Opiate Receptor Inhibition Improves the Blunted Baroreflex Function in Conscious Dogs With Right-Sided Congestive Heart Failure

Susumu Sakamoto, MD, and Chang-seng Liang, MD, PhD

The endogenous opiate system is activated in congestive heart failure. Because endogenous opioids are known to depress the baroreflex function, we conducted studies to determine whether the increased endogenous opioids play a role in causing the reduced baroreflex function that occurs in heart failure. Right-sided congestive heart failure was produced in 16 dogs by tricuspid avulsion and progressive pulmonary artery constriction. Seven sham-operated dogs were included for comparison. Baroreflex function was measured in the conscious dogs after pretreatment with either normal saline or an opiate-receptor antagonist by bolus administration of phenylephrine. The slope of the regression line relating systolic blood pressure to cardiac cycle (R-R) interval was taken as an index of baroreflex sensitivity. Plasma \( \beta \)-endorphin was elevated in the dogs with heart failure (15.3±2.5 pmol/l) compared with the sham-operated dogs (4.2±0.4 pmol/l, \( p<0.001 \)). The dogs with heart failure also exhibited a reduced baroreflex sensitivity (3.84±0.19 msec/mm Hg) after saline pretreatment when compared with the sham-operated dogs (10.86±1.20 msec/mm Hg, \( p<0.001 \)). Administration of naloxone hydrochloride increased the baroreflex sensitivity of dogs with heart failure to 5.16±0.26 msec/mm Hg (\( p<0.01 \)) but produced no significant effects in sham-operated dogs (11.36±1.42 msec/mm Hg). To further study the site of action for the effect of naloxone, we measured baroreflex sensitivity in the dogs with heart failure after pretreatment with naloxonazine, a selective \( \mu \)-receptor antagonist, with ICI 154,129, a selective \( \Delta \)-receptor antagonist, or with naloxone methobromide, a quaternary analogue of naloxone that does not penetrate the blood-brain barrier. Like naloxone hydrochloride, naloxonazine increased baroreflex sensitivity (5.31±0.44 msec/mm Hg, \( p<0.01 \)) in heart failure. However, this effect was not produced by ICI 154,129 or naloxone methobromide. The results indicate that the activated endogenous opiate system in heart failure plays a role in modulating the baroreflex. Naloxone probably exerts its effects on improving baroreflex function via an action on central \( \mu \)-opiate receptors.

(Circulation 1989;80:1010-1015)

Diminished baroreceptor reflexes have been well described in cases of congestive heart failure.\(^1\)\(^-\)\(^4\) However, the mechanisms by which this baroreflex dysfunction occurs are not

---


From the Cardiology Research Laboratories (Cardiology Unit), Department of Medicine, University of Rochester Medical Center, Rochester, New York.

Supported in part by the United States Public Health Service Grant HL-35194 and a grant from the American Heart Association, New York State Affiliate.

Address for correspondence: Chang-seng Liang, MD, PhD, Cardiology Research Laboratories, Cardiology Unit, Box 679, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642.

Received January 20, 1989; revision accepted May 16, 1989.
inhibit baroreflex function by impairing the central integration of the baroreflexes in the solitary tract nucleus.6,7 In the present study, we hypothesized that increased endogenous opioids play a role in modulating the baroreflex function in heart failure and that the reflex could be restored by administering the nonselective opiate antagonist naloxone hydrochloride. In addition, we used the relatively selective μ-receptor antagonist naloxazone, the selective Δ-receptor antagonist ICI 154,129,6,9 and the quaternary nalozone methobromide (MeBr), which does not cross the blood-brain barrier,10,11 to determine the site of action of the opioids on baroreflexes.

Methods

Surgical Preparation

Adult healthy mongrel dogs (weight, 20.3–30.7 kg) underwent a modified two-staged procedure for the production of right-sided congestive heart failure.12,13 Animals were anesthetized with pentobarbital sodium (25 mg/kg i.v.) and ventilated with room air using a respirator (Harvard Apparatus Co., South Natick, Massachusetts). In the first operation, a sterile right thoracotomy was performed through the fifth intercostal space and an index finger was inserted via a right atriotomy to rupture the anterior chordae tendineae of the tricuspid valves. A Tygon catheter (1.02 mm i.d.; Norton Co., Plastics and Synthetics Division, Akron, Ohio) was then placed in the right atrium, and the wound closed. Two weeks later, a second sterile thoracotomy was performed through the left fifth intercostal space. A silicone rubber Jones hydraulic occluder (R.E. Jones, Silver Springs, Maryland) was placed around the main pulmonary artery. Tygon catheters were placed in the pulmonary artery, left atrium, and descending thoracic aorta. All catheters were exteriorized at the nape of the neck.

After 2 weeks of recuperation, the dogs were brought to the laboratory for acclimatization and progressive inflation of the pulmonary occluders once or twice a week. Stable congestive heart failure developed in 3–4 weeks and was characterized by ascites, tachycardia, and elevated right atrial pressure. Sham-operated dogs underwent two surgical procedures identical to those described above for the heart failure animals, except neither tricuspid valve avulsion nor the pulmonary artery constriction was included.

The study protocol was approved by the University Committee of Animal Resources and conformed with the guiding principles approved by the American Physiological Society in the use and care of animals and with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Measurements and Protocol

Studies were performed in seven sham-operated dogs and 16 right-sided heart failure (RHF) dogs. Dogs were trained to lie quietly in a lateral decubitus position. The previously implanted catheters were connected to Statham P23Db pressure transducers and a multichannel Brush model 480 recorder (Gould Inc, Instrument Systems Division, Cleveland, Ohio) for measuring pressures. Atraumatic electrocardiographic electrodes were attached to the limbs for measuring R-R intervals. After the animals had rested for 30 minutes, arterial blood was taken for measuring basal plasma β-endorphin by radioimmunoassay44 after extraction on an SP-Sephadex C-25 column, with use of reagents purchased from Immuno Nuclear Corp (Stillwater, Minnesota).

RHF dogs were divided into two groups of eight dogs each. Baroreflex sensitivity was determined in each animal on three sessions conducted at least 2 days apart. Baroreflex sensitivity was examined on the three occasions in group 1 after pretreatment with normal saline, naloxone hydrochloride (1.0 mg/kg), and naloxone MeBr (1.2 mg/kg). In group 2, baroreflex sensitivity was determined after pretreatment with saline, naloxazone (2 mg/kg), and ICI 154,129 (10 mg/kg). The seven sham-operated dogs were studied twice, the two studies separated by at least 2 days, after pretreatment with either normal saline or naloxone hydrochloride (1.0 mg/kg). All agents were dissolved in normal saline and infused intravenously over a 2-minute period. Previous studies8,10,11,15 have shown that these antagonists at the doses chosen produce hormonal and hemodynamic evidence of specific opiate-receptor inhibition in intact animals. Likewise, we have found that the dose of naloxazone administered attenuates the hypotensive effect of the μ-receptor agonist fentanyl (unpublished data). The sequence of the drug pretreatments was randomized in sham-operated and group 1 heart failure dogs. In group 2, the sequence of saline and ICI 154,129 administrations was randomized, but naloxazone was given last because it is a noncompetitive irreversible antagonist.9

Systemic hemodynamics were measured before and after the saline or drug administration. Then, baroreflex sensitivity was determined 10–20 minutes after each pretreatment with a bolus injection of phenylephrine (5–12.5 μg/kg i.v.). The dose of phenylephrine was adjusted in each animal to raise systolic aortic pressure 25–30 mm Hg. The aortic pressure signal and electrocardiographic lead were recorded continuously during the ascending phase of the pressor response to phenylephrine at a paper speed of 100 mm/sec. The R-R intervals were plotted against their respective peak systolic aortic pressures of preceding aortic pulses on a beat-to-beat basis, and the slope of the linear portion of the relation was taken as an index of baroreflex sensitivity.16

Statistics

Data were analyzed with RS/1 Research System (Bolt, Beranek and Newman Software Products
Corp, Cambridge, Massachusetts), and values are given in mean±SEM. The statistical significance of differences among groups was determined by analysis of variance and Student’s t test. The relations between systolic aortic pressure and R-R interval were determined by correlation and regression analyses. A p value of less than 0.05 was considered statistically significant.

**Results**

Table 1 shows the baseline hemodynamic characteristics and plasma β-endorphin levels in seven sham-operated and 16 RHF dogs. The measurements, taken before saline or opiate-receptor antagonist administration, were reproducible during repeated studies; the repetitive measurements were averaged and used for statistical analyses. The RHF dogs exhibited ascites, elevated right atrial pressure, higher heart rates, and lower systolic and mean aortic pressure than sham-operated dogs. The difference of diastolic aortic pressure, however, did not differ between the two groups. In addition, plasma β-endorphin was elevated to levels threefold to fourfold those of the sham-operated dogs.

Normal saline administration produced no significant effects on the systemic hemodynamics in the sham-operated and RHF dogs. Nor did naloxone hydrochloride affect aortic pressure, heart rate, or right atrial pressure in the sham-operated dogs. Naloxone hydrochloride and ICI 154,129 administration in RHF dogs significantly increased mean aortic pressure by 6.1±1.9 and 4.5±1.9 mm Hg, respectively. In contrast, naloxone MeBr caused a transient 5–10 mm Hg decrease in mean aortic pressure, which returned to baseline values within 10 minutes after its administration. Naloxonazine had no effect on mean aortic pressure, heart rate, and right atrial pressure. Furthermore, despite the significant changes produced by naloxone hydrochloride, naloxone MeBr, and ICI 154,129 on mean aortic pressure compared with baseline, systolic aortic pressure, heart rate, and right atrial pressure did not differ significantly by analysis of variance among the repeated studies before phenylephrine administration (Table 2).

Significant correlation coefficients (>0.80) were found for the relations between the phenylephrine-induced increase in systolic aortic pressure and R-R interval in each animal. The doses of phenylephrine we used did not differ between the 16 RHF (5.1±0.1 μg/kg) and seven sham-operated (6.4±0.9 μg/kg) dogs. Nor did the magnitude of increase in aortic pressure produced by phenylephrine differ in the RHF (26.6±1.1 mm Hg) and sham-operated (25.1±1.4 mm Hg) dogs. The doses of phenylephrine needed to produce the target increase in aortic pressure also were not altered by naloxone hydrochloride in sham-operated dogs (6.8±0.9 μg/kg) or by naloxone hydrochloride (5.3±0.3 μg/kg), naloxone MeBr (5.3±0.3 μg/kg), naloxonazine (5.6±0.6 μg/kg), or ICI 154,129 (5.6±0.6 μg/kg) in RHF dogs. To determine the difference in baroreflex sensitivity between the sham-operated and RHF animals, we combined the slopes of the regression lines obtained after saline pretreatment in both RHF groups and compared them with those from sham-operated dogs. The slope was significantly lower in the RHF dogs (3.84±0.21 msec/mm Hg, 

### Table 1. Baseline Hemodynamics and Plasma β-Endorphin

<table>
<thead>
<tr>
<th></th>
<th>Sham-operated (n=7)</th>
<th>Heart failure (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right atrial pressure (mm Hg)</td>
<td>4±1</td>
<td>15±1*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>114±6</td>
<td>146±3*</td>
</tr>
<tr>
<td>Systolic aortic pressure (mm Hg)</td>
<td>127±3</td>
<td>115±3*</td>
</tr>
<tr>
<td>Diastolic aortic pressure (mm Hg)</td>
<td>92±3</td>
<td>84±2</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td>103±3</td>
<td>97±2*</td>
</tr>
<tr>
<td>Plasma β-endorphin (pmol/l)</td>
<td>4.2±0.4</td>
<td>15.3±2.5*</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM. *p<0.001, †p<0.02, ‡p<0.01 vs. sham-operated dogs by Student’s t test.

### Table 2. Heart Rate and Aortic Pressure After Saline and Opiate-Receptor Antagonist Administration

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (beats/min)</th>
<th>Systolic (mm Hg)</th>
<th>Mean (mm Hg)</th>
<th>Right atrial pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sham-operated dogs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>112±5</td>
<td>126±3</td>
<td>104±3</td>
<td>4±1</td>
</tr>
<tr>
<td>Naloxone hydrochloride</td>
<td>111±6</td>
<td>129±4</td>
<td>105±4</td>
<td>4±1</td>
</tr>
<tr>
<td><strong>RHF (group 1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>147±4</td>
<td>115±4</td>
<td>96±3</td>
<td>14±1</td>
</tr>
<tr>
<td>Naloxone hydrochloride</td>
<td>147±5</td>
<td>121±4</td>
<td>102±4</td>
<td>14±1</td>
</tr>
<tr>
<td>Naloxone methobromide</td>
<td>151±5</td>
<td>113±4</td>
<td>94±4</td>
<td>14±1</td>
</tr>
<tr>
<td><strong>RHF (group 2)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>149±5</td>
<td>116±4</td>
<td>98±3</td>
<td>16±1</td>
</tr>
<tr>
<td>Naloxonazine</td>
<td>153±5</td>
<td>115±3</td>
<td>98±4</td>
<td>16±1</td>
</tr>
<tr>
<td>ICI 154,129</td>
<td>152±5</td>
<td>118±3</td>
<td>100±2</td>
<td>17±1</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM. Analysis of variance showed no statistical differences among the sessions within the same group of animals.
addition, they have been shown to have impaired myocardial contractility; low cardiac output; depleted myocardial norepinephrine store; elevated plasma levels of catecholamines, arginine vasopressin, and β-endorphin; and reduced myocardial β-receptor density. These baroreflex, hemodynamic, neurohumoral, and β-receptor changes are similar to those that occur in patients with congestive heart failure. However, our animal preparation differs from clinical left ventricular failure in that left atrial pressure is not elevated.

Our present study further shows that although naloxone hydrochloride exerted no effect on baroreflex sensitivity in sham-operated dogs, it partially restored the blunted baroreflexes in RHF dogs. These effects of naloxone hydrochloride correlated significantly with plasma β-endorphin concentration. The results suggest that although the endogenous opiate system does not normally participate in the regulation of baroreflex function, the activated endogenous opiate system may play a role in modulating the baroreflex function during chronic circulatory failure. Similarly, activated endogenous opioids have been shown to modify baroreflexes in rats during electroconvulsive shock.

Gordon has also shown that endogenous opioids do not play a functionally important role in modulating baroreflexes in normal animals. However, other investigators have shown that naloxone hydrochloride reduces baroreflex sensitivity in pentobarbital-anesthetized normal dogs, whereas it increases baroreflex sensitivity in normal cats and humans. The reasons for the discrepant results are not known but may be related, at least in part, to the use of anesthetics, differences in animal species, or relative basal levels of endogenous opioids among the studies.

The endogenous opiate system is comprised of a group of structurally related peptides (β-endorphin, enkephalins, and dynorphins) and at least three distinct opiate receptors (μ, Δ, and K). These peptides, differing from each other in their precursors and in their affinities for receptors, are present near the cardiovascular centers of the ventrolateral surface of the brain, in the autonomic integratory center of the hypothalamus, and in the peripheral sympathoadrenomedullary system. The endogenous opiate system exerts a variety of potent cardiovascular effects through the sympathetic nervous system. Studies that use specific receptor agonists and antagonists have shown that activation of central μ-receptors causes circulatory stimulation, whereas activation of central Δ-receptors inhibits sympathetic activities and decreases arterial pressure. Our findings that aortic pressure increased after the nonselective opiate antagonist naloxone hydrochloride and the selective Δ-receptor antagonist ICI 154,129 are consistent with the reversal of Δ-receptor-mediated hypotension.

On the other hand, the effects of opioid peptides on baroreflex function probably are mediated via

Figure 1. Bar chart of changes in baroreflex sensitivity (from control after saline pretreatment) produced by naloxone hydrochloride (HCl), naloxone methobromide (MeBr), naloxonazine, and ICI 154,129. Bars indicate SEM. *Values differ significantly from the control at p<0.01. Analysis of variance showed the results differ among the groups at p<0.05.

n=16) than in sham-operated dogs (10.86±1.20 msec/mm Hg, t=5.74, df=21, p<0.001).

Compared with saline pretreatment, naloxone hydrochloride pretreatment produced no significant effect on the baroreflex sensitivity in sham-operated dogs (11.36±1.41 msec/mm Hg, t=0.43). However, baroreflex sensitivity was increased significantly by naloxone hydrochloride in RHF dogs (5.16±0.26 msec/mm Hg, p<0.01). The slope was also increased by naloxonazine in RHF, but it was unaffected by either naloxone MeBr or ICI 154,129 (Figure 1). The increases in baroreflex sensitivity produced by naloxone hydrochloride and naloxonazine in heart failure correlated significantly with the plasma β-endorphin levels (Figure 2) but not with systolic aortic pressure before phenylephrine injection.

Discussion

The present study shows that baroreflex sensitivity was reduced in this model of RHF. The RHF animals showed ascites, high right atrial pressure, increased heart rate, and low aortic pressure. In

Figure 2. Plot of correlation between naloxone- and naloxonazine-induced changes in baroreceptor sensitivity and plasma β-endorphin levels in dogs with right heart failure. r, correlation coefficient.
central $\mu$-receptors at the solitary tract nucleus. Early studies have shown that the baroreflex function is reduced by intracisternal administration of specific $\mu$-receptor agonists but not by $\Delta$-receptor agonists. Similarly, we have found in the present study that the baroreflex sensitivity in RHF increased after the relatively selective $\mu$-receptor antagonist naloxonazine but was affected by neither ICI 154,129 nor naloxone MeBr. The changes in baroreflex sensitivity did not correlate with baseline systolic blood pressure before phenylephrine administration. Furthermore, because acute changes in atrial pressure have been shown to alter the arterial baroreceptor reflex control of heart rate, we also measured right atrial pressure before and after antagonist administration. Our results indicate that right atrial pressure was not altered by any of the opiate-receptor antagonists used. Thus, it appears that the increases in baroreflex sensitivity produced by naloxone hydrochloride and naloxonazine cannot be explained by baseline systolic aortic or right atrial pressures. Most likely, the agents act to increase baroreflex sensitivity by blocking the inhibitory action of the activated endogenous opiate system on the central $\mu$-receptors.

Acute volume loading that elevates right atrial pressure has been shown to increase heart rate and suppress the baroreceptor–heart rate reflex. This finding suggests that an increase in right atrial pressure may cause a tonic inhibitory input via vagal afferent fibers and contribute to the reduction of the baroreceptor-mediated changes of heart rate. However, the activity of atrial vagal afferents during volume expansion is markedly reduced in RHF, suggesting that cardiac baroreceptors are impaired in heart failure. Furthermore, a significant correlation is lacking between the baroreflex sensitivity and left ventricular filling pressure in patients studied after a myocardial infarction. Thus, contrary to the acute elevation of right atrial pressure, the chronically elevated atrial pressure in heart failure may not play an important role in modulating the arterial baroreflexes.

The doses of naloxone hydrochloride, naloxone MeBr, naloxonazine, and ICI 154,129 we used were similar to those used by previous investigators who demonstrated significant opiate-receptor blockade. In the present study, aortic pressure and baroreflex sensitivity increased after administration of naloxone hydrochloride, naloxonazine, or ICI 154,129, suggesting the doses we used were large enough to produce central opiate-receptor blockade. In contrast, neither aortic pressure nor baroreflex sensitivity was increased by naloxone MeBr, probably because of the relative impermeability of blood-brain barrier to the quaternary naloxone. Nevertheless, the dose of naloxone MeBr was probably adequate to produce significant peripheral opiate-receptor blockade because this dose has been demonstrated to abolish the $\Delta$-receptor-mediated duodenal spike potentials produced by morphine.

Our results further show that although opiate-receptor antagonists significantly increased baroreflex sensitivity, they did not normalize the vagally mediated reflex slowing of heart rate in RHF. Our findings may suggest that the endogenous opioids play only a minor role in baroreflex control of heart rate in heart failure. However, the functional importance of the activated endogenous opiate system in the central regulation of baroreflex sensitivity probably cannot be fully established in our animals with a markedly diminished vagal activity. A much greater effect of opiate-receptor antagonists on baroreflexes could have been masked by the markedly diminished vagal tone in our animals. Further studies are warranted to study whether opiate-receptor-blocking agents produce a greater improvement of baroreflex sensitivity in heart failure patients who have lower heart rates and less diminished vagal tone than our animals.

In summary, these results indicate that the activated endogenous opiate system contributes to the depressed baroreflex function in heart failure and that the baroreflex dysfunction in heart failure may be partly restored by naloxone via an inhibitory action on the $\mu$-opiate receptors within the central nervous system.

Acknowledgments

We thank Dr. J. Franklin Richeson for his constructive comments and Lisa B. Guthinger and Amy Miller for their technical assistance.

References


**Key Words** • naloxone • naloxonazine • ICI 154,129 • phenylephrine • reflexes, cardiovascular
Opiate receptor inhibition improves the blunted baroreflex function in conscious dogs with right-sided congestive heart failure.
S Sakamoto and C S Liang

Circulation. 1989;80:1010-1015
doi: 10.1161/01.CIR.80.4.1010

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/80/4/1010