized, the thoracotomy was closed in layers, and the intrapleural space was evacuated. Cephalixin (500 mg) was administered postoperatively for 7 days after surgery. Control experiments were initiated 2–3 wk after surgery when the dogs were healthy, i.e., body temperature, blood cell count, and chemistry were within normal limits. The study was approved by the Lilly Institutional Animal Care and Use Committee, and all animals were maintained in accordance with the guidelines in the Guide for Care and Use of Laboratory Animals [DHHS Pub. No.(NIH) 83-23, Revised 1985].

Canine Model of Chronic Heart Failure

After the initial control study, heart failure was induced by chronic rapid right ventricular pacing at 240 beats/min for 3–4 wk with a programmable pacemaker (model EV4543; Pace Medical, Waltham, MA) that was worn externally in a vest.

Measurements and Data Analysis

Dogs were studied in the conscious state while lying quietly on their right side. Hemodynamic measurements were recorded in sinus rhythm after a 20- to 30-min stabilization period after the pacemaker was turned off. All signals were collected online and analyzed on a beat-to-beat basis with a digital data acquisition system (Ponemah; Gould Instrument System). The sampling rate was 250 Hz for arterial pressure (AP) and left atrial pressure (LAP) and 500 Hz for LVP and LVID. AP and LAP were measured with strain gauge transducers (P23 ID; Gould Statham, Valley View, OH) previously calibrated by a mercury manometer and connected to the fluid-filled aortic and left atrial catheters. LVP was measured with a solid-state miniature pressure gauge and calibrated in vivo against the measurement of AP by 10.22 ± 0.32.246 on July 14, 2017

Hemodynamics and LV Function Before and After

Development of Heart Failure

Hemodynamics and LV function were measured in the conscious state, and heart failure was induced by chronic cardiac pacing. Compared with presleeping baseline, there were significant decreases in MAP, LV dP/dt, LVFS, $V_{cfc}$, minimum LV dP/dt (LV dP/dt(min)), LV stroke work, and minute work accomplished by increases in LAP, LVEDP, relaxation time constant (τ), and HR after 3–4 wk of rapid ventricular pacing (Table 1). Exertional dyspnea and ascites were also observed. All hemodynamic and cardiac functional data and clinical signs indicated the development of severe CHF by cardiac pacing, consistent with previous studies (29, 30, 31).
Table 1. Effects of LY-368052 (20 μg·kg⁻¹·min⁻¹) on hemodynamics and LV function in conscious dogs before and after development of heart failure

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LY-368052, %change</th>
<th>CHF</th>
<th>LY-368052, %change</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>82±5</td>
<td>7±8</td>
<td>113±7†</td>
<td>-25±7†</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>103±3</td>
<td>5±5</td>
<td>84±2†</td>
<td>4±4</td>
</tr>
<tr>
<td>Left atrial pressure, mmHg</td>
<td>4±1</td>
<td>-12±20</td>
<td>21±1†</td>
<td>-12±6</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mmHg</td>
<td>8±1</td>
<td>-12±10</td>
<td>24±1†</td>
<td>-9±8</td>
</tr>
<tr>
<td>LV systolic pressure, mmHg</td>
<td>130±4</td>
<td>29±*</td>
<td>97±2†</td>
<td>24±6*</td>
</tr>
<tr>
<td>LV dP/dt max, mmHg/s</td>
<td>3,124±15</td>
<td>123±25*</td>
<td>1,399±85†</td>
<td>137±33*</td>
</tr>
<tr>
<td>LV end-diastolic diameter, mm</td>
<td>34.9±2.3</td>
<td>-2.5±1.1*</td>
<td>41.1±1.5†</td>
<td>-0.1±0.7</td>
</tr>
<tr>
<td>LV fractional shortening, %</td>
<td>25.4±1.1</td>
<td>29±*</td>
<td>9.5±0.8†</td>
<td>117±35*</td>
</tr>
<tr>
<td>LV Vcf, s⁻¹</td>
<td>1.09±0.03</td>
<td>41±9*</td>
<td>0.39±0.03†</td>
<td>158±46*</td>
</tr>
<tr>
<td>LV ejection time, ms</td>
<td>191±5</td>
<td>-14±2*</td>
<td>177±4†</td>
<td>2±3†</td>
</tr>
<tr>
<td>LV dP/dt min, mmHg/s</td>
<td>2.749±76</td>
<td>-2±5</td>
<td>1.584±72†</td>
<td>40±6†</td>
</tr>
<tr>
<td>LV relaxation τ, s⁻¹</td>
<td>24.2±1.05</td>
<td>2±12</td>
<td>35±3±0.71†</td>
<td>-28±6*</td>
</tr>
<tr>
<td>Stroke work, dyn·cm⁻¹·min⁻¹×10³</td>
<td>146±12</td>
<td>47±13*</td>
<td>41±6†</td>
<td>206±61†</td>
</tr>
<tr>
<td>Cardiac work, dyn·cm⁻¹·min⁻¹×10⁶</td>
<td>11.9±1.1</td>
<td>65±18*</td>
<td>5.0±0.6†</td>
<td>123±43*</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, number of animals; LV, left ventricle; LV dP/dt max, LV dP/dt min, maximum and minimum first derivative of LV pressure; Vcf, velocity of circumferential fiber shortening; τ, time constant; bpm, beats per minute. *P < 0.05 vs. baseline; †P < 0.05 vs. control (preparing).

Inotropic, Lusitropic and Bradycardic Effects of LY-368052 in Absence or Presence of CHF

Inotropic, lusitropic, and bradycardic effects of the Na⁺ channel enhancer were examined in seven dogs before and after the development of heart failure induced by cardiac pacing. LY-368052 caused an insignificant change in HR in normal dogs (preparing) but a marked bradycardic response (−26 ± 8%; P < 0.05) in heart failure (Table 1, Fig. 1).

LY-368052 (2–20 μg·kg⁻¹·min⁻¹) resulted in a dose-dependent increase in cardiac contractile performance in both the control state and heart failure (Table 1, Figs. 1 and 2). Compared with preparing, LY-368052 (20 μg·kg⁻¹·min⁻¹) caused a similar increase in LV dP/dt max (137 ± 32% vs. 123 ± 25%) in heart failure. However, without a significant change in LVEDD, LY-368052 resulted in a greater increase in LV FS (117 ± 35% vs. 29 ± 8%; P < 0.05) and in LV Vcf (158 ± 46% vs. 41 ± 9%; P < 0.05) and was accompanied by a longer LV ejection time (2 ± 3% vs. −14 ± 2%; P < 0.05). Thus compared with control, LY-368052 markedly increased both LV stroke work (206 ± 61% vs. 47 ± 13%; P < 0.05) and LV minute work (123 ± 43% vs. 65 ± 18%) in heart failure (Figs. 1 and 2, Table 1). These data therefore clearly demonstrate that the positive cardiac inotropic effect of LY-368052 is preserved in conscious dogs with heart failure.

LY-368052 did not significantly alter LV dP/dt min and relaxation τ in normal dogs (preparing) but caused a significant increase in LV dP/dt max by 40 ± 6% (P < 0.05) and reduction of LV relaxation τ by 28 ± 6% (P < 0.05) in dogs with heart failure (Table 1, Fig. 3), demonstrating an improvement in LV diastolic relaxation.

Inotropic and Bradycardic Effect of LY-368052 in Dogs with Heart Failure in Presence of β-Adrenergic Receptor or Ganglionic Blockade

The inotropic and bradycardic effects of LY-368052 were examined in conscious dogs with heart failure before and after β-adrenergic receptor blockade with propranolol. Compared with baseline, pretreatment with propranolol resulted in a significant decrease in LV dP/dt max by 27 ± 3%, without a significant change in HR. β-Adrenoreceptor blockade was confirmed by the complete inhibition of the response to isoproterenol (Fig. 4, left). LY-368052 increased LV dP/dt max and...
LY-368052 RESTORES BAROREFLEX IN HEART FAILURE

H1511

Fig. 2. Dose-dependent effects of LY-368052 on LV stroke work and LV minute work in conscious dogs (n = 7) before (control) and after development of CHF. All data are means ± SE. *P < 0.05 vs. prepacing control.

 decreased HR to the same extent in the absence and presence of propranolol (1,110 ± 326 vs. 1,349 ± 154 mmHg/s and −43 ± 10 vs. −44 ± 8 beats/min, respectively; Fig. 4, middle and right). The cardiac inotropic and bradycardic effects of LY-368052 were also examined before and after pretreatment with ganglionic blockade. Although the inotropic effect of LY-368052 was unaltered by inhibiting central autonomic outflow to the heart by hexamethonium alone or combined with atropine, its bradycardic effect was nearly abolished (Fig. 5). Thus, in conscious dogs, LY-368052 directly enhances contractility by a β-adrenergic receptor-independent mechanism, but its bradycardic activity is dependent on intact autonomic pathways via an enhancement of parasympathetic tone.

Alteration of Baroreflex Sensitivity by LY-368052

Baroreflex sensitivity was evaluated as the PI/MAP slopes constructed for the HR response to temporary reflex change of blood pressure induced by phenylephrine, nitroglycerin, or IVC occlusion. Compared with the pre-heart failure response, the PI/MAP slopes obtained with phenylephrine (13.6 ± 2.4 vs. 6.1 ± 1.5 ms/mmHg; P < 0.05), nitroglycerin (7.6 ± 0.7 vs. 3.0 ± 0.5 ms/mmHg; P < 0.05) and IVC occlusion (7.4 ± 1.3 vs. 1.5 ± 1.7 ms/mmHg; P < 0.05) were all significantly reduced after the development of heart failure. Treatment of heart failure animals with LY-368052 (10 μg·kg⁻¹·min⁻¹ for 20 min) completely restored the PI/MAP slopes to the control level under each experimental condition (Fig. 6), indicating an acute and specific restoration of baroreflex sensitivity by LY-368052 in heart failure.

Effects of LY-368052 on Electrocardiographic Parameters

The possible impact of Na⁺ channel enhancers on electrocardiographic parameters in dogs with heart failure was examined in the conscious state. LY-368052 caused a significant decrease in heart rate, resulting in increased PR and QT intervals; however, there was no change in QRS duration or QTc interval (Table 2).

DISCUSSION

Decreased baroreflex sensitivity is a hallmark of heart failure and is associated with increased mortality and morbidity in experimental and clinical settings (5, 23). Consistent with previous observations (36, 38), depressed baroreflex sensitivity was demonstrated in the present study by blunt reflex change of HR in response to pharmacological and mechanical alterations of arterial pressure in conscious dogs with pacing-induced heart failure. The most important finding in our study was that the depressed PI/MAP slopes in response to either pharmacological or mechanical alteration of arterial pressure in heart failure were restored completely to pre-heart failure levels after acute treatment with the Na⁺ channel enhancer. The effect of LY-368052 on baroreflex sensitivity in heart failure was similar to that reported for the Ca²⁺ channel promoter BAY y 5959 (36). The Ca²⁺ channel promoter enhanced baroreflex sensitivity in heart failure through a direct central neural mechanism presumably mediated by increased intracellular Ca²⁺ (36). Because increased Na⁺ flux secondarily results in increased intracellular Ca²⁺ stores, it may be reasonable to invoke a similar mechanism for increased baroreceptor sensitivity in the case of the Na⁺ channel enhancer. Alternatively, peripheral aortic and carotid sinus baroreceptor afferent function is known to be depressed in heart failure (22, 38), and enhanced receptor sensitivity due to local alterations in the Na⁺ content of the vessel wall by the Na⁺ channel enhancer might also be involved (18).

LY-368052 also caused a dose-dependent bradycardic effect in heart failure, consistent with our previous findings with the less potent analog LY-34311 (30). The specific bradycardic effect accompanying the positive cardiac inotropic response in heart failure is characteristic for Na⁺ channel enhancers and distinguishes them from catecholamines, such as dobutamine, which are often associated with tachycardia. The bradycardic effect of the Na⁺ channel enhancer was eliminated after ganglionic blockade, indicating that the reduction of HR was mainly mediated by the autonomic nervous system. The bradycardic effect of LY-368052 was completely preserved in the presence of β-adrenergic blockade, demonstrating that the reduction of HR was mediated by increased parasympathetic activity and not due to changes in sympathetic outflow. Interestingly, the bradycardic effect with LY-368052 occurred only

Fig. 3. Dose-dependent effects of LY-368052 on minimum first derivative of LV pressure (LV dP/dtmin) and LV relaxation time constant (τ) in conscious dogs (n = 7) before (control) and after development of CHF. All data are means ± SE. *P < 0.05 vs. prepacing control.
in dogs with heart failure and not in normal dogs. Because vagal withdrawal is a major component of the tachycardia of heart failure (15), this observation suggests that the Na\(^+\)/H\(^+\) channel enhancer specifically corrects the high sympathetic and low parasympathetic tone imbalance in heart failure. It remains unclear whether the bradycardic effect results from the recovery of baroreflex sensitivity induced by the Na\(^+\)/H\(^+\) channel enhancer. However, depressed baroreflex control of HR has been suggested as one of the major mechanisms contributing to the elevated sympathetic tone and depressed vagal activity in heart failure (37, 39). Thus rapid restoration of the depressed baroreflex sensitivity and the rebalancing of sympathetic and vagal tone in heart failure with LY-368052 could lead to an autonomically mediated reduction in HR. However, it is worth noting that the Ca\(^{2+}\)/H\(^+\) channel promoter BAY y 5959 resulted in bradycardia through a direct central effect that was independent of enhanced baroreflex sensitivity (36). Regardless of the precise mechanisms, our data clearly demonstrate that the Na\(^+\)/H\(^+\) channel enhancer was able to rapidly restore impaired baroreflex function.

---

Fig. 4. Effects of LY-368052 on maximum LV dp/dt (LV dp/dt\(_{max}\)) and HR after pretreatment with β-adrenergic receptor blockade (propranolol, 0.5 mg/kg iv) in conscious dogs with heart failure (n = 3). The efficacy of blockade was confirmed by the absence of an isoproterenol response (left). The positive inotropic and negative chronotropic responses to LY-368052 were unaffected by β-adrenergic receptor blockade. All data are means ± SE. *P < 0.05 vs. baseline. MAP, mean arterial pressure; Prop, propranolol; BL, baseline.

Fig. 5. Effects of LY-368052 on LV dp/dt\(_{max}\) and HR after pretreatment with ganglionic blockade, hexamethonium (30 mg/kg iv) alone (n = 4) or with atropine methyl bromide (0.1 mg/kg iv) (n = 5), in conscious dogs with heart failure. After autonomic block, the negative chronotropic response to LY-368052 was abolished (right), but the increase in LV dp/dt\(_{max}\) was maintained (left). All data are means ± SE. *P < 0.05 vs. baseline.
baroreflex sensitivity and permit expression of a negative chronotropic response in heart failure. The combination of bradycardia and positive inotropy produces direct benefit for myocardial energy consumption, especially in the failing heart, because HR is a critical factor contributing to cardiac energy consumption. We showed previously (30) that LY-341311 caused little change in oxygen consumption during its inotropic response in conscious dogs with heart failure, unless the bradycardic effect of the agent was prevented by atrial pacing.

The preservation of the bradycardic response in heart failure also contributes to the cardiac inotropic response to Na+ channel enhancement in heart failure. As expected, LV end-diastolic volume, estimated from LVEDD, did not significantly change with LY-368052 even though HR decreased (Table 1), an observation consistent with the exhaustion of the Frank-Starling mechanism in canine heart failure (17). However, with a slower HR, LV ejection time becomes longer, allowing LV contraction to be more complete during the ejection phase as evidenced as a greater change in LVFS (Fig. 1). Consequently, the Na+ channel enhancer produced larger increases in LV stroke work and LV minute work in heart failure than in control animals.

Catecholamines are used clinically to improve the depressed LV function of heart failure, especially during acute decompensation. However, desensitization due to down-regulation of β-adrenergic receptor and cAMP pathway limits the efficacy of these agents in the therapy of advanced heart failure (7, 16). For example, the cardiac positive inotropic responses to dobutamine, as reflected by LV systolic pressure, LV dp/dt, and LVFS, were reduced by 65% after the development of heart failure (2, 16). The cardiac inotropic responses to LY-368052 in dogs before and after development of heart failure were not different, suggesting that the Na+ channel enhancer may be devoid of desensitization in heart failure. In the current study, LY-368052 significantly increased myocardial contractile performance to the same extent in the presence and the absence of β-adrenergic receptor blockade, indicating that the positive inotropic response is β-adrenergic independent, consistent with earlier in vitro findings with these agents (24, 28).

Of particular interest was the finding that in CHF LV systolic relaxation, as reflected by an increase in LV dp/dt by 40 ± 6% and a reduction of LV t by 28 ± 6%, but caused little change in normal dogs. This was consistent with earlier findings with the less potent analog LY-341311 (13). Thus Na+ channel enhancers appear to exert not only positive inotropic but also positive lusitropic effects in heart failure. Because Na+ channel enhancers indirectly increase intracellular Ca2+ availability, this intracellular Ca2+ gain might have been expected to slow sarcoplasmic reticulum (SR) Ca2+ uptake and have an adverse effect on cardiac diastolic function. The mechanism(s) for the positive lusitropic effect in the failing heart is not known. It has been suggested, however, that a reduction of the Na+ gradient might also cause an increase in both Ca2+ influx and efflux; the latter being secondary to an enhanced release of Ca2+ from the SR (4). Clearly, additional work characterizing this effect at the cellular level is warranted.

Cardiac glycosides produce positive cardiac inotropic effect by increasing intracellular Na+ concentration through inhibition of the Na+-K+ATPase. However, cardiac glycosides are well known to induce or aggravate arrhythmia (14). In the current study, LY-358052 did not significantly prolong the QRS duration or the QTc interval, although there was an increase in the QT interval that accompanied the bradycardia. These ECG interval data from conscious dogs with heart failure were consistent with earlier observations with a less potent Na+ channel enhancer (30). As in anesthetized dogs with infarction (3), we saw no overt arrhythmogenicity; nevertheless, our study was not specifically designed to address the arrhythmogenic potential of LY-368052, and further investigation in appropriate models seems warranted.

In summary, the Na+ channel enhancer LY-368052 restored impaired baroreflex sensitivity in CHF, thereby improving the balance between sympathetic and parasympathetic tone while maintaining potent β-adrenergic-independent inotropic effects. The enhanced myocardial efficiency of this agent compared with catecholamines may be useful in preventing further deterioration of myocardial energy stores in the setting of acute heart failure.

ACKNOWLEDGMENTS

We are grateful to Dr. Gerald D. Smith, Jennifer K. Hochstetler, and Allison Renee Cook, who provided excellent veterinary care throughout the course of the study.
this study. We also acknowledge Karen M. Zimmerman for assistance in providing the drug solutions. We also thank Tanya Wood for expert assistance in the preparation of this manuscript.

REFERENCES


