Intrarenal Effects of Ecadotril during Acute Volume Expansion in Dogs with Congestive Heart Failure

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ABSTRACT

Neutral endopeptidase 24.11 (NEP) inhibitors are known to have vascular, diuretic, and natriuretic effects that may be helpful in the treatment of congestive heart failure (CHF). Most NEP inhibitors may act principally through intrarenal mechanisms, which are not completely understood. The purpose of this study was to determine the principal renal effects of the NEP inhibitor eca-
dotril in dogs with progressive CHF induced by rapid ventricular pacing. Renal function was measured before, during, and after acute i.v. infusion of normal saline in a total of six dogs during normal cardiac function, early left ventricular dysfunction, and overt CHF. During overt CHF, each dog was treated with either eca-
dotril or placebo orally for 1 week. Parameters measured included glomerular filtration rate, renal blood flow, urine output, sodium clearance, sodium frac-
tional excretion, and proximal and distal sodium reabsorption. Ecadotril treatment resulted in increased urine output, sodium clearance, and renal sodium excretion relative to placebo-treated controls. The principal intrarenal effect of eca-
dotril was decreased distal renal tubular sodium reabsorption. Both glog-
merular filtration rate and renal blood flow declined during overt CHF and were unaffected by eca-
dotril treatment. The results of this study are consistent with the principal action of eca-
dotril occurring by way of intrarenal events as opposed to changes in renal hemodynamics. The principal effect of eca-
dotril on distal tubular sodium reabsorption suggests that inhibition of NEP activity in the proximal renal tubules may allow increased bind-
ing of filtered atrial natriuretic peptide to natriuretic peptide receptor sites in the distal renal tubules and collecting ducts.

Inhibitors of neutral endopeptidase 24.11 (NEP; EC 3.4.24.11) are being considered in the treatment of congestive heart failure (CHF) either as single therapeutic agents or in combination therapy (Campbell et al., 1998; Lisy et al., 1998). NEP is active against several neuropeptides, including na-
triuretic peptides, angiotensins, and bradykinins (Richards et al., 1992; Yamamoto et al., 1992; Margulies and Burnett, 1993; Roques et al., 1993; Lisy et al., 1998). In addition to cardio-
vascular effects, inhibitors of NEP activity cause diure-
sis and natriuresis (Margulies et al., 1990). At this time, the mechanism by which NEP inhibition induces these effects during CHF is only partially understood. Gaining such an understanding should be helpful in determining the most effective uses of NEP inhibitors as single therapeutic agents or in combination therapy.

Although NEP inhibitors can augment the activity of many natriuretic peptides and enzymes, potentiation of endoge-
nous atrial natriuretic peptide (ANP) activity has been po-
sulated for some time as the principal target of such therapy (Borgeson et al., 1998; Cavero et al., 1990; Margulies et al., 1990). Inhibition of NEP is thought to decrease the degrada-
tion of atrial natriuretic peptide (ANP) and thereby augment its activity through increased natriuretic peptide receptor binding, primarily by way of the membrane guanyllyl cyclase-
A/natriuretic peptide receptor-A (NPR-A). However, in-
creased plasma ANP concentrations that could result in in-
creased delivery of ANP to the nephron are an inconsistent finding with NEP inhibition (Cavero et al., 1990; Willenbrock et al., 1996). This suggests that the most important effects of NEP inhibition in the induction of natriuresis may be the effects of NEP inhibition within the kidney itself. Renal NEP activity is highest on the luminal membrane in the brush border of proximal renal tubule epithelial cells (Berg et al., 1988; Olins et al., 1987; Shima et al., 1988). In contrast, NPR-A is prevalent not only in the proximal regions of the nephron but also within distal tubules and inner medullary collecting ducts (Healy and Fanestil, 1986; Grandclement and Morel, 1998). Hence, there are several potential intrarenal sites of ANP-receptor interaction.

The NEP inhibitor eca-
dotril [{N-(S)-[2-[(acetylthio)methyl]-

ABBREVIATIONS: NEP, Neutral endopeptidase 24.11; CHF, congestive heart failure; ANP, atrial natriuretic peptide; NPR-A, natriuretic peptide receptor-A; bpm, beats per minute; PAH, para-aminohippurate; VE, volume expansion; PCV, packed cell volume; GFR, glomerular filtration rate; RBF, renal blood flow.
1-oxo-3-phenyl propyl-glycine benzylester; sinorphan] has been shown to be effective in the treatment of CHF (Varin et al., 1991; Wegner et al., 1996; Kimura et al., 1998). The active metabolite of ecadotril is S-thiorphan, which is known to potentiate the natriuretic activity of exogenous ANP (Trapani et al., 1989). However, the principal intrarenal effects of ecadotril during CHF have not been determined. The purpose of this study was to determine how renal function is altered by ecadotril during CHF by comparing the relative contribution of various aspects of renal function on sodium excretion by the kidneys. For this purpose, a model of progressive left ventricular dysfunction, induced by rapid ventricular pacing in combination with acute sodium volume expansion was used to accentuate any effects on renal sodium handling that might be caused by ecadotril treatment.

Materials and Methods

Animals. Six conditioned, male mixed-breed dogs weighing ~25 kg each were housed at the University of Illinois at Urbana-Champaign Laboratory Animal Care Facility for at least 1 week before beginning the study. Each dog was screened for physical abnormalities that could interfere with the study by a complete physical examination, echocardiogram, and electrocardiogram. All dogs were fed a fixed sodium diet (58 mEq/day; Hills ID dog food) and evaluated throughout the study for advanced signs of heart failure and general malaise (e.g., anorexia, reluctance to move) with the contingency plan that dogs showing such signs would be removed from the study.

After evaluation, each dog was anesthetized and a permanent transvenous ventricular pacemaker was implanted. A permanent arterial catheter also was placed into a femoral artery of each dog. The patency of the arterial line was maintained by biweekly heparin flushes. Each dog was allowed to recover for a minimum of 14 days before ventricular pacing was initiated.

Ventricular Pacing. Progressive ventricular dysfunction was induced by rapid ventricular pacing (Luchner et al., 1996, 1998; Jougasaki et al., 1997). After a 14-day postimplantation recovery period (termed study day 0), each dog’s heart rate was set at 180 beats per minute (bpm) for 10 days. This rate and duration of tachycardia cause early left ventricular dysfunction, characterized by decreased cardiac function and increased plasma ANP and nor-epinephrine concentrations; however, there are no measurable effects on renal function, the renin/angiotensin/aldosterone system, or sodium excretion by the kidneys. For this purpose, a model of progressive left ventricular dysfunction, induced by rapid ventricular pacing in combination with acute sodium volume expansion was used to accentuate any effects on renal sodium handling that might be caused by ecadotril treatment.

Renal Function Studies. Renal function was assessed in each dog on day 0 (the onset of ventricular pacing), day 24 (early ventricular dysfunction), and day 32 (7 days of treatment with either ecadotril or placebo during overt CHF). On the afternoon before assessing renal function, each dog was given 300 to 600 mg of lithium carbonate orally. Lithium reabsorption by the kidney occurs principally in the proximal renal tubules, and in parallel with sodium and water, thereby allowing the measurement of lithium clearance to reflect proximal tubule sodium clearance (Thomsen et al., 1981; Burnett et al., 1986; Skott, 1994; Lisy et al., 1998). Food, but not water, was withheld overnight. On the day of each study, the dogs were lightly sedated with acepromazine and buprenorphine and a flow-directed balloon tip pulmonary artery catheter was placed via an external jugular vein for measurement of cardiac output and filling pressures. Indwelling i.v. catheters were placed in a saphenous vein for infusion of inulin and para-aminOHippurate (PAH) and in a cephalic vein for saline infusion. A urinary catheter was placed for the collection of urine samples.

Following recovery from sedation, each dog was placed in a sling harness to maintain a standing position and given an i.v. bolus (0.05 ml/kg) of a 200-mg/ml solution of PAH (Merck & Co., Inc., West Point, PA) and a 100-mg/ml solution of inulin (0.25 ml/kg; Cypress Pharmaceutical Corporation, West Carlsbad, CA). A maintenance i.v. drip of normal saline containing PAH and inulin was begun immediately after the bolus at a rate of 100 ml/h with a Harvard infusion pump. The PAH and inulin concentrations of the i.v. drip were adjusted to maintain a continuous flow of 0.25 mg/kg/min PAH and 0.375 mg/kg/min inulin. At the end of a 45-min equilibration period, the urinary bladder was emptied and a 30-min baseline urine collection period began. An arterial blood sample for EDTA plasma was taken 15 min into the urine collection period. After the 30-min baseline sample was collected, normal saline was infused i.v. at 10% of the dog’s body weight over a 60-min period. The 60-min volume expansion period was divided into two 30-min periods (termed VE-1 and VE-2). Total urine was collected separately over each 30-min period and an arterial blood sample for EDTA plasma and packed cell volume (PCV) was taken 15 min into each 30-min period. After the completion of VE-2, the saline infusion was stopped and urine was immediately collected for an additional 30-min “recovery” period with an additional arterial blood sample taken 15 min into this final period.

Plasma and urine sodium concentrations were determined with ion selective electrodes with an automated chemistry analyzer (Hitachi 911; Boehringer Mannheim, Indianapolis, IN). Serum and urine creatinine concentrations also were done with the automated analyzer. Plasma and urine lithium concentrations were determined by atomic emission spectroscopy with lithium carbonate standards. The PCV was determined by centrifugation of microhematocrit tubes.

Plasma and urine PAH concentrations were determined based on a previously described procedure (Waugh and Beall, 1974). Briefly, 25 μl of urine was incubated at room temperature for at least 15 min in 225 μl of a type III jack bean urease (Sigma Chemical Co., St. Louis, MO) solution prepared by dissolving 5,000 to 10,000 U of urease in 100 ml of 0.05 M potassium phosphate, pH 7.0. After the incubation, 250 μl of the urease-treated urine, or 250 μl of plasma, was mixed with 2.5 ml of a protein precipitation solution consisting of 129 g of p-toluene sulfonic acid and 57 g of dichloroacetic acid in 1 liter of deionized H2O adjusted to a pH of 4.1 ± 0.03. After 10 min, each sample was centrifuged at 2500g for ~5 min and 1.0 ml of supernatant fluid mixed with 1.0 ml of a solution containing 5.0 g of p-dimethylaminobenzaldehyde in 300 ml of 95% ethanol and 200 ml of deionized H2O. The absorbance of each solution was measured at 450 nm and the PAH concentration was determined from a standard curve.

Plasma and urine inulin concentrations were determined based on a previously described procedure (Davidson and Sackner, 1963). Briefly, 250 μl of urine diluted up to 1:100 with deionized H2O or plasma was mixed with 2.25 ml of 0.44 N trichloroacetic acid to precipitate proteins. The solutions were allowed to stand at room temperature for at least 30 min and then centrifuged at 2500g for 5 min. After centrifugation, 500 μl of the supernatant fluid was layered over 3 ml of ice-cold anthrone solution consisting of 500 mg of anthrone, 500 ml of concentrated H2SO4, and 130 ml of deionized
H₂O. Each sample was thoroughly vortexed and placed on ice for 1 min and then incubated at 37°C for 50 min. The absorbance at 620 nm was read and the inulin concentrations determined from a standard curve.

Data Analysis. The following formulae were used for the calculation of renal function parameters: glomerular filtration rate (GFR) = (urine inulin concentration × urine flow) ÷ plasma inulin concentration; renal blood flow (RBF) = [urine PAH concentration × urine flow ÷ plasma PAH concentration] ÷ (1 - PCV) ÷ body weight; lithium clearance (LiCl) = (urine lithium concentration × urine flow) ÷ plasma lithium concentration; sodium clearance (NaCl) = (urine sodium concentration × urine flow) ÷ plasma sodium concentration; fractional excretion of sodium (FENa) = [(urine sodium concentration × plasma sodium concentration) ÷ (plasma sodium concentration × urine sodium concentration)] × 100%; proximal fractional sodium reabsorption (PFNa) = [1 - (LiCl ÷ GFR)] × 100%; and distal fractional sodium reabsorption (DFNa) = [(LiCl - NaCl) ÷ LiCl] × 100%.

Mean values ± S.E. were calculated for each parameter. The calculated data were evaluated for significant differences between study periods and between the ecadotril- and placebo-treated groups by two-way ANOVA and one-way ANOVA with Bonferroni's post-test of multiple comparisons, respectively (Motulsky, 1995). The level of significant differences of all analyses was set at P ≤ .05.

Results

The main signs of overt CHF observed included reduced exercise tolerance, ascites, and pulmonary congestion. Symptoms tended to be most severe in the placebo-treated dogs. The GFR significantly decreased (P < .0001) with the development of overt heart failure, decreasing from an overall mean value of 6.2 ± 0.59 ml/kg/min on day 0 and 7.1 ± 0.70 ml/kg/min on day 24 to an overall mean of 3.7 ± 0.39 ml/kg/min on day 32 of the study (Fig. 1). Ecadotril administration had no significant effect on GFR compared with dogs receiving placebo. RBF (Fig. 2) showed comparable effects to GFR, with a significant decrease in RBF (P < .0001) with overt CHF from 25.6 ± 1.58 ml/kg/min on day 0 and 23.5 ± 1.86 ml/kg/min on day 24 to 13.6 ± 0.45 ml/kg/min on day 32. As with GFR, a difference in RBF between dogs treated with ecadotril and dogs treated with placebo during overt CHF was not observed.

Urine output increased in response to sodium volume expansion during normal cardiac function (day 0) and early CHF (day 24), averaging once saline infusion was begun at 14.9 ± 5.12 and 16.8 ± 4.97 ml/kg/h, respectively (Fig. 3). In contrast, urine output did not increase substantially with saline infusion in the dogs with overt CHF (day 32) that were treated with placebo, averaging 1.6 ± 0.41 ml/kg/h. This was significantly different (P < .0001) from urine output in dogs with overt CHF that were treated with ecadotril. Urine output in the ecadotril-treated dogs was comparable with that observed on days 0 and 24 of the study, averaging 14.2 ± 2.61 ml/kg/h after saline infusion was begun.

The effect of acute sodium volume expansion on days 0 and 24 of the study was to increase NaCl (Fig. 4) and FE Na (Fig. 5). However, by day 32, neither NaCl nor FE Na significantly changed in response to acute sodium volume expansion in the dogs receiving placebo. In contrast, in the ecadotril-treated group, both NaCl and FE Na increased significantly (P < .0001) and to a comparable degree to that observed in the two earlier studies.

Acute sodium volume expansion also resulted in a significa-

![Fig. 1. Measurement of GFR during rapid ventricular pacing and i.v. saline infusion. A. GFR values in dogs with normal cardiac function, with early asymptomatic left ventricular dysfunction and with CHF induced by rapid ventricular pacing. GFR was measured just before beginning the i.v. infusion of saline (baseline). GFR was then measured during two consecutive time points (VE-1 and VE-2) during which each dog was infused with 10% of its body weight in normal saline. The saline infusion was then stopped, and GFR again determined (recovery). The mean GFR was significantly decreased (P < .0001) from control values by the third study. B, GFR with ecadotril administration versus placebo. There was no significant difference in GFR observed between ecadotril- and placebo-treated dogs (mean ± S.E.).](image-url)

Discussion

The principal intrarenal response to ecadotril observed in this study was decreased distal tubular sodium reabsorption.
Similar results have been obtained in other studies, including those studies performed with a competitive inhibitor of NEP (SQ 28,603) infused intrarenally to anesthetized dogs with normal cardiac function, with early asymptomatic left ventricular dysfunction, and with CHF induced by rapid ventricular pacing. RBF was measured during saline infusion over the same time points as described in Fig. 1. Mean RBF was significantly decreased ($P < .0001$) from control values by the third study. B, RBF with ecadotril administration versus placebo. There was no significant difference in RBF between ecadotril-treated and placebo-treated dogs (mean ± S.E.).

Fig. 2. Measurement of RBF during rapid ventricular pacing and i.v. saline infusion. A, RBF values in dogs with normal cardiac function, with early asymptomatic left ventricular dysfunction, and with CHF induced by rapid ventricular pacing. RBF was measured during saline infusion over the same time points as described in Fig. 1. Mean RBF was significantly decreased ($P < .0001$) from control values by the third study. B, RBF with ecadotril administration versus placebo. There was no significant difference in RBF between ecadotril-treated and placebo-treated dogs (mean ± S.E.).

Similar results have been obtained in other studies, including those studies performed with a competitive inhibitor of NEP (SQ 28,603) infused intrarenally to anesthetized dogs with normal cardiac function (Margulies et al., 1990) or to dogs with experimentally induced acute heart failure and natriuretic peptide receptor inhibitors (HS-142-1; Stevens et al., 1996). For the current study, it was evident that the major site of effect on sodium reabsorption was after passage of filtrate through the proximal renal tubule. The primary renal tubule-binding site of ANP is guanylyl cyclase A/NPR-A. Although ANP receptor binding occurs in glomerular and proximal renal tubular sites, ANP-binding sites have been localized in high concentration to the inner medullary collecting ducts (Healy and Fanestil, 1986; Koseki et al., 1986). In addition, ANP has been found to inhibit tubular reabsorption primarily between the superficial late distal tubule and papillary collecting duct base and the papillary collecting duct (Fried et al., 1988). However, infusion of ANP results in not only distal renal tubule effects but also proximal renal tubule effects, glomerular effects, and increased GFR and RBF (Takeda et al., 1986; Ballermann and Brenner, 1987). Similar effects are observed with other members of the ANP family of peptides, including brain natriuretic peptide and urodilatin, when administered systemically or by way of the renal blood system and in spite of the fact that under physiologic conditions, urodilatin is probably more involved in the regulation of electrolyte excretion than blood pressure regulation (Forssmann et al., 1998; Meyer et al., 1998).

Fig. 3. Urine output with rapid saline infusion. Urine production was measured during saline infusion over the same time points and conditions as described in Fig. 1. A, urine production in dogs with either normal cardiac function or with early asymptomatic left ventricular dysfunction induced by rapid ventricular pacing. B, effect of ecadotril administration on dogs with CHF versus those who received placebo. Mean ± S.E., **$P < .01$ and ***$P < .001$ compared with urine output in the placebo-treated dogs at each time point.
filtered ANP to distal tubule sites that it normally does not access.

The prolonged and progressive method of induction of CHF used in this study may have accentuated the distal effects observed due to down-regulation of more proximal binding sites. With time, one effect of CHF is elevation of circulating ANP concentrations (Wilkins et al., 1990; Wilkins and Needleman, 1992). Prolonged exposure to increased exogenous ANP concentrations results in a suppressed renal response due to down-regulation of receptor numbers. With inhibition of NEP in the proximal tubules, a larger percentage of the ANP filtered through the glomerulus would gain access to receptor sites in the distal tubules and collecting ducts. Compared with proximal tubule sites, these sites are relatively isolated from systemic ANP and therefore their response to ANP is anticipated to be less suppressed. It had been proposed that inhibition of NEP by thiorphan allows filtered ANP to gain access to renal tubule sites that are normally inaccessible (Wilkins et al., 1990). It was further proposed that NEP inhibition would allow filtered ANP to reach normally inaccessible renal tubule sites where receptors would not have been down-regulated due to their isolation from the high circulating ANP levels. The findings of our study are consistent with this hypothesis. It also has been shown that the concentrations of ecadotril are relatively high in the kidneys for prolonged periods.4 This and regional differences in the intrarenal concentration of NEP 24.11 also may have been a factor.

This study has shown that ecadotril administered orally for 1 week can effectively induce diuresis and natriuresis in dogs with progressive CHF caused by rapid ventricular pacing. The use of the progressive left ventricular dysfunction pacing model in conjunction with acute sodium volume expansion enhanced greatly our ability to detect the effects of ecadotril on renal function during overt CHF. The rapidity

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4 Steinke W and Schwarz T. [14-C]BAY y 7432: Distribution of the radioactivity in rats after single oral and l.v. administration (whole body autoradiography). PH-report 23165, April 11, 1994, Bayer AG.
and degree of the response in the ecadotril-treated group to saline infusion was comparable with that observed during periods of normal cardiac function and early asymptomatic ventricular dysfunction. In contrast, ecadotril almost completely prevented pathophysiologic changes as a consequence of CHF induced by rapid pacing.

Our data also have shown that during CHF ecadotril administration is not associated with alterations to either RBF or GFR. This finding is similar to that of an earlier study that examined the effects of a different NEP inhibitor (SQ 28,603) during CHF and acute volume overload and that concluded that induction of natriuresis by NEP inhibition under such conditions may be independent of alterations to systemic or renal hemodynamics (Cavero et al., 1990).

In conclusion, the findings of this study demonstrate that the oral administration of ecadotril for 1 week during progressive CHF induced by rapid ventricular pacing results in a significant diuretic and natriuretic effect in response to acute sodium volume expansion. The effects of ecadotril occur by way of intrarenal events as opposed to changes in renal hemodynamics. The principal renal effect of ecadotril was on distal tubular sodium reabsorption. This is consistent with the hypothesis that the chain of events leading to increased natriuresis by the metabolite of ecadotril, S-thiorphan, occur through inhibition of NEP 24.11, diminished natriuretic peptide degradation, enhanced NPR-A receptor binding in the distal tubules and collecting ducts, and activation of NPR-A receptors.

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References


