Short-term effects of ecadotril in dogs with induced congestive heart failure

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Objective—To evaluate short-term hemodynamic effects of ecadotril in a model of congestive heart failure in dogs.

Animals—6 conscious adult male dogs.

Procedures—Instruments were placed in dogs to measure left ventricular, aortic, and atrial blood pressures. Heart failure was induced by repeated coronary embolization with latex microspheres. Four times, and in random order, dogs were given vehicle or active drug (3, 10, or 30 mg/kg of body weight) orally. Hemodynamic variables, urine flow, and urinary electrolyte excretion were measured before and 30, 90, and 150 minutes, and 10 and 21 hours after drug administration.

Results—Changes in urine flow, heart rate, mean arterial pressure, or peak positive and negative rate of change in ventricular pressure were not apparent. Urinary sodium excretion significantly increased in response to the low and high doses of ecadotril but not in response to the 10 mg/kg dose. Left ventricular end diastolic pressure (LVEDP) consistently decreased in dose- and time-dependent manner. Maximal group-averaged reductions in LVEDP were 5.2, 8.1, and 10 mm Hg for the low, middle, and high doses, respectively. The magnitude of the decrease in LVEDP was not related to cumulative change in urine flow.

Conclusions and Clinical Relevance—Orally administered ecadotril reduced left ventricular filling pressures in these dogs by a mechanism that does not require a substantial diuretic effect. Ecadotril may be effective for alleviating clinical signs in dogs with left-sided heart failure and may be particularly beneficial for use in dogs that are refractory to traditional diuretic therapy. (*Am J Vet Res* 2000;61: 333–338)

Treatment of congestion and edema is often necessary to control the clinical signs of heart failure. Diuretics, vasodilators, and sodium restriction are commonly used for this purpose. Treatment failures are encountered owing to variable and suboptimal responses to some venodilators, electrolyte-related complications, or inadequate renal function.

The **natriuretic peptides (NP)** are a family of hormones produced and released by heart, kidney, and other tissues. As a group, they have vasodilatory, natriuretic, and diuretic properties, each of which could be beneficial in relieving edema. The effects of NP reflect a balance between types and concentration of circulating NP as well as the number of clearance receptors and the activity of **neutral endopeptidases (NEP)** that catalyze the cleavage of NP to inactive forms.[1-4]

An emerging treatment strategy for congestive heart failure is directed at enhancing the physiologic effects of endogenous NP by inhibiting NEP. The purpose of the study reported here was to evaluate acute hemodynamic effects of ecadotril (formerly sinorphan), an NEP inhibitor, in a model of congestive heart failure in dogs.

Materials and Methods

Animals—Data were collected from 6 adult male hound-type dogs (27 to 33 kg). Dogs were housed separately, with water and a commercial dry food formulated for dogs[a] available ad libitum, in accordance with USDA guidelines.

Instrumentation—Dogs were anesthetized for left lateral thoracotomy, during which a previously calibrated 6.5-mm Konigsberg pressure transducer was secured in the **left ventricle (LV)** through an apical stab incision. A fluid-filled catheter[b] (inside diameter, 0.50 in) was secured within the left atrial appendage to assist in drift correction of the Konigsberg transducer. The catheter and transducer wires were tunneled through tissues and exteriorized behind the skull. After induction of heart failure, each dog again was briefly anesthetized for implantation of fluid-filled catheters in the thoracic aorta and right atrium via the omocervical artery and vein.

Induction of heart failure—We used the coronary embolization model of heart failure described by Sabbah et al.[5] Anesthesia was induced by IV administration of thiopental and maintained by inhalation of a mixture of isoflurane and oxygen. Through percutaneous femoral arterial access, the left coronary arteries (circumflex and cranial descending) were selectively catheterized by use of fluoroscopic guidance to enable slow administration of a bolus injection of between 0.75 and 1.5 ml of latex microspheres (90 to 110-µm diameter) in an aqueous 2.5% suspension. Microsphere embolization was performed once every 7 to 10 days until heart failure developed. For this study, heart failure was defined as ≥ 25% reduction in echocardiographic fractional shortening, ≥ 25% reduction in LV **peak-positive rate of change in pressure (dP/dt)**, and **LV end-diastolic pressure (LVEDP)** that was ≥ 12 mm Hg and double the pre-embolization value. Echocardiographic and LV pressure measurements were recorded before embolization and ≥ 5 days after the last embolization. Echocardiographic images were obtained from conscious dogs positioned in lateral recumbency. Pressure measurements were obtained while dogs were suspended in a sling.

Data measurements—Analog signals were amplified and digitized at 500 Hz for storage on a microcomputer, using commercial software.[c] Heart rate was calculated from a lead-II ECG. Left ventricular end-diastolic, maximal dP/dt (positive and negative), and peak systolic pressures were derived from the Konigsberg micromanometer signal after baseline correction against left atrial pressure, aortic

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pressure, or both. An exception was made for 1 dog that had excess and uncorrectable drift in LV pressure measurements. The dP/dt pressure signals were retained for this dog, but the absolute pressure measurements were discarded. Left atrial, right atrial, and aortic pressures were recorded, using a mercury-calibrated transducer[d] connected to the respective catheters.[b] An atmospheric (zero) reference pressure was calibrated for each dog in the sling, using the height above the floor to the major tubercle of the shoulder. End-diastolic pressures were identified at the moment the LV dP/dt signal rapidly crossed zero in a positive direction. Reported values were the average of ≥ 15 randomly selected sinus-rhythm beats during each recording segment. During periods of data collection, an 8-F balloon-tipped urinary catheter was advanced into the bladder for urine collection. Collected urine was stored at –70 C for subsequent quantification of sodium and potassium concentrations and cGMP excretion.[c]

Protocol—Experiments were conducted, using a repeated-measures design. Each dog was given vehicle and 3 doses (3, 10, and 30 mg/kg of body weight) of active compound orally on separate days and in random order. Experiments were separated by an interval of ≥ 3 days. Food was withheld on the morning of each experiment, and dogs were restrained in a sling. After urinary bladder catheterization and connection of recording instruments, 2,000 U of sodium heparin was administered IV as a systemic anticoagulatant. A supplemental dose of 1,000 to 2,000 U was given approximately 2 hours later. Hemodynamic data and urine samples were collected for 30 minutes immediately before administration of the test agent (baseline data) and again 30, 90, 120, and 150 minutes, and 10 and 21 hours after its administration. Test agents (vehicle and active drug) were given orally, followed by forced ingestion of 30 ml of distilled water.

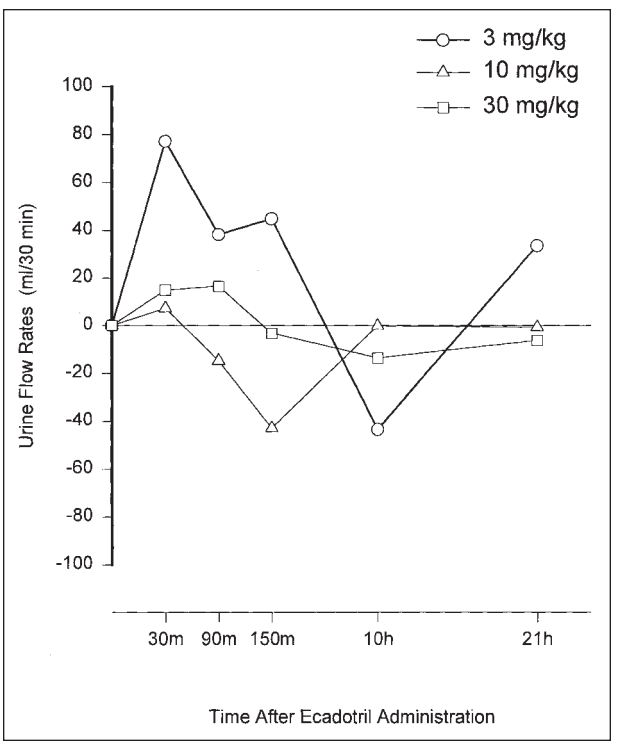

Figure 1—Group-averaged urine flow rates expressed as the vehicle-subtracted change from baseline (time 0) as a function of time after administration of ecadotril (mg/kg of body weight) to 6 dogs. m = Minutes. h = Hours.

Statistical analyses—Data were expressed as mean ± SEM. Comparisons of paired data, such as the hemodynamic and echocardiographic measurements made before and after coronary embolizations, were performed, using a two-tailed paired *t*-test. The effects of ecadotril on hemodynamic values and urinary excretion were determined by use of a Dunnett test for comparison of treatment means with the control (vehicle) mean values at differing time. Urine flow and urinary excretion of sodium and potassium were transformed as the vehicle-subtracted percentage change from baseline and as the sum or cumulative change from baseline for the first 3 collection periods after treatment.

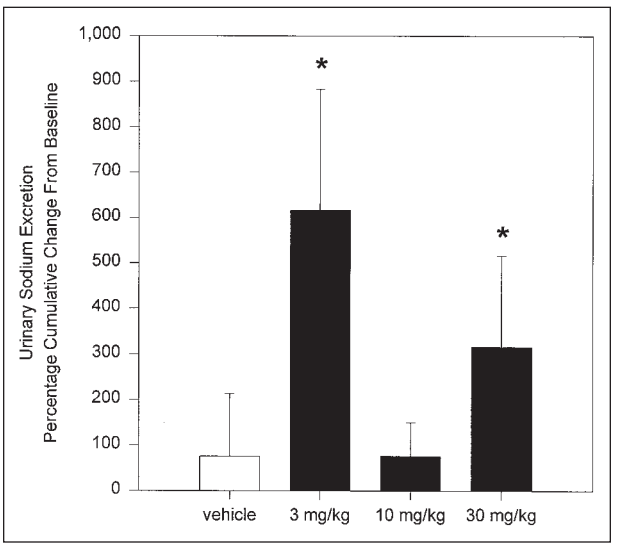

Figure 2—Urinary sodium excretion expressed as the 150-minute cumulative percentage change from baseline after administration of ecadotril to 6 dogs. * = Values are significantly ($P < 0.05$) different from values for the vehicle.

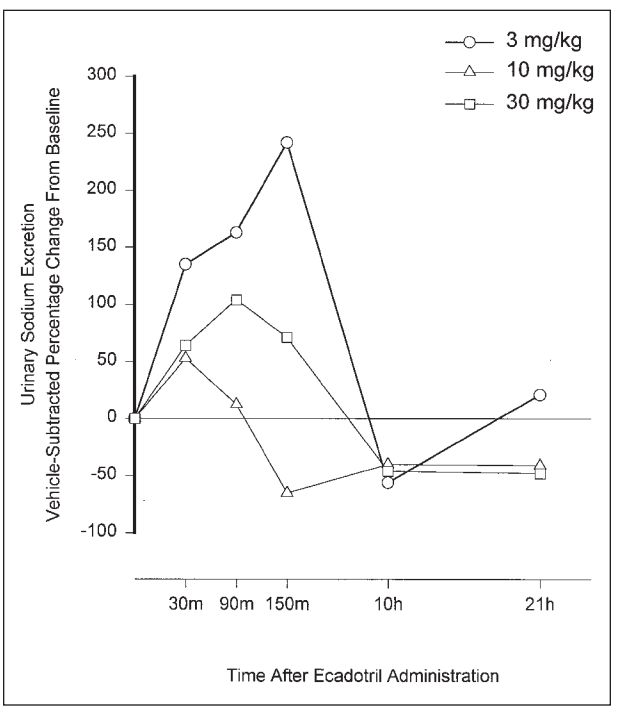

Figure 3—Urinary sodium excretion expressed as the vehicle-subtracted percentage change from baseline as a function of time after administration of ecadotril to 6 dogs.

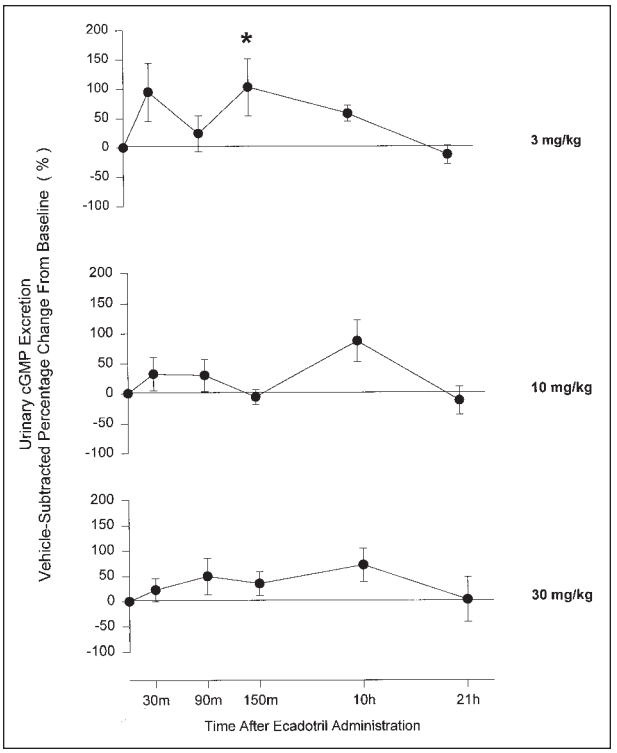

Figure 4—Urinary cGMP excretion expressed as the vehicle-subtracted percentage change from baseline as a function of time after administration of ecadotril to 6 dogs.

Percentage changes were justified on the basis of a high degree of inter- and intra-dog variability in the magnitude of the baseline values for these variables. Cumulative values were used to minimize the slurring effect of repeated measurements in which the response to the drug can peak at differing but closely spaced time points. For these 3 urine excretion variables, a one-way Dunnett test for differences was used. Statistical significance was defined to provide a nominal type-I error rate of 5%. Data for 1 dog could not be collected at 1 time point. Estimates for these missing values were calculated, using the method of Yates.[6]

Results

Severity of heart failure—The 6 dogs available for data collection developed a degree of LV dysfunction characterized by significant reductions in global indices of LV contractile performance, including peak positive dP/dt (4,364 ± 605 to 2,610 ± 307 mm Hg/s), fractional shortening (34.8 ± 2.4 to 20.7 ± 2.3%) and increase in end-systolic LV internal dimensions (27 ± 0.9 to 35.9 ± 1.4 mm). End-diastolic LV diameter did not change significantly (41.9 ± 0.6 to 45.4 ± 1.9 mm); LVEDP increased significantly from 5.3 ± 1 to 16.9 ± 0.8 mm Hg after embolizations in association with a 35% decrease in peak negative dP/dt (3,832 ± 372 to 2,414 ± 255 mm Hg/s). Neither heart rate (106 ± 11 to 121 ± 8 beats/min) nor systolic arterial blood pressure (151 ± 9 to 116 ± 10 mm Hg) was significantly affected by this embolization model of heart failure.

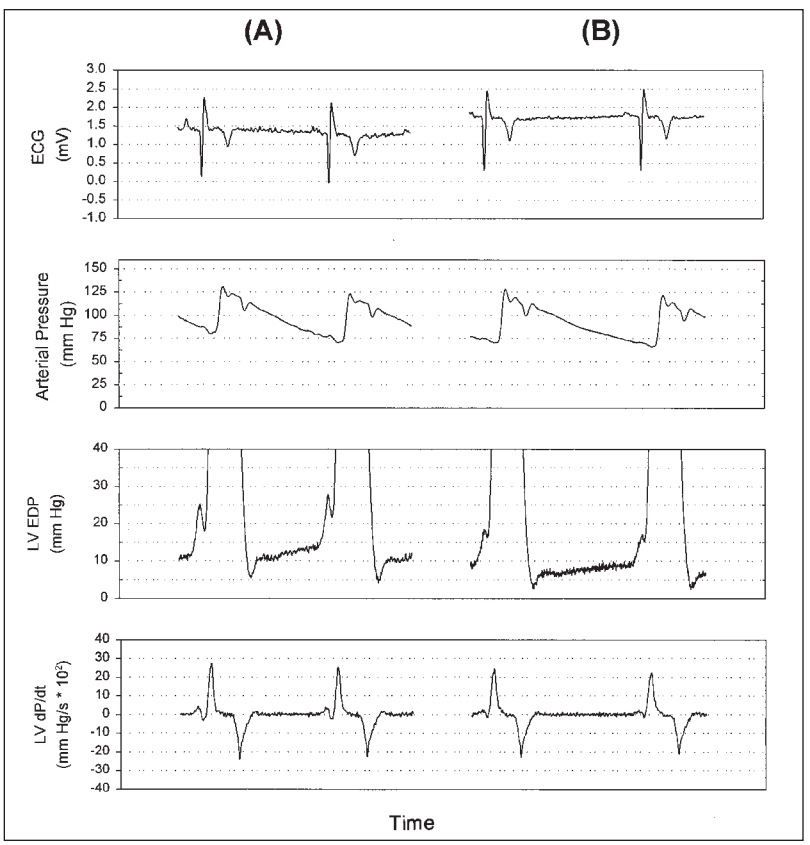

Figure 5—Representative sample of the hemodynamic data from a single dog. Period A depicts data collected before administration of ecadotril (30 mg/kg), whereas period B depicts data collected 90 minutes after ecadotril administration. LV dP/dt = Rate of change in left ventricular pressure. LVEDP = Left ventricular end-diastolic pressure.

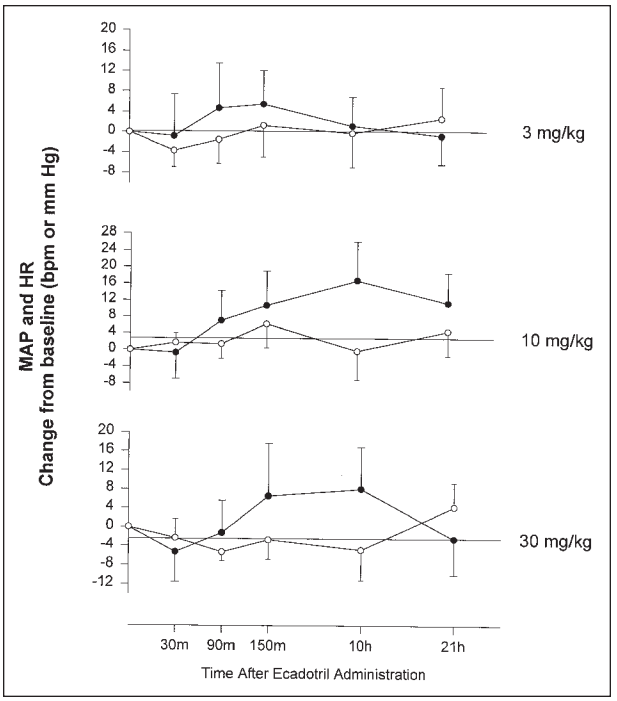

Figure 6—Absolute change from baseline data for heart rate (HR; filled circles) and mean arterial pressure (MAP; open circles) as a function of time after administration of ecadotril to 6 dogs. Data are expressed as mean ± SEM. bpm = Beats per minute.

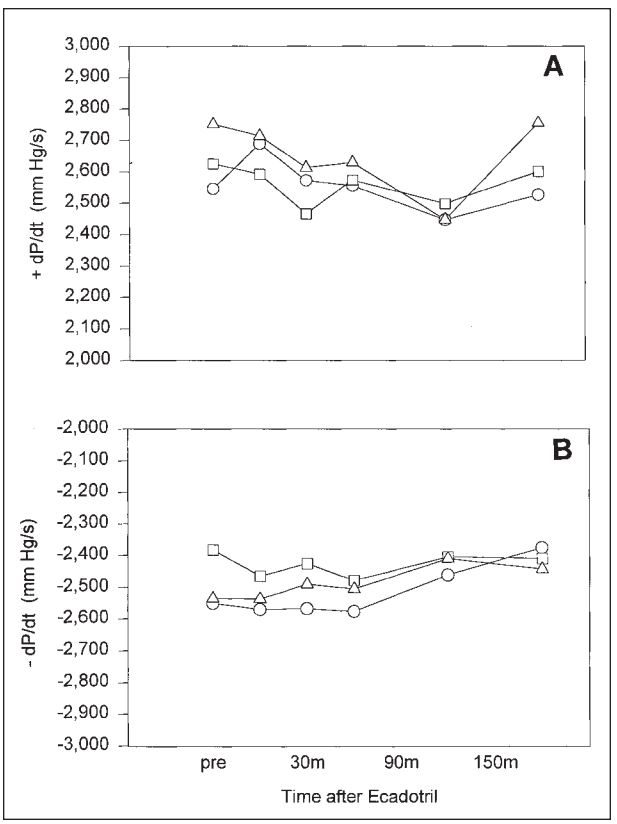

Figure 7—Group-averaged values for left ventricular peak positive dP/dt (A) and peak negative dP/dt (B) as a function of time after administration of ecadotril to 6 dogs. *See* Figure 1 for key.

Urine flow and excretion—A high degree of variation was evident in baseline urine flow among dogs and within each dog on several days. When the cumulative changes from baseline for the initial 3 sample collection periods were calculated, 4 dogs had an increased urine flow at the low dose, 3 dogs had increased flow after the middle dose, and only 2 dogs had an increased flow after the high dose (**Fig 1**). None of the urine flow changes was significantly different from baseline values. The effect of ecadotril on urinary sodium excretion was similar but more consistent among dogs, with a significant natriuretic response associated with the 3 and 30 mg/kg doses but not the 10 mg/kg dose (**Figs 2 and 3**). We did not detect significant effects of ecadotril at any dose on urinary potassium excretion. A significant increase in urinary cGMP excretion was observed only after the low dose of ecadotril was administered (**Fig 4**).

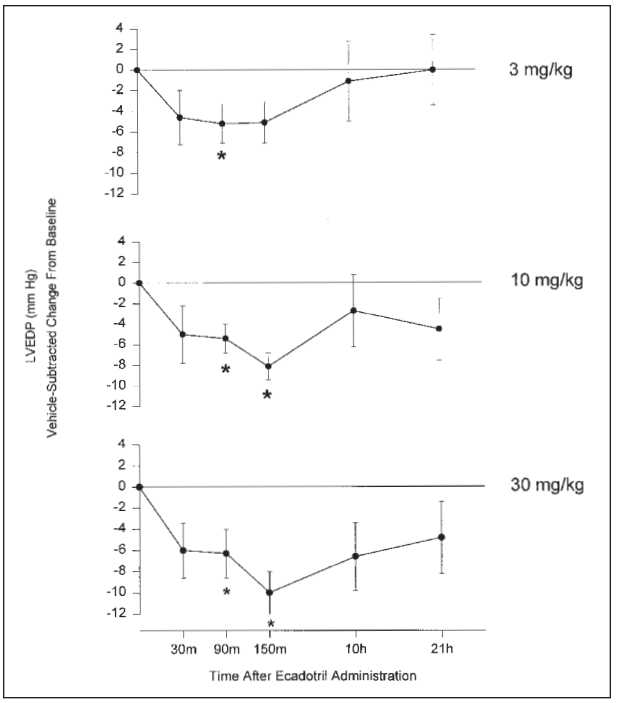

Figure 8—Vehicle-subtracted change from baseline in LVEDP as a function of time after ecadotril administration to 6 dogs. Data are expressesd as group mean ± SEM. * = Values are significantly ($P < 0.05$) different from baseline values.

Hemodynamic changes—A slight but nonsignificant reduction in mean arterial pressure was evident after ecadotril administration (**Fig 5**). Similar to those in arterial pressure, significant changes in heart rate were not observed for the 6 dogs of this study. Small-magnitude changes (5 to 10 beats/min) were frequently observed but not with regularity or any clear dose- or time-dependent pattern (**Fig 6**).

Changes in positive and negative peak LV dP/dt after drug administration were generally small, inconsistent, and unpredictable in direction (**Fig 7**). We did not detect significant changes in either variable attributable to drug administration. The only significant hemodynamic effect of ecadotril in this study was on LVEDP (**Fig 8**). Maximal group-averaged reductions in LVEDP were 5.2 ± 1.9, 8.1 ± 1.3 , and 10 ± 2.0 mm Hg for the low, middle, and high doses, respectively. The

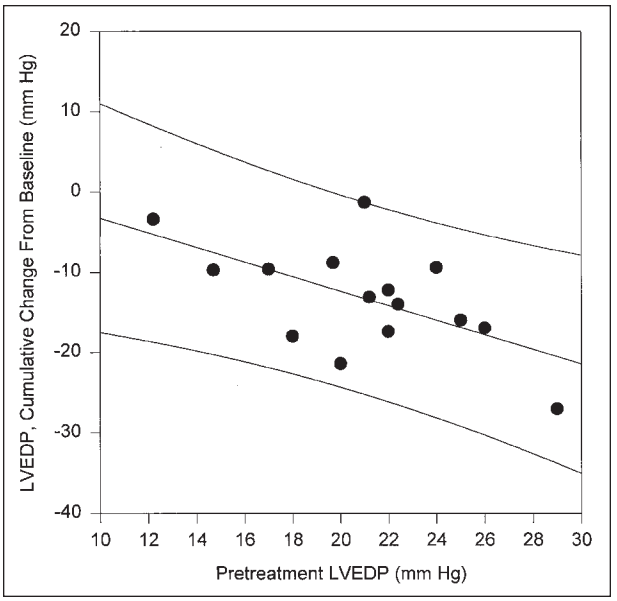

Figure 9—Cumulative change in LVEDP from baseline during the first 150 minutes of data collection, plotted as a function of pretreatment LVEDP. A significant linear correlation is evident ($r = 0.593$, $P = 0.02$).

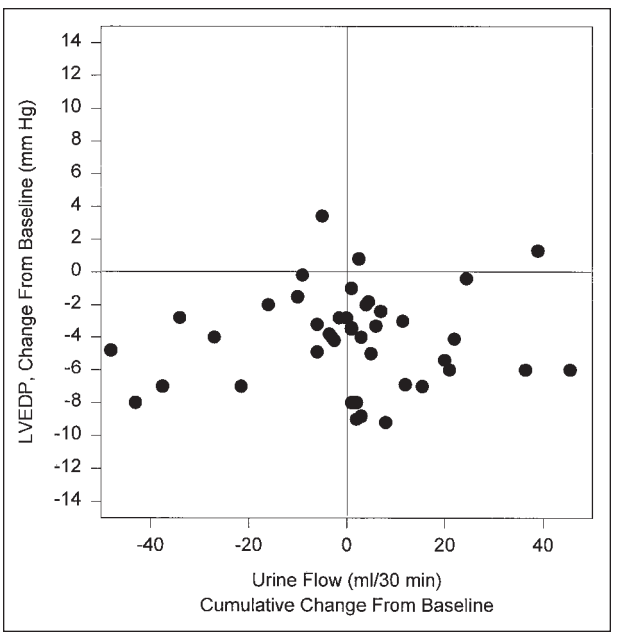

Figure 10—Scattergram of the absolute value of the change from baseline in LVEDP as a function of cumulative change from baseline in urine flow rate.

magnitude of the decrease in LVEDP was linearly correlated to the pretreatment value ($r = 0.593$, $P = 0.02$; **Fig 9**). There was not a recognizable correlation between the decrease in LVEDP and change in urine flow rate (**Fig 10**).

Discussion

The major finding of the study reported here was the decrease in LV filling pressures in response to ecadotril administration, which was rapid, sustained, and of a magnitude likely to be clinically relevant. Reductions after administration of the 10 and 30 mg/kg doses were similar to those observed after enalapril administration during an earlier study.[7]

Potential mechanisms—The effects of ecadotril presumably are associated with the effects of endogenously released natriuretic peptides. The design of this study did not permit thorough evaluation of mechanisms responsible for the reduction in LVEDP. A direct lusitropic, inotropic, or chronotropic effect on the heart or an indirect effect through changing cardiac loads may be involved. A chronotropic mechanism is unlikely, because changes in heart rate of the order observed in this study have been documented to have little or no effect on LVEDP.[8] For similar reasons, changes in systolic loads on the LV are unlikely to be important contributing factors, because consistent and large changes in arterial pressure were not observed after drug administration. Peak positive dP/dt is an imperfect index of contractility, but lack of a significant increase in this variable makes an inotropic mechanism unlikely as an explanation for the substantial decrease in LVEDP. A beneficial lusitropic effect of endopeptidase inhibition cannot be excluded, although we did not observe a significant change in LV peak negative dP/dt, a crude index of ventricular relaxation. Changes in venous return may be the most likely explanation for the reduction in LVEDP observed after ecadotril administration. This could have resulted from a diuretic effect, venodilating effect, or reduction in effective circulating blood volume secondary to an atrial natriuretic peptide-mediated increase in vascular permeability and transcapillary fluid flux. Diuresis as a major, or at least consistent, explanation for the decrease in filling pressure can be discounted in this study, because all dogs had a decrease in filling pressure, whereas only 4 had a noticeable diuretic effect. In 2 dogs, filling pressure decreased despite reduction in urine flow rate after drug administration. This is collectively illustrated as a lack of a recognizable association between the decrease in LVEDP and the cumulative change in urine volume (Fig 10).

Endopeptidase inhibitors typically induce a profound (1.5- to sixfold) increase in urine flow and sodium excretion.[9-11] The average diuretic effect observed in this study was not significantly different from pretreatment values and was quantitatively smaller than expected; maximal percentage change was only 70% above pretreatment values. The natriuretic effect was also less marked than that of most reports, with maximal increases being only 50 to 250%. The modest renal responses to endopeptidase inhibition in the study reported here are unusual but not unique. Varin et al[12] reported findings similar to ours in a study of humans with heart failure treated with ecadotril. Munzel et al[13] also reported variable degrees of diuresis and natriuresis and correlated this to pretreatment cardiac index. They concluded that low cardiac output, by reducing renal perfusion, diminishes renal efficacy of treatment. In our study, we considered the related possibility that the short-term reduction in LV filling pressure decreased cardiac output and renal blood flow and, as a result, decreased the diuretic response. Though blood flow was not measured in this study, we did not observe a significant decrease in arterial blood pressure

or the reflex increase in heart rate expected for this hypothesis.

Cardiac filling pressures have been inconsistently measured in studies evaluating endopeptidase inhibition. Munzel[13] and Northridge[14] reported 21 and 30% reductions in pulmonary capillary wedge pressure, respectively, after endopeptidase inhibition; such values are similar to the maximal reductions in LVEDP in the dogs of our study (22, 28, and 37% for the low, middle, and high doses of ecadotril, respectively).

The microembolization model of cardiac dysfunction induces a mild degree of heart failure, at least initially. Left ventricular filling pressures were clearly high but rarely became so high that overt respiratory distress was evident in any dog at the time of drug evaluation. Even greater drug effects may have been observed in dogs with more severe heart failure, such as those typically hospitalized for treatment. The small sample size and high degree of variability between and within dogs acted in concert to limit the statistical power of some of the calculations. Several urinary excretion responses followed recognizable trends that did not meet the 5% type-I threshold for significance. Although a threshold must be stated a priori, the customary but stringent 5% distinction may have increased the risk of a type-II error. Such a reduction in statistical power is a potential explanation for the qualitative differences in the urinary responses of this study, compared with those of most other reports.

Ecadotril acts consistently and potently to reduce cardiac filling pressures. In this model of failure, it exerted this effect with little or no effect on arterial blood pressure, heart rate, or urinary excretion of potassium. Collectively, these actions are similar to the effect of nitrate venodilators and, to a lesser degree, diuretics. There may be important differences in the mechanism of action and consequences of these 3 preload reducing treatments. Endopeptidase inhibition differs from effects of nitrates and diuretics by virtue of its indirect action to unmask the effects of the naturally released NP vasodilators. Because the circulating concentrations of NP are correlated with the severity of heart failure, maximal endopeptidase inhibition could be an autoregulating form of treatment, with the magnitude of effect titrated to physiologic need. Analysis of our results provide some support for this concept, because the reduction in LVEDP was generally greatest for dogs with the highest pretreatment pressure, one of several measures of disease severity (Fig 9). Another potentially important difference involves the effect of treatment on angiotensin and adrenergic control of circulation, both of which are stimulated by diuretics and nitrates but inhibited by NP.[4,15-17] Preload reduction with neutral or suppressive neuroendocrine effects could make ecadotril an attractive alternative as the sole therapy for mild to moderate degrees of congestive heart failure. On the basis of the sustained responses to long-term infusions of NP, ecadotril also may be free of the tolerance problems typical of long-term nitrate therapy.[18,19] Finally, because analysis of our results suggested that the preload reducing effects of ecadotril do not require a diuretic effect, clinical benefits may be extended to a broad range of clinical situations, including dogs with impaired renal blood flow or intrinsic renal function.[20]

[a]Science Diet Maintenance, Hills Pet Nutrition Inc, Topeka, Kan.
[b]Tygon, Norton Performance Plastics Corp, Akron, Ohio.
[c]ATCodasa, Dataq Instruments Inc, Akron, Ohio.
[d]P23XL, Gould Instruments, Valley View, Ohio.
[e]NEX-133 radioimmunoassay kit, Dupont New England Nuclear Research, Boston, Mass.

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