Journal of the American College of Cardiology
© 2004 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 44, No. 6, 2004
ISSN 0735-1097/04/$30.00
doi:10.1016/j.jacc.2004.04.059

**PRECLINICAL RESEARCH**

# Furosemide and the Progression of Left Ventricular Dysfunction in Experimental Heart Failure

John M. McCurley, MD,* Stephen U. Hanlon, MD,* Shao-kui Wei, MD,* Erich F. Wedam, MD,† Michael Michalski, MD,‡ Mark C. Haigney, MD*

*Bethesda, Maryland; and San Diego, California*

**OBJECTIVES** We tested the hypothesis that furosemide accelerates the progression of left ventricular systolic dysfunction in a tachycardia-induced porcine model of heart failure.

**BACKGROUND** Furosemide activates the renin-angiotensin-aldosterone system in patients with congestive heart failure (CHF). Such activation may contribute to CHF progression, but prospective data are lacking.

**METHODS** Thirty-two Yorkshire pigs were randomized to furosemide (1 mg/kg intramuscularly daily, mean 16.1 ± 0.9 mg) or placebo. Thereafter, a pacing model of heart failure was utilized to produce systolic dysfunction in both sets of animals (fractional shortening <0.16 by echocardiogram). The goal was to determine if furosemide would accelerate the progression of left ventricular dysfunction in the "treated" group. After sacrifice, sodium-calcium exchanger currents and their responsiveness to isoproterenol were measured during voltage clamp. All investigators were blinded to treatment assignment.

**RESULTS** Furosemide shortened the time to left ventricular dysfunction (35.1 ± 5.1 days in placebo versus 21.4 ± 3.2 days for furosemide animals; $p = 0.038$, log-rank test). By day 14, aldosterone levels were significantly higher in furosemide animals (43.0 ± 11.8 ng/dl vs. 17.6 ± 4.5 ng/dl; $p < 0.05$). Serum sodium was reduced (133.0 ± 0.9 mmol/l furosemide vs. 135.7 ± 0.8 mmol/l placebo; $p < 0.05$), but no difference in norepinephrine, potassium, magnesium, creatinine, or urea nitrogen was present. Basal sodium-calcium exchanger currents were significantly increased and isoproterenol responsiveness depressed by furosemide.

**CONCLUSIONS** Tachycardic pigs given furosemide had significant acceleration of both contractile and metabolic features of CHF, including left ventricular systolic dysfunction, elevated serum aldosterone levels, and altered calcium handling in a controlled experimental model of heart failure. (J Am Coll Cardiol 2004;44:1301–7) © 2004 by the American College of Cardiology Foundation

Furosemide is a non–potassium-sparing loop diuretic prescribed for the majority of patients with heart failure (1), and American College of Cardiology guidelines recommend the use of loop diuretics for the treatment of fluid overload in congestive heart failure (CHF) (2). Although furosemide

**See page 1308**

provides an immediate benefit to the patient in reducing fluid overload, its effect on the progression of left ventricular systolic dysfunction is not known. In a recent retrospective analysis of the Studies Of Left Ventricular Dysfunction (SOLVD), the use of non–potassium-sparing diuretics (including furosemide) was associated with increased risk of hospitalization for progression of CHF, increased risk of death from progressive CHF, and increased cardiovascular and all-cause mortality when compared with no diuretic or combination therapy (i.e., combination of a potassium-sparing with a non–potassium-sparing diuretic) (3). Furosemide activates the renin-angiotensin-aldosterone system (RAAS) in patients and animals in heart failure (4,5). Activation of the RAAS is associated with the progression of heart failure (6,7); in patients with symptomatic CHF, plasma aldosterone levels are chronically elevated and prognostically significant (8). A mechanistic role for aldosterone in heart failure was implied in the Randomized Aldactone Evaluation Study (RALES), which demonstrated that an aldosterone antagonist substantially reduced the risk of death and hospitalization when added to standard therapy (9). Despite their widespread use and concerns regarding their detrimental potential in heart failure, no prospective randomized controlled trial of the effect of loop diuretics has ever been performed in humans or experimental animals. We conducted a blinded, randomized, controlled trial in an experimental animal model of heart failure to test the primary hypothesis that furosemide accelerates the progression of systolic dysfunction. Using the tachycardic pacing model, we randomized 32 Yorkshire pigs to daily injections

From the *Division of Cardiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland; †Division of Cardiology, National Naval Medical Center, Bethesda, Maryland; and the ‡Division of Cardiology, Naval Medical Center, San Diego, California. Drs. McCurley and Hanlon contributed equally to this work. The views expressed in this paper reflect the opinions of the authors only and are not the official policy of the Uniformed Services University, the United States Navy, or the Department of Defense.

Manuscript received December 23, 2003; revised manuscript received March 31, 2004, accepted April 20, 2004.

JACC Vol. 44, No. 6, 2004
September 15, 2004:1301–7

**Abbreviations and Acronyms**

BUN = blood urea nitrogen
CHF = congestive heart failure
NCX = sodium-calcium exchanger
RAAS = renin-angiotensin-aldosterone system
RALES = Randomized Aldactone Evaluation Study
SOLVD = Studies Of Left Ventricular Dysfunction

of furosemide or placebo and assessed ventricular chamber size and systolic performance with blinded serial echocardiograms. Neurohumoral activation, an important contributor to heart failure progression, was assessed using serum aldosterone and norepinephrine levels (10). Finally, downregulation of the beta-adrenergic receptor system has been recognized as an important predictor of heart failure progression. A tonic increase in the phosphorylation state of the calcium-handling proteins contributing to the depressed contractile response to catecholamines in CHF has recently been described (11). We have reported that heart failure is associated with an increase in the sodium-calcium exchanger (NCX) current and a reduction in responsiveness to beta-adrenergic receptor stimulation due to augmented phosphorylation of the exchanger by protein kinase A (12). We asked whether furosemide use would be associated with a further increase in NCX current and a greater reduction in responsiveness to isoproterenol than placebo.

The purpose of this study, therefore, was to assess whether the capacity of furosemide to accelerate the activation of the RAAS during incipient CHF was accompanied by deleterious changes in contractile performance and calcium handling.

## METHODS

**Study population.** Thirty-two Yorkshire pigs of either gender (Sus scrofa domesticus: males; 5 furosemide and 4 placebo vs. females; 11 furosemide and 12 controls) between 7 to 10 weeks of age underwent permanent transvenous pacemaker implantation. There was no sham treatment arm. Before the initiation of pacing, the animals were randomized in blocks of four to treatment with furosemide (1 mg/kg intramuscularly) or intramuscular saline (placebo). The dose of furosemide was similar to or less than that given in previous studies using dogs (13). Animals were given ad libitum access to water, were not restricted with respect to salt, and were weighed every five days. All investigators were blinded to treatment assignment throughout the study; veterinary technicians administered the study drug and observed the animals for lethargy, inappetance, vocalization, or other signs of symptomatic heart failure. Animals were sacrificed within 24 h of developing systolic dysfunction, as defined by a fractional shortening of <16% (see the following text). The protocol was reviewed and approved by the Institutional Animal Care and Utilization Committee of the Uniformed Services University of the Health Sciences and conforms with the guidelines endorsed in the "Position of the American Heart Association on Research Animal Use" adopted November 11, 1984 by the American Heart Association.

**Pacemaker implantation.** Animals were initially sedated with ketamine (10 mg/kg, intramuscularly) for placement of an ear vein intravenous catheter (20 gauge). Anesthesia was induced with thiopental sodium (10 mg/kg, intravenously) to allow tracheal intubation and then maintained with isoflurane. The right external jugular vein was isolated and cannulated. An active-fixation pacing lead was advanced under fluoroscopy to the apex of the right ventricle. Adequate positioning was confirmed by pacing threshold and R-wave sensing, and the lead was sutured to underlying fascia. Prepectoralis fascia lateral to the cutdown was bluntly dissected to create a pocket for the pacemaker (courtesy of St. Jude Medical, Sylmar, California). The inactive pacemaker was connected to the lead, and the wound closed using two layers (2-0 vicryl, 3-0 vicryl). Veterinary personnel observed the animals in a warming cage until ambulatory. After 48 h of recovery, pacing was initiated at 200 beats/min.

**Echocardiography.** All animals underwent transthoracic echocardiography every 5 ± 0.5 days, using an Acuson (Mountain View, California) 128 XP/10 imaging system with a 4-MHz phased array transducer. Pacing was discontinued 60 min before imaging. During imaging, animals were sedated with tiletamine 2 to 3 mg/kg combined with zolazepam 2 to 3 mg/kg intramuscularly (Telazol, Lederle Parenterals, Carolina, Puerto Rico). Weight and heart rate were recorded for each animal. Echocardiographic measurements were made from the M-mode recording of the right parasternal short-axis view (14), and included left ventricular wall thickness, end-systolic and end-diastolic dimensions, and fractional shortening. Normal values for echocardiographic measurements were derived from normal swine of the same species, strain, and age range imaged before this study (mean fractional shortening, 0.32; standard deviation 0.08; n = 27). Significant left ventricular systolic dysfunction was defined prospectively as a fractional shortening <0.16, or two standard deviations below the mean value in this normal sample.

**Laboratory analysis.** Blood samples were obtained at the time of pacer implantation, day 14 and 21 of pacing (if the end point was not yet reached), and at time of sacrifice. Samples were analyzed for serum sodium, magnesium, potassium, blood urea nitrogen (BUN), creatinine, aldosterone, and norepinephrine. Electrolyte assays were evaluated by direct potentiometry (VITROS system 250, Johnson and Johnson, Rochester, New York). Neurohormonal levels were obtained utilizing extraction chromatography immunoassays (Quest Diagnostics, Baltimore, Maryland). Baseline samples were obtained immediately after cannulation of the right external jugular vein. Before echocardiographic assessment, blood samples were obtained from sedated animals by direct puncture of the retrosternal venous plexus.

Sacrifice samples were obtained from the pulmonary artery after the animals were sedated and before euthanization.

**Euthanasia and myocyte isolation.** Upon the development of significant left ventricular systolic dysfunction, paced animals were euthanized with sodium pentobarbital (50 mg/kg intravascularly) and saturated potassium chloride (20 ml intraventricular via left ventricular puncture with an 18 gauge needle). Additionally, 11 unpaced control animals were sacrificed to provide nonfailing myocytes. The hearts were harvested by left lateral thoracotomy, then immersed in ice-cold saline. The region of ventricle perfused by the left anterior descending coronary artery was excised, cannulated, and perfused at 15 ml/min for 10 min with nominally $Ca^{2+}$-free modified Tyrode's solution (in mmol/l, NaCl 138, KCl 4, $MgCl_2$ 1, $NaH_2PO_4$ 0.33, glucose 10, and HEPES 10 [pH 7.3 with NaOH] at 37°C and oxygenated with 100% $O_2$). Perfusion was continued for 12 min with the same solution but with added 0.24% (w/v) collagenase type I (Sigma, St. Louis, Missouri) and 0.028% protease XIV (Sigma), and then for 10 min with washout solution with 0.1 mmol/l $CaCl_2$ and 0.02% albumin. Sections of well-digested ventricular tissue from the midmyocardial layer of the ventricle were dissected out, and cells mechanically dissociated and resuspended in buffers of gradually increasing [$Ca^{2+}$]. To remove dead myocytes and residual contaminating cell types, the myocyte suspension was centrifuged through a discontinuous Percoll gradient, usually resulting in over 90% rod-shape cells. To allow the myocytes to recover from enzymatic digestion, the cells were cultured overnight at 37°C in serum-free medium 199 supplemented with 5 mmol/l carnitine, 5 mmol/l creatine, 5 mmol/l taurine, 100 $\mu$/ml penicillin, 100 $\mu$g/ml streptomycin, and 0.25 $\mu$g/ml amphotericin.

**Measurement of the NCX current.** Whole-cell recordings were obtained at 37°C using standard patch-clamp techniques. Membrane current was assessed by use of an Axopatch-100A amplifier and a 1/100 CV-3 headstage (Axon Instruments, Union City, California). Experimental control, data acquisition, and data analysis were accomplished by use of the software package Pclamp 8.0 with the Digidata 1200 acquisition system (Axon Instruments). Patch-pipettes were pulled from thin-walled glass capillary tubes and heat-polished; electrode resistance ranged from 1 to 2 MΩ. The external solution contained (mmol/l): NaCl 145, $MgCl_2$ 1, HEPES 5, $CaCl_2$ 2, CsCl 5, and glucose 10 (pH 7.4, adjusted with NaOH). Ouabain (0.02 mmol/l) and nifedipine (0.01 mmol/l) were added to the solution. The effects of full-scale beta-adrenergic stimulation were achieved by adding isoproterenol (2 $\mu$mol/l). The internal solution contained (mmol/l): CsCl 65, NaCl 20, $Na_2ATP$ 5, $CaCl_2$ 6 or 13, $MgCl_2$ 4, HEPES 10, tetraethyl ammonium chloride 20, EGTA 21, and ryanodine 0.05 (pH 7.2 adjusted with CsOH). Membrane currents were elicited by using a standard voltage ramp protocol. From a holding potential of −40 mV, a 100-ms step depolarization to +80 mV was followed by a descending voltage ramp (from +80 mV to −120 mV at 100 mV/s). The protocol was applied every 10 s; $Ni^{2+}$ (5 mmol/l) was added to assess the fraction of current that derives from NCX (total current remaining after subtraction of post-$Ni^{2+}$ trace), the NCX current being defined as the Ni-sensitive current. Membrane capacitance was directly read from the membrane test function of Pclamp 8.0 before compensating series resistance and membrane capacitance.

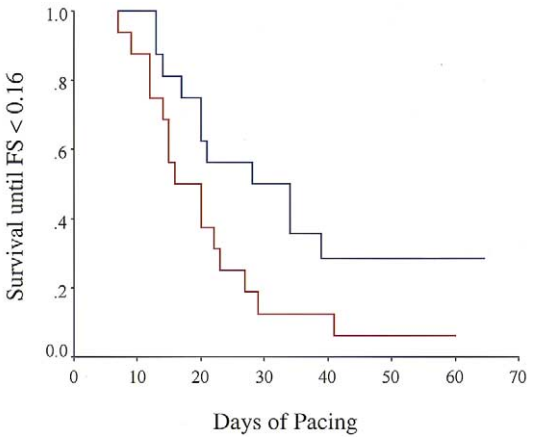


**Figure 1.** Cumulative probability of the development of severe systolic dysfunction as defined as a fractional shortening (FS) of <0.16 in placebo-treated **(blue line)** and furosemide-treated animals **(red line)** (n = 16 for both groups). p = 0.038 from log-rank statistics.

**Statistical analysis.** The primary prespecified comparison in this study was Kaplan-Meier survival until the development of significant left ventricular systolic dysfunction as defined by a fractional shortening less than 0.16. Serum electrolyte and neurohormonal levels were also compared at pacemaker implant, day 14 of pacing, and sacrifice. Continuous variables were compared by Student *t* test or analysis of variance. Due to the small size of the study and to avoid type 2 errors, adjustment was not made for multiple comparisons. Significance was defined as $p < 0.05$. The sample size of 32 animals was estimated to achieve a power of >80% to detect a 40% reduction in time until systolic dysfunction with a two-tailed $p < 0.05$.

## RESULTS

**Progression of systolic dysfunction.** Mean time until the development of significant left ventricular dysfunction was 35.1 ± 5.1 days (n = 16) in placebo animals compared with 21.4 ± 3.2 days (n = 16) for animals treated with furosemide (p = 0.038, log-rank test) (Fig. 1). Five animals in the placebo group and one furosemide-treated animal did not develop systolic dysfunction by 42 days (p = 0.07 by chi-square).

**Neurohormones, electrolytes, and echocardiographic dimensions.** Animals treated with furosemide manifested a significant increase in serum aldosterone compared with the day of implantation, and at day 14 had significantly higher levels compared with those receiving placebo (Fig. 2A). Serum aldosterone levels at day 14 correlated inversely with

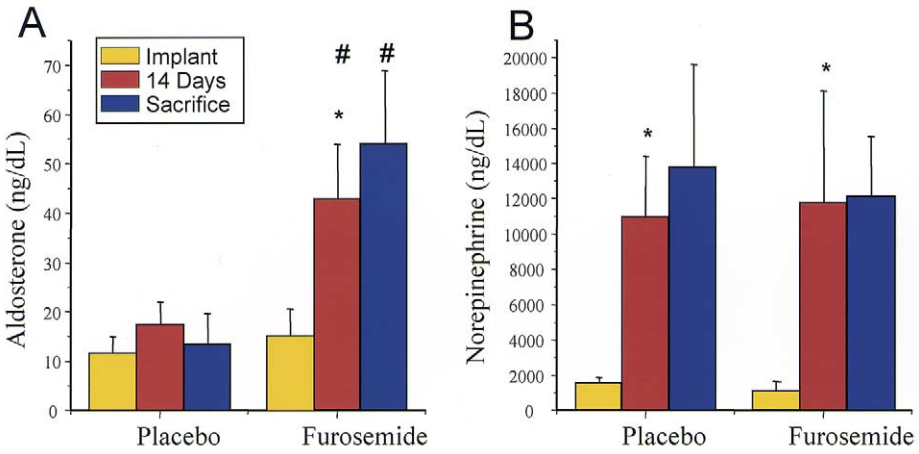


**Figure 2.** **(A)** Comparison of mean ± SEM of serum aldosterone measured in both groups at implantation **(yellow)**, at day 14 of pacing **(red)**, and at sacrifice **(purple)**. Serum aldosterone in placebo animals did not increase at day 14 (n = 11), while aldosterone increased significantly in furosemide-treated animals both compared with implant and compared with placebo (n = 9, *$p < 0.05$ compared with implantation; #$p < 0.05$ with respect to placebo). **(B)** Comparison of mean ± SEM of serum norepinephrine measured in both groups at implantation **(yellow)**, at day 14 of pacing **(red)**, and at sacrifice **(purple)**. Serum norepinephrine increased equally in both placebo- and furosemide-treated animals at day 14 (n = 11, *$p < 0.05$ compared with implantation).

fractional shortening in animals receiving furosemide ($R = -0.72$, $p < 0.05$), but not in placebo animals ($R = 0.05$, $p = 0.89$), suggesting that aldosterone levels may have an impact on fractional shortening in the furosemide group. Serum norepinephrine, however, increased significantly in both groups, with no difference between placebo and furosemide animals (Fig. 2B). Serum sodium was slightly (but significantly) lower in animals receiving furosemide compared with placebo animals (Table 1). No difference in serum potassium, magnesium, creatinine, or urea nitrogen was observed between the two groups. Animals treated with placebo tended to have a larger weight gain by day 14 (26.9 ± 4.23%, n = 14) compared with furosemide animals (16.1 ± 4.3% n = 14, $p = 0.09$). Because a significant reduction in preload will decrease end-diastolic volume and depress the extent of systolic fiber shortening through the Starling mechanism, chronic dehydration could be an explanation for the reduction in fractional shortening in the furosemide group. Left ventricular end-diastolic dimension indices measured between days 12 and 18, however, were similar in the two groups (furosemide, 7.48 ± 0.38 cm/M$^2$; placebo, 7.93 ± 0.42 cm/M$^2$; $p = 0.45$). Furthermore, the relationship between the reduction in fractional shortening and increase in end-diastolic dimension index (expressed as a change from baseline) was similar for the two groups at day 13 to 14 and at sacrifice (Fig. 3). End-systolic dimension indexes were also not significantly different between treat-

**Table 1.** Electrolyte and Renal Data

| | Furosemide | Placebo | p Value |
|---|---|---|---|
| Day 0 | | | |
| Potassium, mmol/l | 3.9 ± 0.12 | 4.1 ± 0.08 | 0.16 |
| Magnesium, mg/dl | 1.8 ± 0.03 | 1.9 ± 0.08 | 0.42 |
| Sodium, mmol/l | 136.7 ± 1.0 | 137.5 ± 1.1 | 0.56 |
| Urea nitrogen, mg/dl | 6.1 ± 1.3 | 7.1 ± 1.3 | 0.61 |
| Creatinine, mg/dl | 1.0 ± 0.04 | 1.0 ± 0.04 | 0.71 |
| Day 14 | | | |
| Potassium, mmol/l | 4.0 ± 0.09 | 4.0 ± 0.07 | 0.83 |
| Magnesium, mg/dl | 2.2 ± 0.015 | 2.1 ± 0.07 | 0.41 |
| Sodium, mmol/l | 133.0 ± 0.9 | 135.7 ± 0.8 | 0.043 |
| Urea nitrogen, mg/dl | 13.9 ± 1.9 | 11.8 ± 1.5 | 0.39 |
| Creatinine, mg/dl | 1.3 ± 0.09 | 1.3 ± 0.04 | 0.44 |
| Sacrifice | | | |
| Potassium, mmol/l | 4.2 ± 0.22 | 3.9 ± 0.15 | 0.30 |
| Magnesium, mg/dl | 2.3 ± 0.11 | 2.0 ± 0.08 | 0.08 |
| Sodium, mmol/l | 131.8 ± 1.6 | 135.1 ± 1.1 | 0.09 |
| Urea nitrogen, mg/dl | 14.2 ± 1.8 | 12.3 ± 1.9 | 0.47 |
| Creatinine, mg/dl | 1.3 ± 0.08 | 1.2 ± 0.02 | 0.15 |

Comparison of mean ± SEM ratio of serum electrolytes, urea nitrogen, and creatinine in both groups at implant, day 14, and sacrifice. Furosemide was associated with a statistically lower sodium compared with placebo at day 14. No other statistically significant differences were seen in serum electrolytes or serum markers of renal function.

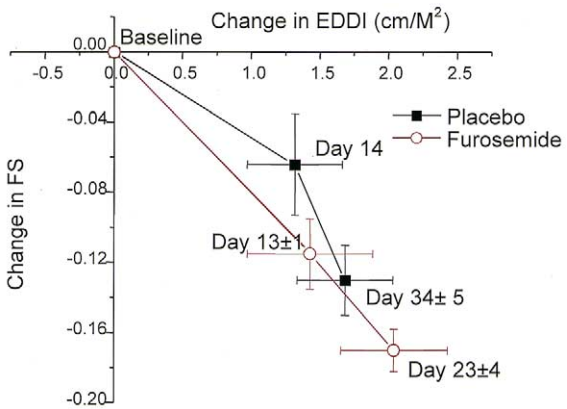


**Figure 3.** The mean change in fractional shortening (FS) as a function of the change in end-diastolic dimension index (EDDI) from baseline to day 14 and to sacrifice (day 34 ± 5, n = 15) in placebo-treated **(black squares)** versus furosemide animals **(red circles)**. Furosemide group measurements were made at day 13 (±1) and sacrifice (day 23 ± 4, n = 14). Despite its diuretic action, furosemide was associated with similarly increased end-diastolic dimension and reduced FS compared with placebo, indicating that the reduced systolic performance in the furosemide group was not due to a drop in presystolic chamber volume. The change in FS at sacrifice was marginally greater in the furosemide group ($p = 0.07$). Sacrifice occurred at different time points due to the earlier development of systolic dysfunction in the furosemide group.

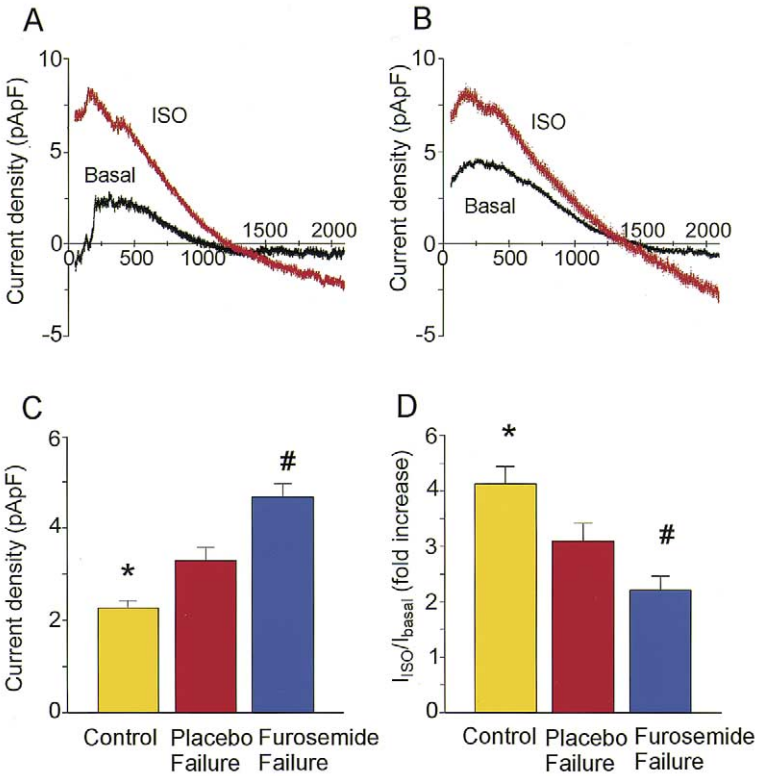


**Figure 4. (A and B)** Representative traces of the sodium-calcium exchange current elicited by voltage ramp in a myocyte from a failing placebo-treated animal **(A)** and a failing furosemide-treated animal **(B)**. Basal currents are seen in **black**, and the current in the same cell after stimulation with 2 $\mu$M isoproterenol (ISO) are presented in **red**. **(C)** Comparison of mean ± SEM peak outward sodium-calcium exchanger currents elicited from myocytes from nonfailing controls (**yellow**, n = 42 cells from 11 pigs), failing placebo-treated (**red**, n = 40 cells from 8 pigs), and furosemide-treated animals (**purple**, n = 50 cells from 9 pigs). Heart failure was associated with a significantly greater peak current, but furosemide use was associated with significantly greater current compared with placebo (*different compared with both failure groups, $p < 0.05$; #different from placebo cells, $p < 0.05$). **(D)** Comparison of mean ± SEM ratio of peak outward sodium-calcium exchanger currents elicited in response to ISO divided by basal current from failing myocytes from placebo- and furosemide-treated animals. Furosemide use was associated with significantly less inducible current compared with placebo and nonfailing controls, consistent with a reduction in responsiveness to beta-adrenergic stimulation (*different compared with both failure groups, $p < 0.05$; #different from placebo cells, $p < 0.05$).

ment groups (furosemide, 6.01 ± 0.37 cm/M$^2$; placebo, 6.01 ± 0.41 cm/M$^2$; p = 0.99). At sacrifice, mean fractional shortening was depressed to a similar extent in both groups as well, falling from 0.28 ± 0.01 at baseline to 0.15 ± 0.01 at sacrifice in the placebo group, versus 0.30 ± 0.01 at baseline to 0.13 ± 0.01 at sacrifice in the furosemide animals (p = NS between groups). The change in fractional shortening from baseline to sacrifice was greater in the furosemide group despite the shorter duration of pacing, although this did not reach significance (p = 0.07) (Fig. 3). It should be emphasized, however, that the animals were sacrificed once they developed systolic dysfunction, and, therefore, the present study was not intended to compare the extent of heart failure between the groups. Instead, the study assessed the effect of furosemide on the rate of heart failure progression, and found that furosemide accelerated the development of systolic dysfunction.

**NCX current.** We have previously reported that myocytes from failing animals manifest significantly increased basal NCX currents but depressed beta-adrenergic responsiveness when compared with cells from nonfailing animals due to an increase in the phosphorylation state of the exchanger (12). We predicted that furosemide would further increase the basal NCX current compared with placebo; NCX current was compared between unpaced control animals and those that developed severe systolic dysfunction in which adequate myocyte yield was achieved (n = 11 nonfailing control, 8 failing placebo, and 9 failing furosemide pigs). Peak outward NCX currents were significantly greater in both failing groups compared with nonfailing controls, while the furosemide-treated animals had significantly greater peak currents compared with placebo (Figs. 4A to 4C). When compared with failing placebo-treated animals, the responsiveness of the NCX currents to isoproterenol stimulation in furosemide animals was significantly reduced (Fig. 4D).

## DISCUSSION

To the best of our knowledge, this study represents the first randomized controlled trial to assess the effect of furosemide on the progression of heart failure. The principle findings are: 1) animals treated with furosemide developed left ventricular systolic dysfunction significantly faster than animals receiving placebo; 2) furosemide treatment was asso-

ciated with a statistically significant increase in serum aldosterone levels compared with placebo, but norepinephrine was increased equally in both groups; 3) furosemide treatment was associated with significantly greater NCX current than placebo; and 4) furosemide-treated animals manifested significantly reduced beta-adrenergic responsiveness in the NCX current. Based on our previous work, we believe this may be due to a greater degree of phosphorylation of that protein.

**Mechanisms of furosemide effect.** Putative detrimental effects of non–potassium-sparing diuretics include wasting of potassium and magnesium (15), activation of the RAAS (4,6), and increased sympathetic nervous system activity (16). We found that furosemide use was associated with increased serum aldosterone levels, and propose that a likely mechanism by which furosemide hastens the development of systolic dysfunction is activation of the RAAS. Furosemide activates the RAAS by wasting sodium and reducing circulating volume (17). Interestingly, although serum sodium was slightly decreased in animals receiving furosemide, there was no difference in serum potassium or magnesium levels, and, although weight gain after two weeks trended higher in the placebo group, there was no difference between the two groups in BUN, creatinine, or indexed ventricular dimensions. Systolic dysfunction as a result of dehydration and a concomitant decrease in preload and renal perfusion, therefore, seems unlikely to be the cause of the early failure in the furosemide group.

Contrary to our expectation, we did not observe an increase in aldosterone in our placebo group despite a significant increase in norepinephrine. Given the ubiquitous use of diuretics in heart failure, few studies report aldosterone levels in diuretic-naive patients with symptomatic heart failure. Bayliss et al. (4) reported that neither plasma renin activity nor aldosterone were increased in 12 subjects with untreated symptomatic heart failure; both increased significantly after one month's treatment with furosemide. Similarly, asymptomatic subjects in the SOLVD with left ventricular dysfunction did not manifest an increase in plasma renin activity unless they received a diuretic, despite an increase in norepinephrine (5). In a tachycardia pig model of heart failure similar to ours, Spinale et al. (18) described an increase in aldosterone at three weeks comparable with that seen in the furosemide arm of our study; however, all animals in that study received a relatively high dose of furosemide for the last week of pacing (100 mg daily) (Francis Spinale, personal communication, November 24, 2003). In a recent study of dogs with surgically induced mitral regurgitation, heart failure was not associated with a rise in aldosterone unless the diuretic torasemide was administered (19).

Other groups have found that furosemide augments RAAS activation, increasing plasma renin activity and serum aldosterone levels (6,7). Recent data have suggested that prolonged aldosterone exposure has deleterious effects on left ventricular function, causing reactive perivascular and interstitial myocardial fibrosis as well as replacement fibrosis secondary to myocyte necrosis (20,21). In a substudy of RALES, addition of the aldosterone antagonist spironolactone significantly decreased the levels of serum markers of extracellular matrix protein turnover (22,23).

While we did not assess the impact of furosemide on extracellular matrix protein turnover, we investigated its effect on the beta-adrenergic modulation of an important calcium-handling protein, the NCX. Recent work has identified chronic hyperphosphorylation of the L-type calcium channel (24) and ryanodine receptor (25) by protein kinase A in failing human myocardium, resulting in diminution in the heart's ability to augment cytosolic calcium in response to stress. We have recently described a similar increase in phosphorylation state of the NCX in this heart failure model, resulting in greater basal activity and depression of beta-adrenergic responsiveness (12). An increase in NCX activity could contribute to inefficient calcium cycling, depletion of sarcoplasmic reticular calcium load, and reduced contractile reserve as well as proarrhythmic late depolarizations (26,27). Furosemide use appears to further augment the basal exchanger current and reduce its responsiveness to isoproterenol, consistent with increased phosphorylation of the protein.

We observed no difference in serum potassium or magnesium levels between the two groups, though previous studies indicate that aldosterone significantly alters potassium and magnesium homeostasis (28). However, serum electrolyte levels are insensitive measures of total body content, and a decrease in intracellular electrolyte levels may yet contribute to the accelerated progression to left ventricular dysfunction in the furosemide group (29,30).

**Clinical implications.** Despite the lack of a prospective study on the effect of furosemide on the progression of heart failure (31), this loop diuretic is routinely used in the treatment of patients with heart failure, and American College of Cardiology/American Heart Association guidelines recommend using loop diuretics for fluid overload in CHF (2). In current practice, loop diuretics are rarely used as monotherapy for heart failure, but are commonly used together with other medications that antagonize the neurohormonal activation seen in heart failure, such as angiotensin-converting enzyme inhibitors, beta-adrenergic antagonists, and spironolactone. Concomitant therapy may mitigate the detrimental effect of furosemide; however, aldosterone levels have been shown to "escape" in heart failure patients treated with long-term angiotensin-converting enzyme inhibitor therapy after initial suppression, suggesting incomplete blockade of the RAAS (32). Consistent with the results of our study, continued furosemide therapy has been noted to contribute to this phenomenon because sodium depletion can result in angiotensin-independent secretion of aldosterone (33), with the associated detrimental effects of such increased aldosterone exposure. Clearly, a clinical trial to evaluate the impact

of diuretic use (and the modifying role of aldosterone blockade) on the progression of heart failure is warranted.

**Study limitations.** Furosemide treatment was initiated simultaneously with ventricular pacing in this study, rather than after the onset of ventricular dysfunction. An objection could be raised that such an approach may have exposed the animals to dehydration and other toxic effects of furosemide. The animals were, however, given access to unlimited water and food, however, and were examined daily by veterinary technicians for evidence of dehydration or heart failure. Furthermore, there was no evidence of significant electrolyte or renal abnormality in any animal. While it would perhaps have been optimal to delay therapy, the investigators felt that the additional complexity in study design was not justified, given that furosemide therapy was well-tolerated at these doses.

**Reprint requests and correspondence:** Dr. Mark C. Haigney, Division of Cardiology, Uniformed Services University of the Health Sciences, Room A3060, 4301 Jones Bridge Road, Bethesda, Maryland 20814. E-mail: mhaigney@usuhs.mil.

## REFERENCES

1. McAlister FA, Teo KK, Taher M, et al. Insights into the contemporary epidemiology and outpatient management of heart failure. Am Heart J 1999;138:87–94.
2. Hunt SA, Baker DW, Chin MH, et al. ACC/AHA guidelines for the evaluation and management of chronic heart failure in the adult. J Am Coll Cardiol 2001;38:2101–13.
3. Domanski M, Norman J, Pitt B, et al. Diuretic use, progressive heart failure, and death in patients in the Studies Of Left Ventricular Dysfunction (SOLVD). J Am Coll Cardiol 2003;42:705–8.
4. Bayliss J, Norell M, Canepa-Anson R, et al. Untreated heart failure: clinical and neuroendocrine effects of introducing diuretics. Br Heart J 1987;57:17–22.
5. Francis GS, Benedict C, Yusuf S, et al., for SOLVD Investigators. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. Circulation 1990;82:1724–9.
6. Benedict CR, Weiner DH, Yusuf S, et al., for SOLVD Investigators. Comparative neurohormonal responses in patients with preserved and impaired left ventricular ejection fraction: results of the Studies Of Left Ventricular Dysfunction (SOLVD) registry. J Am Coll Cardiol 1993;22 Suppl A:146A–53A.
7. Dzau VJ, Colucci WS, Hollenberg NK, Williams GH. Relation of the renin-angiotensin-aldosterone system to clinical state in congestive heart failure. Circulation 1981;63:645–51.
8. Levine TB, Francis GS, Goldsmith SR, Simon AB, Cohn JN. Activity of the sympathetic nervous system and renin-angiotensin system assessed by plasma hormone levels and their relation to hemodynamic abnormalities in congestive heart failure. Am J Cardiol 1982;49:1659–66.
9. Pitt B, Zannad F, Remme WJ, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. N Engl J Med 1999;341:709–17.
10. Schrier RW, Abraham WT. Hormones and hemodynamics in heart failure. N Engl J Med 1999;341:577–85.
11. Marks AR, Reiken S, Marx SO. Progression of heart failure: is protein kinase A hyperphosphorylation of the ryanodine receptor a contributing factor? Circulation 2002;105:272–5.
12. Wei SK, Ruknudin A, Hanlon SU, et al. PKA hyperphosphorylation increases basal current but decreases beta-adrenergic responsiveness of the sarcolemmal $Na^+/Ca^{2+}$ exchanger in failing pig myocytes. Circ Res 2003;92:897–903.
13. Lovern CS, Swecker WS, Lee JC, Moon ML. Additive effects of a sodium chloride restricted diet and furosemide administration in healthy dogs. Am J Vet Res 2001;62:1793–6.
14. Mashiro I, Nelson RR, Cohn JN, Franciosa JA. Ventricular dimensions measured noninvasively by echocardiography in the awake dog. J Appl Physiol 1976;41:953–9.
15. Laragh JH, Sealey JE. K+ depletion and the progression of hypertensive disease or heart failure: the pathogenic role of diuretic-induced aldosterone secretion. Hypertension 2001;37:806–10.
16. Kimmerly DS, Shoemaker JK. Hypovolemia and neurovascular control during orthostatic stress. Am J Physiol Heart Circ Physiol 2002;282: H645–655.
17. Weber KT. Aldosterone in congestive heart failure. N Engl J Med 2001;345:1689–97.
18. Spinale FG, de Gasparo M, Whitebread S, et al. Modulation of the renin-angiotensin pathway through enzyme inhibition and specific receptor blockade in pacing-induced heart failure. Circulation 1997; 96:2385–96.
19. Uechi M, Matsuoka M, Kuwajima E, et al. The effects of the loop diuretics furosemide and torasemide on diuresis in dogs and cats. J Vet Med Sci 2003;65:1057–61.
20. Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium; fibrosis and renin-angiostensin-aldosterone system. Circulation 1991;83:1849–65.
21. Lijnen P, Petrov V. Induction of cardiac fibrosis by aldosterone. J Mol Cell Cardiol 2000;32:865–79.
22. Zannad F, Dousset B, Alla F. Treatment of congestive heart failure: interfering the aldosterone-cardiac extracellular matrix relationship. Hypertension 2001;38:1227–32.
23. Zannad F, Alla F, Dousset B, et al., on behalf of the RALES Investigators. Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insights from the Randomized Aldactone Evaluation Study (RALES). Circulation 2000;102:2700–6.
24. Schroder F, Handrock R, Beuckelmann DJ, et al. Increased availability and open probability of single L-type calcium channels from failing compared with nonfailing human ventricle. Circulation 1998;98:969–76.
25. Marx SO, Reiken S, Hisamatsu Y, et al. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. Cell 2000;101:365–76.
26. Schillinger W, Janssen PML, Emami S, et al. Impaired contractile performance of cultured rabbit ventricular myocytes after adenoviral gene transfer of $Na^+$-$Ca^{2+}$ exchanger. Circ Res 2000;87:581–7.
27. Pogwizd SM, Schlotthauer K, Li L, Yuan W, Bers DM. Sodium-calcium exchange contributes to mechanical dysfunction and triggered arrhythmias in heart failure. Circ Res 2001;88:1159–67.
28. Douban S, Brodsky MA, Whang DD, Whang R. Significance of magnesium in congestive heart failure. Am Heart J 1996;132:664–71.
29. Laragh JH. Renin-angiotensin-aldosterone system for blood pressure and electrolyte homeostasis and its involvement in hypertension, in congestive heart failure and in associated cardiovascular damage (myocardial infarction and stroke). J Hum Hypertens 1995;9:385–90.
30. Haigney MCP, Wei SK, Kaab S, et al. Loss of cardiac magnesium in experimental heart failure prolongs and destabilizes repolarization in dogs. J Am Coll Cardiol 1998;31:701–6.
31. Cody RJ. Clinical trials of diuretic therapy in heart failure: research directions and clinical considerations. J Am Coll Cardiol 1993;22:165–71A.
32. Staessen J, Lijnen P, Fagard R, Verschueren LJ, Amery A. Rise in plasma concentration of aldosterone during long-term angiotensin II suppression. J Endocrinol 1981;91:457–65.
33. Okubo S, Niimura F, Nishimura H, et al. Angiotensin-independent mechanism for aldosterone synthesis during chronic extracellular fluid volume depletion. J Clin Invest 1997;99:855–60.