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Systemic Hemodynamics, Renal Function and Hormonal Levels during Inhibition of Neutral Endopeptidase 3.4.24.11 and Angiotensin-Converting Enzyme in Conscious Dogs with Pacing-Induced Heart Failure

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ABSTRACT

The systemic hemodynamic, renal and hormonal responses to SQ 28,603 (N-[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]-β-alanine) the selective inhibitor of neutral endopeptidase 3.4.24.11, the angiotensin-converting enzyme inhibitor captopril and their combination were determined in conscious dogs after 1 or 3 weeks of rapid ventricular pacing. Coadministration of captopril (100 or 10 μmol/kg i.v.) and SQ 28,603 (10 μmol/kg i.v.) significantly reduced mean arterial pressure, systemic vascular resistance and renal vascular resistance and increased cardiac output, stroke volume and renal blood flow in the conscious dogs paced for 1 week. This pattern of hemodynamic improvement was not predicted by the activity of the individual inhibitors. The combination of inhibitors did not significantly increase sodium excretion because of the variability introduced by the depressor activity; however, the pressure-natriuresis curve was steeper and shifted leftward, indicating that sodium excretion was maintained at lower renal perfusion pressures. The increases in urinary and plasma levels of cyclic GMP and atrial natriuretic peptide stimulated by SQ 28,603 were not affected by captopril. The data indicated that the hemodynamic and renal responses produced by SQ 28,603, presumably by elevating atrial natriuretic peptide levels, were enhanced by suppression of angiotensin II or that the combination of inhibitors protected other vasodilator/natriuretic peptides from degradation. Qualitatively similar responses to SQ 28,603, captopril and the combination of inhibitors were obtained in dogs paced for 3 weeks. In summary, the combined angiotensin-converting enzyme and neutral endopeptidase 3.4.24.11 inhibitors improved systemic hemodynamics and maintained renal function in conscious dogs with pacing-induced heart failure.

ANP 99–126 is a hormone released from the cardiac atria in response to atrial stretch. Accordingly, plasma ANP concentrations increase as atrial pressures increase in heart failure (Bates *et al.*, 1986; Abraham *et al.*, 1992). The biological activities of circulating ANP, *i.e.*, relaxation of vascular smooth muscle, stimulation of natriuresis and suppression of the renin-angiotensin-aldosterone axis, should theoretically counter the increases in SVR and the sodium retention that develop as heart failure worsens. However, the renal responsiveness to exogenous ANP is reduced in heart failure patients (Cody *et al.*, 1986; Moe *et al.*, 1992) and in several experimental models, including rats with healed myocardial infarctions (Lee *et al.*, 1991; Kohzuki *et al.*, 1989a,b; Tikkanen *et al.*, 1989), dogs (Villarreal *et al.*, 1992) and rats (Hoffman *et al.*, 1988; Abassi *et al.*, 1990) with arteriovenous fistula, dogs with constriction of the thoracic vena cava (Freeman *et al.*, 1985; Scriven and Burnett, 1985; Redfield *et al.*, 1989) and dogs undergoing rapid ventricular pacing (Riegger *et al.*, 1988; Margulies *et al.*, 1991a). Interestingly, interruption of the renin-angiotensin cascade with an ACE inhibitor partially or wholly restored the natriuretic and diuretic responses to the exogenous ANP in rats with myocardial infarctions (Lee *et al.*, 1991) and rats (Abassi *et al.*, 1990) and dogs with arteriovenous fistula. Therefore, activation of the renin-angiotensin system contributes to the renal refractiveness to exogenous ANP in several experimental models of heart failure.

NEP 3.4.24.11 is a ubiquitous membrane-bound metalloendopeptidase that metabolizes ANP (Olins *et al.*, 1987; Stephenson and Kenny, 1987; Sonnenberg *et al.*, 1988) to biologically inactive products (Seymour *et al.*, 1988). Specific inhibitors of

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ABBREVIATIONS: ANP, atrial natriuretic peptide; SVR, systemic vascular resistance; ACE, angiotensin-converting enzyme; NEP, neutral endopeptidase 3.4.24.11; SQ 28,603, N-[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]-β-alanine; cGMP, cyclic GMP; LAP, left atrial pressure; RAP, right atrial pressure; MAP, mean arterial pressure; RBF, renal blood flow; CO, cardiac output; RVR, renal vascular resistance; PRA, plasma renin activity; GFR, glomerular filtration rate; TFA, trifluoroacetic acid; PAH, *para*-aminohippuric acid; SV, stroke volume; BNP, brain natriuretic peptide.

NEP 3.4.24.11 administered intravenously potentiated the natriuretic, diuretic and depressor responses to exogenous ANP in rats (Seymour *et al.*, 1989a; Webb *et al.*, 1989; Sybertz *et al.*, 1989; Olins *et al.*, 1989), monkeys (Seymour *et al.*, 1991a, 1992) and dogs (Margulies *et al.*, 1990). Furthermore, SQ 28,603, a selective competitive inhibitor of NEP 3.4.24.11 (Seymour *et al.*, 1991b), increased urinary excretion of ANP and its second messenger cGMP in conscious monkeys (Seymour *et al.*, 1991a) and in anesthetized dogs (Margulies *et al.*, 1990) infused with exogenous ANP, indicating that the inhibitor protected the peptide *in vivo*. Doses of SQ 28,603 that promoted natriuresis and diuresis in anesthetized (Cavero *et al.*, 1990) and conscious (Seymour *et al.*, 1993a) dogs with pacing-induced heart failure also increased the urinary excretion of ANP and cGMP, presumably by protecting endogenous ANP from renal degradation.

Like other experimental heart failure models, the dogs subjected to 1 week of rapid ventricular pacing are hyporesponsive to exogenous ANP (Riegger *et al.*, 1988, Margulies *et al.*, 1991a). If the natriuretic response to NEP inhibitors is mediated by protection of ANP, then the maximal natriuretic potential of the NEP inhibitor may not have been realized in previous studies of SQ 28,603 in dogs with pacing-induced heart failure (Cavero *et al.*, 1990; Seymour *et al.*, 1993a). Indeed, chronic inhibition of ACE with 3 days of oral captopril treatment enhanced the natriuretic response to SQ 28,603 in anesthetized dogs with heart failure induced by 1 week of pacing (Margulies *et al.*, 1991b). The present study was designed to determine the effects of acute intravenous administration of SQ 28,603, captopril or the combination of inhibitors in conscious dogs undergoing rapid ventricular pacing for 1 week for comparison with the previous results obtained in anesthetized dogs paced for the same length of time (Margulies *et al.*, 1991b). The same treatments were also given to conscious dogs after 3 weeks of continuous rapid ventricular pacing. As we (Seymour *et al.*, 1993b) and others (Armstrong *et al.*, 1986; Howard *et al.*, 1988; Moe *et al.*, 1988, 1989) have reported, ventricular dilation progresses, renal function declines and systemic hemodynamics worsen when rapid ventricular pacing is prolonged. The present use of dogs paced for 1 and 3 weeks allowed examination of the effects of the ACE and NEP inhibitors at the two times during development of pacing-induced heart failure. In this conscious model in which pacing was sustained for 1 to 3 weeks, the combination of SQ 28,603 and captopril produced a unique and beneficial profile of systemic vasodilation and natriuresis that was not obtained with either inhibitor alone.

Methods

The systemic symptoms of congestive heart failure were induced in conscious, chronically instrumented dogs by rapid ventricular pacing. These animals were treated with vehicle, the selective NEP inhibitor SQ 28,603, the selective ACE inhibitor captopril and the combination of the two inhibitors after the first week of pacing, a time at which the effects of SQ 28,603 alone had been studied in anesthetized (Cavero *et al.*, 1990) and conscious (Seymour *et al.*, 1993a) dogs with pacing-induced heart failure. SQ 28,603 and captopril and the combination of the inhibitors were also administered after 3 weeks of pacing, a time at which there is greater ventricular dilation and systemic symptoms worsen (Seymour *et al.*, 1993b, Armstrong *et al.*, 1986; Howard *et al.*, 1988; Moe *et al.*, 1988, 1989). In the interest of using the smallest number of animals possible, several of the animals received more than one treatment. These treatments were administered randomly and at least 2 days were allowed between tests to ensure that all parameters had returned to base line. The surgical methods used to instrument the dogs, the procedures used to obtain hemodynamic, renal and hormonal measurements and the experimental protocols are given below.

Surgical procedures and routine care of the colony. Fifteen dogs of either sex were selected from a colony of canines (11–23.6 kg) that were chronically instrumented for hemodynamic measurements according to the techniques outlined below. A catheter was implanted into one femoral artery under sterile conditions during an initial surgical procedure. The free end of the tubing was attached to the reservoir of a vascular access port (Access Technologies, Skokie, IL) that was positioned subcutaneously on the dog's hip. A thoracotomy was performed 1 to 2 weeks later to allow implantation of a sutureless pacing lead (Medtronic Inc., Minneapolis, MN) into the apex of the left cardiac ventricle. The lead was connected to a programmable pacing generator that was held in a subcutaneous pocket located dorsally at the level of the sixth intercostal space. On the same day, a Transonic flow probe was positioned around the ascending aorta to estimate cardiac output by aortic flow. The cable from the flow probe was anchored above the fifth intercostal space using a Konigsberg skin button. Silastic catheters (5 French) were inserted directly into the right and left atrial appendages and connected to separate vascular access ports located subcutaneously in the midscapular region. Some of the dogs were subjected to a third surgical procedure in which the left renal artery was isolated through a flank incision, a Transonic (Transonic Systems, Ithaca, NY) flow probe was applied and the flow probe cable was anchored dorsally *via* a Konigsberg skin button.

For all surgical procedures, the dogs were fasted and premedicated with acepromazine (1 mg/kg) and atropine (0.02 mg/kg) injected intramuscularly. Anesthesia was induced with surital (12.5 mg/kg, i.v.) and maintained with 2% isoflurane/98% oxygen. Sterile technique was observed throughout each surgery. Prophylactic antibiotic treatment began preoperatively with Keflex (1 g i.v.) and was continued for 1 week with oral treatment of Keflex (250 mg p.o.) twice a day.

The animals were housed individually in a room maintained on a 12-hr light-dark cycle. Routine observation of appetite, activity level and the condition of the skin surrounding the implanted devices were conducted daily. During rapid ventricular pacing, regular evaluations also included measurement of heart rate to ensure pacing capture and auscultation to check for pulmonary rales. The color of the gums, capillary refilling time and the presence of ascites or dyspnea were determined empirically. Chest radiographs were obtained before and at weekly intervals after increasing the pacing rate.

Each dog was given free access to tap water to drink *ad libitum* and was fed canned food (Hill's Performance Diet) containing 0.05 mEq Na^+/g twice a day. The daily sodium intake was approximately 40 mEq. Sodium balances were determined in eight of the dogs that were housed in metabolism cages. Twenty-four-hour sodium balances were calculated as the difference between daily sodium excretion and 24-hr sodium consumption. Before each of the day-long studies described below, the dogs were fasted overnight.

Measurements of hemodynamic, renal and hormonal parameters. The base-line renal ($n = 14$ for renal clearances and $n = 10$ for renal blood flow), hemodynamic and hormonal levels ($n = 15$ except where samples were lost due to technical difficulties) were determined as follows before pacing was initiated. The dog was lightly restrained in a sling in a quiet laboratory. The skin above each of the vascular access ports was shaved and disinfected by scrubbing with chlorhexidine (Nolvasan, Neco, Fort Dodge, IA). Each port was punctured using a 22-gauge Huber point needle (Access Technologies Inc., Skokie, IL) that was attached *via* sterile tubing to a Gould-Statham pressure transducer (Spectramed, Oxnard, CA). Patency of each catheter was insured by continuous infusion of 50 μl/min of sterile saline using Sorenson pressure cuffs (Abbott Laboratories, Chicago, IL) inflated to 300 mm Hg. An extension cable attached each flow probe to a dual-channel Transonic flow meter. In some cases, the signal was obtained through a scanner (Transonics model TM04) that alternated between the two flow probes at 30-sec intervals. LAP and RAP, MAP, RBF and aortic flow (an estimate of CO) were recorded on a Grass polygraph

and stored at 10-sec intervals using the PO-NE-MAH data acquisition system. Heart rate was derived on-line from the pulsatile arterial blood pressure signal. SVR was calculated as (MAP − RAP) ÷ CO and RVR as (MAP − RAP) ÷ RBF. The data collected during a 30-min period were averaged to yield a single base-line value for each variable.

Each dog was allowed to rest quietly for 45 to 60 min, then a 12-ml sample of arterial blood was collected in EDTA for measurement of PRA and the plasma concentrations of ANP and cGMP. The arterial blood sample was immediately divided. Aprotinin (1000 U/ml) and sodium azide (2 mg/ml) were added to the blood reserved for measurement of plasma concentrations of ANP and cGMP. All blood samples for hormonal determinations were immediately chilled and the plasma was separated by centrifugation at 4°C. Each sample was stored frozen until processed as described below. Additional arterial blood samples for assessment of arterial hemoglobin, oxygen saturation (IL482 Instrumentation Laboratory Co-oximeter, Lexington, MA), hematocrit (capillary tube method) and plasma electrolyte concentrations were drawn in heparin; a venous blood sample was obtained from a peripheral vein, usually the cephalic, for determination of venous oxygen saturation.

Base-line renal function was evaluated in 14 of the dogs before pacing. Each animal was lightly restrained in a sling and a Foley catheter (8 French for females or 7 French for males) was inserted into the urinary bladder for timed urine collections. Creatinine (50 mg/kg + 1 mg/kg/min, i.v.) was administered *via* a percutaneous venous catheter. One hour later the urinary bladder was emptied and flushed with 20 ml of sterile distilled water. Two 30-min urine samples were then collected during the next hour and arterial blood samples were drawn in heparin at the midpoint of each 30-min period. The concentrations of creatinine in plasma and urine were determined by spectrophotometric assay and the renal clearance of creatinine was calculated as an estimate of the GFR. Sodium and potassium concentrations in plasma and urine were measured by ion-selective electrodes (Instrumentation Laboratories Phoenix Chemistry Analyzer) and the urinary electrolyte excretion rates (μEq/min) were calculated. A portion of each urine sample was also reserved for measurement of cGMP concentration, and in some cases, ANP levels as described below.

Plasma samples collected for measurement of PRA were stored at −20°C until analyzed using a radioimmunoassay kit purchased from Biotecx (Friendswood, TX). Urine and plasma samples obtained for ANP and cGMP determinations were stored at −80°C until extracted on preconditioned reversed-phase Sep-Pak C_{18} cartridges. Each column was washed three times with 2 ml of 0.1% TFA before ANP was eluted with 2 ml of 0.1% TFA in 100% methanol. The solvent was evaporated in a Speed-Vac concentrator, then the residue was reconstituted in the assay buffer. Recovery of a trace of [^{125}I]ANP from this extraction procedure averaged 80%. ANP concentrations were measured in the extracted plasma and urine samples according to the procedures of the radioimmunoassay purchased from Peninsula Laboratories (San Carlos, CA). cGMP content in each plasma extract and in each unextracted urine sample was determined according to the instructions provided with the New England Nuclear Products (Boston, MA) radioimmunoassay kit. Urinary excretion rates of cGMP and ANP were calculated by multiplying the urinary volume flow rate (ml/min) by the concentration of cGMP (nmol/ml) and ANP (fmol/ml) and were expressed as nanomoles per minute and fentomole per minute, respectively.

Effects of NEP and ACE inhibitors in conscious paced dogs. Once base-line values had been established, the pacemaker generator was programmed for rapid ventricular pacing (rate = 260 beats/min; amplitude = 5 V, pulse width = 2 msec). Capture was verified by checking heart rate and the aortic flow traces. Each animal was evaluated clinically on a daily basis and the hemodynamic parameters were monitored periodically. If any animal appeared to be in jeopardy at any time during the pacing episode, the pacing rate was reduced by 20 to 40 beats/min. This procedure was necessary to prevent untimely deaths and resulted in more homogenous groups of animals. At least 3 days were allowed to re-establish stable base lines (Seymour *et al.*, 1993b) if the pacing rate was slowed before an experiment. Each of the 15 dogs was subjected to one to four of the following treatments during a 3-week pacing period. A minimum of 2 days was allowed between successive studies if an animal was included in more than one test.

The pacemaker remained activated throughout each study in order to maintain stable base lines for renal and hemodynamic measurements for the 6 hr required to conduct each test. Previous experience had indicated that deactivation of the pacemaker caused unacceptable changes in basal renal function, hormonal levels and systemic hemodynamics (Seymour *et al.*, 1993b; Mizelle *et al.*, 1989) during the first 24 hr.

On the morning of each experiment, the dogs were prepared for measurement of hemodynamic, renal and hormonal parameters as described above. After a 1-hr stabilization period, two 30-min control periods were observed, a blood sample was obtained for hormonal measurements and the dog was treated with 1 ml/kg of 0.84% $NaHCO_3$, 10 μmol/kg i.v. of SQ 28,603, captopril or the combination of NEP and ACE inhibitors. Thereafter, urine collections and midpoint blood samples were obtained at 30-min intervals whereas blood for hormonal assays was drawn 2 and 4 hr after treatment. The hemodynamic data for each 30-min period were averaged and presented as a single value for that time.

The following treatments were administered after 1 week of pacing: 1 ml/kg i.v. of the 0.84% $NaHCO_3$ vehicle ($n = 6$), 10 μmol/kg i.v. (~2.8 mg/kg) of SQ 28,603 ($n = 8$), 100 μmol/kg i.v. of captopril ($n = 5$) and the combination of 100 μmol/kg i.v. of captopril plus 10 μmol/kg i.v. of SQ 28,603 ($n = 4$). After 3 weeks of ventricular tachycardia, the dogs were treated with: 1 ml/kg i.v. of the 0.84% $NaHCO_3$ vehicle ($n = 5$), 10 μmol/kg i.v. of SQ 28,603 ($n = 5$), 10 μmol/kg i.v. of captopril ($n = 4$) and the combination of 10 μmol/kg i.v. of captopril plus 10 μmol/kg i.v. of SQ 28,603 ($n = 8$). When the NEP and ACE inhibitors were administered in the same study, captopril was delivered 30 min before injection of SQ 28,603. The protocols described above were approved by the Bristol-Myers Squibb Institutional Animal Care and Use Committee as being in full compliance with appropriate care and use of conscious dogs.

In our initial studies, renal plasma flow was estimated by clearance of PAH. Concern that captopril and/or SQ 28,603 (both organic acids) may compete with PAH for the same organic acid secretory pathway led us to implant flow probes on the renal arteries for renal blood flow determinations. Consequently, we do not have complete data for RBF and RVR in all treatment groups. Where the number of measurements was less than three the RBF data were not included in our statistical analyses. The average values for those groups are given for reference only.

All other data were collected from every animal except where catheter failures precluded pressure measurements or blood samples were lost due to technical difficulties. In those cases, the losses are indicated by a lower n. Because base-line values varied somewhat among treatment groups, the data were expressed as changes (Δ) from control to facilitate statistical analysis and graphic presentations. Analysis of variance and Fishers' least significant difference test were applied to the Δ to identify significant differences among treatment groups at each time point. All data are given as the mean ± S.E.M.

Results

Effects of rapid ventricular pacing. The changes in systemic hemodynamics, renal function, hormonal levels and cardiac structure that occurred during 3 weeks of rapid ventricular pacing in the 15 dogs included in the present study are presented in table 1. The number of animals vary because some base-line measurements were not made due to equipment or time restraints. CO, SV and MAP were significantly reduced after 1 or 3 weeks of pacing, whereas atrial pressures and SVR increased (table 1). RBF and GFR declined during the pacing period, whereas RVR gradually increased (table 1). Although base-line sodium excretion that was measured over a 1-hr

TABLE 1

Effects of rapid ventricular pacing in conscious dogs

Data are expressed as mean ± S.E.M. Significant difference ($P < .05$) from *Control or †Week 1 were identified by analysis of variance and the Fisher's protected least significant difference test.

Measurement	Control	n	Week 1	n	Week 3	n
CO (l/min)	2.1 ± 0.1	15	1.0 ± 0.1*	15	0.9 ± 0.2*	14
SV (ml/beat)	22.5 ± 1.5	15	3.8 ± 0.3*	15	3.4 ± 0.5*	14
MAP (mm Hg)	116 ± 3	15	91 ± 2*	15	93 ± 3*	14
LAP (mm Hg)	8.4 ± 0.6	15	21.7 ± 1.2*	14	28.6 ± 1.8*†	13
SVR (mm Hg/l/min)	57 ± 5	15	98 ± 16*	15	123 ± 17*	14
RBF (ml/min)	149 ± 18	10	85 ± 8*	9	59 ± 5*	10
GFR (ml/min)	66 ± 6	14	60 ± 6	15	44 ± 5*	14
RVR (mm Hg/ml/min)	0.84 ± 0.10	10	0.99 ± 0.08	9	1.44 ± 0.12*†	10
Sodium balance (mEq/24 hr)	29.4 ± 4.0	8	25.2 ± 4.2	8	4.1 ± 8.9*†	7
PRA (pmol A I/ml/hr)	2.99 ± 0.58	15	2.61 ± 0.44	14	7.74 ± 3.04	14
Plasma ANP concentration (fmol/ml)	20 ± 3	13	104 ± 12*	14	91 ± 15*	13
Plasma cGMP concentration (pmol/ml)	8.8 ± 1.3	15	15.6 ± 3.8	14	20.2 ± 4.4	14
Urinary cGMP excretion (nmol/min)	0.78 ± 0.08	14	2.64 ± 0.32*	15	1.60 ± 0.22*†	14
Urinary ANP excretion (fmol/min)	2.95 ± 0.60	4	13.24 ± 9.30	5	7.00 ± 1.63	6
Arterial O_2 saturation (%)	94.4 ± 0.4	9	95.7 ± 0.2*	7	96.0 ± 0.2*	5
Venous O_2 saturation (%)	71.5 ± 2.4	9	58.6 ± 5.0*	7	41.1 ± 3.5*†	5
Arteriovenous O_2 difference (ml/dl)	4.5 ± 0.4	9	6.9 ± 0.9*	7	8.9 ± 0.6*	5
O_2 consumption (ml/min)	86.9 ± 10.9	9	63.8 ± 8.6	7	56.3 ± 7.5*	5
Arterial hemoglobin (ng/dl)	14.7 ± 0.4	9	13.8 ± 0.5	7	12.1 ± 0.4*†	5
Plasma sodium concentration (mEq/l)	150.3 ± 0.4	15	150.0 ± 0.5	15	147.7 ± 0.9*†	13
Body weight (kg)	18.3 ± 1.0	15	18.1 ± 0.9	15	17.9 ± 1.0	14

period in fasted dogs did not change significantly during pacing (data not shown), daily sodium balances (table 1) indicated an avid retention of sodium during the third week of pacing. PRA was not significantly affected by the sustained ventricular tachycardia, whereas plasma ANP concentration and urinary cGMP excretion were significantly elevated (table 1). However, urinary ANP excretion was not significantly affected at any time, suggesting that the increase in renal delivery of ANP during pacing did not surpass the capacity for renal degradation or clearance.

Venous oxygen saturation (table 1) continuously decreased so that the difference in arteriovenous oxygen content increased during the 3-week period; however, oxygen consumption decreased due to the reduction in CO produced by prolonged ventricular tachycardia. The reductions of arterial hemoglobin concentration and plasma sodium concentration (table 1) suggested that plasma volume had expanded by the third week of ventricular tachycardia. In summary, rapid ventricular pacing for 1 week produced changes in systemic hemodynamics, renal function and hormonal levels that approximate the peripheral symptoms of clinical heart failure. Greater systemic dysfunction was apparent after 3 weeks of pacing, indicating that the systemic consequences of heart failure had worsened. By examining the activities of SQ 28,603 and captopril after 1 and 3 weeks of sustained tachycardia, the effects of these inhibitors could be ascertained at two levels of hemodynamic and renal dysfunction in this canine model of heart failure.

Hemodynamic and hormonal responses to SQ 28,603 and captopril after 1 week of rapid ventricular pacing. One week of rapid ventricular pacing significantly decreased base-line CO, SV and increased atrial pressures (table 1). Administration of either 10 μmol/kg i.v. of SQ 28,603 ($n = 8$) or 100 μmol/kg i.v. of captopril ($n = 6$) individually did not significantly affect CO or SV (fig. 1) in the paced dogs. In contrast, the combination of NEP and ACE inhibitors ($n = 4$) significantly increased by more than 50% (fig. 1) both CO (0.94 ± 0.15–1.42 ± 0.18 l/min) and SV (3.6 ± 0.06–5.5 ± 0.7 ml/beat). The improvement of CO was mediated, at least in part,

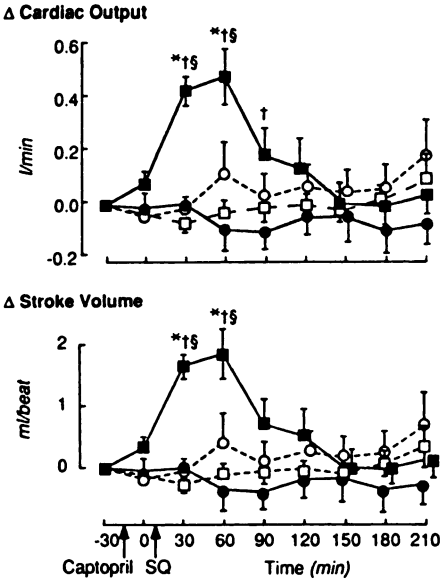

Fig. 1. Hemodynamic responses to NEP and ACE inhibitors in dogs paced for 1 week. The changes in CO (top graph) and SV (bottom graph) were determined in dogs treated with the 0.85% $NaHCO_3$ vehicle ($n = 6$, unfilled circles), 100 μmol/kg i.v. of captopril ($n = 5$, filled circles), 10 μmol/kg i.v. of SQ 28,603 ($n = 8$, unfilled squares) or both inhibitors ($n = 4$, filled squares). As indicated by the arrows at the bottom of the graph, captopril was given 30 min before SQ 28,603. Base-line CO were 1.32 ± 0.09, 1.15 ± 0.18, 0.94 ± 0.06 and 0.94 ± 0.15 min and base-line SV were 5.1 ± 0.3, 4.4 ± 0.4, 3.6 ± 0.2 and 3.6 ± 0.6 ml/beat in dogs receiving vehicle, captopril, SQ 28,603 and captopril + SQ 28,603, respectively. $P < .05$ *vs.* *vehicle, †captopril or §SQ 28,603.

by a reduction in afterload as indicated by the significant decrease in MAP from 96 ± 6 to 79 ± 7 mm Hg and the 50% decline in SVR from 98 ± 27 to 49 ± 13 mm Hg/l/min (fig. 2). These systemic vasodilatory responses to the combination of the ACE inhibitor with SQ 28,603 were significantly greater than the effects of either treatment administered singly.

Captopril alone significantly ($P < .05$) decreased LAP from 26.8 ± 0.9 to 24.4 ± 1.6 mm Hg (Δ = −2.4 ± 1.1 mm Hg) within

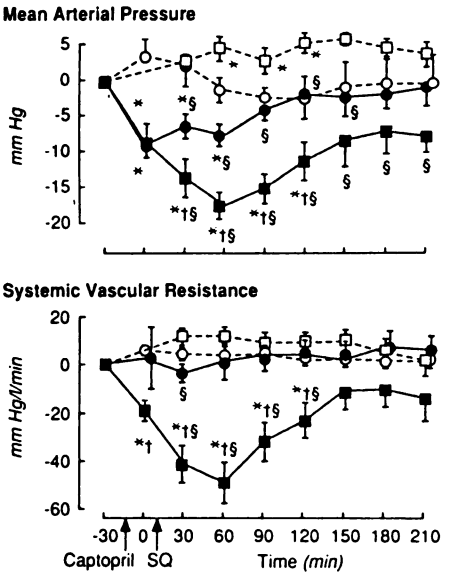

Fig. 2. Hemodynamic responses to NEP and ACE inhibitors in dogs paced for 1 week. The changes in MAP (top graph) and SVR (bottom graph) were determined in dogs treated with the 0.85% $NaHCO_3$ vehicle (n = 6, unfilled circles), 100 μmol/kg i.v. of captopril (n = 5, filled circles), 10 μmol/kg i.v. of SQ 28,603 (n = 8, unfilled squares) or both inhibitors (n = 4, filled squares). As indicated by the arrows at the bottom of the graph, captopril was given 30 min before SQ 28,603. Base-line MAP were 96 ± 3, 94 ± 2, 98 ± 4 and 96 ± 6 mm Hg and base-line SVR were 68 ± 4, 80 ± 13, 96 ± 6 and 98 ± 10 mm Hg/l/min in dogs receiving vehicle, captopril, SQ 28,603 and captopril + SQ 28,603, respectively. $P < .05$ *vs.* *vehicle, †captopril or §SQ 28,603.

the first 30 min, whereas the effects of SQ 28,603 were not significantly different from those produced by vehicle at any time after treatment. (control LAP = 22.2 ± 2.6 and 21.5 ± 3.1 mm Hg, before vehicle and SQ 28,603, respectively.) Unfortunately, LAP could not be measured in all the dogs receiving the combination of NEP and ACE inhibitors because the left atrial catheters had failed in two of the four animals; this group was excluded from further analysis. Base-line RAP were 12.2 ± 1.4, 12.8 ± 1.0, 13.0 ± 1.3 and 11.8 ± 0.9 mm Hg in the animals treated with vehicle, captopril, SQ 28,603 and the combined NEP and ACE inhibitors, respectively, after 1 week of pacing. None of these treatments significantly affected RAP.

Concurrent inhibition of ACE and NEP also increased RBF from 62 ± 12 to 113 ± 15 ml/min and decreased RVR from 1.39 ± 0.28 to 0.58 ± 0.08 mm Hg/ml/min (table 2). These responses were greater than the activity of either inhibitor given alone. In contrast, the combination of inhibitors increased GFR (39 ± 4–56 ± 9 ml/min) to the same extent as did captopril alone (table 2).

SQ 28,603 given alone significantly increased plasma ANP concentrations (table 2) to 178 ± 47 and 124 ± 22 fmol/ml at 90 and 210 min, respectively. Urinary excretion of ANP (fig. 3) increased significantly from 3 ± 1 to a peak of 1129 ± 762 fmol/min and urinary cGMP (fig. 3) increased significantly from 0.75 ± 0.12 to 3.12 ± 0.46 nmol/min. These changes were consistent with the protection of endogenous ANP from *in vivo* degradation. Captopril alone did not affect urinary cGMP (fig. 3), plasma cGMP concentrations (data not shown) or plasma ANP concentrations (table 2). Furthermore, the ACE inhibitor did not augment the cGMP or ANP responses to SQ 28,603 (fig. 3 and table 2). Therefore, further potentiation of ANP by the ACE inhibitor was not responsible for the additional hemodynamic responses to the combination of SQ 28,603 and captopril.

PRA (table 2) tended to increase after captopril and to decrease after SQ 28,603, but neither of these changes was significantly different from the effects of vehicle in dogs paced for 1 week. In contrast, the combination of captopril and SQ 28,603 caused PRA to reach levels that were significantly greater than the effects of any of the other treatments (table 2). It was unclear whether the greater increase in PRA was a result of renin release stimulated by the reduction in renal perfusion pressure or due to the protection of angiotensin I from degradation by NEP 3.4.24.11.

Hemodynamic and hormonal responses to SQ 28,603 and captopril after 3 weeks of rapid ventricular pacing. Base-line CO, SV and MAP were consistently reduced and SVR was elevated after 3 weeks of rapid ventricular pacing (table 1). At that time, the dogs were more sensitive to captopril such that 100 μmol/kg of the ACE inhibitor elicited a profound hypotension (>20 mm Hg) in three dogs tested in a pilot study (data not shown). Even after reducing the dose of captopril to 10 μmol/kg (n = 4), the ACE inhibitor transiently increased CO from 0.7 ± 0.1 to 1.0 ± 0.1 min and SV from 2.8 ± 0.4 to 3.7 ± 0.5 ml/beat (fig. 4) and reduced MAP from 88 ± 5 to 82 ± 7 mm Hg and SVR from 121 ± 14 to 84 ± 9 mm Hg/l/min (fig. 5). Additional increases in CO to 1.1 ± 0.1 l/min (46% above base-line) and SV to 3.9 ± 0.7 ml/beat (47% above base-line) were obtained when 10 μmol/kg of captopril was combined with SQ 28,603 (n = 8) (fig. 4). As observed after 1 week of pacing, the improvements in cardiac performance measured after 3 weeks of sustained tachycardia were accompanied by a proportional reduction in SVR to 72 ± 9 mm Hg/l/min (46% below base-line) and a decrease in MAP to 78 ± 6 mm Hg (fig. 5).

The reduction in LAP from 30 ± 2 to 28 ± 1 mm Hg (Δ = −2.5 ± 1.0 mm Hg) produced by captopril was similar to the response obtained after the first week of pacing. The additional decrease in LAP to 25 ± 2 mm Hg (Δ = −4.6 ± 0.9 mm Hg) produced by the combination of SQ 28,603 with captopril was not significantly different from the effects of captopril alone, but was significantly greater than the activity of vehicle (Δ = 0.1 ± 1.2 mm Hg) or SQ 28,603 (Δ = 0.5 ± 1.0 mm Hg). RBF and GFR increased and RVR declined by 40% (table 3), indicating that renal hemodynamics were not adversely effected even though the combination of ACE and NEP inhibitors had reduced renal perfusion pressure to 78 ± 6 mm Hg (30 min after SQ 28,603 in fig. 4).

Neither vehicle nor captopril effected plasma ANP concentrations (table 3) or urinary excretion of ANP or cGMP (fig. 6) in the dogs paced for 3 weeks. In addition, coadministration of captopril did not alter the ANP and cGMP responses that were stimulated by SQ 28,603 (table 3 and fig. 6). These results were similar to those obtained with SQ 28,603 and captopril in dogs paced for 1 week. Captopril also significantly elevated PRA whether given alone or in combination with SQ 28,603 (table 3).

Effects of SQ 28,603 and captopril on urine volume and electrolyte excretions in paced dogs. In dogs paced for 1 week, vehicle, captopril, SQ 28,603 and the combination of ACE and NEP inhibitors caused cumulative natriuretic responses of 10 ± 3, 25 ± 10, 28 ± 6 and 41 ± 15 mEq, respectively. The cumulative sodium excretion stimulated by vehicle, captopril, SQ 28,603 and captropril + SQ 28,603 in

TABLE 2

Effects of ACE and NEP inhibitors after 1 week of rapid ventricular pacing in conscious dogs

$P < .05$ *vs.* [a] vehicle, [b] captopril or [c] SQ 28,603. Data are expressed as mean ± S.E.M. Abbreviations used are: SQ, SQ 28603; Cap, captopril.

Inhibitor	*n*	Base Line	Captopril	Time after SQ 28,603 (min) 30	60	90	120	150	180	210
				Δ RBF ml/min						
Vehicle	4	89 ± 11	0 ± 4	4 ± 2	10 ± 5	12 ± 9	13 ± 9	11 ± 7	11 ± 7	14 ± 8
Captopril	4	93 ± 7	20 ± 16	21 ± 4[a]	22 ± 5[ac]	18 ± 2	23 ± 7	22 ± 9	19 ± 7	21 ± 5
SQ 28603	6	70 ± 6		−2 ± 2	1 ± 2	5 ± 2	7 ± 2	10 ± 1	14 ± 2	16 ± 2
Cap + SQ	3	62 ± 12	13 ± 5	40 ± 5[abc]	51 ± 4[abc]	41 ± 5[abc]	32 ± 5[ac]	26 ± 6	21 ± 6	21 ± 10
				Δ RVR mm Hg/ml/min						
Vehicle	4	1.05 ± 0.12	0.00 ± 0.04	−0.02 ± 0.05	−0.12 ± 0.07	−0.14 ± 0.10	−0.16 ± 0.11	−0.15 ± 0.10	−0.16 ± 0.09	−0.19 ± 0.10
Captopril	4	0.91 ± 0.06	−0.17 ± 0.09	−0.20 ± 0.06[c]	−0.22 ± 0.07	−0.17 ± 0.04	−0.18 ± 0.08	−0.15 ± 0.10	−0.16 ± 0.08	−0.16 ± 0.08
SQ 28603	6	1.22 ± 0.10		0.06 ± 0.04	0.02 ± 0.05	−0.04 ± 0.06	−0.06 ± 0.06	−1.0 ± 0.07	−0.14 ± 0.04	−0.16 ± 0.02
Cap + SQ	3	1.39 ± 0.28	−0.32 ± 0.01	−0.68 ± 0.14[abc]	−0.81 ± 0.23[abc]	−0.70 ± 0.18[abc]	−0.58 ± 0.18[abc]	−0.49 ± 0.14[abc]	−0.40 ± 0.11	−0.41 ± 0.11
				Δ GFR ml/min						
Vehicle	5	72 ± 9	−3 ± 4	−10 ± 3	11 ± 6	10 ± 4	13 ± 7	9 ± 4	15 ± 6	8 ± 8
Captopril	4	58 ± 6	2 ± 8	15 ± 10	12 ± 8[c]	8 ± 4	18 ± 11	20 ± 2[c]	12 ± 3	30 ± 4[ac]
SQ 28603	8	44 ± 4		3 ± 4	−6 ± 3[a]	−1 ± 4	8 ± 6	3 ± 4	4 ± 5	7 ± 4
Cap + SQ	4	39 ± 4	11 ± 4	9 ± 8	18 ± 6[c]	11 ± 7	3 ± 13	20 ± 6[c]	14 ± 3	10 ± 8[b]
				Δ Plasma ANP Concentration fmol/ml						
Vehicle	5	99 ± 19				−7 ± 5				−3 ± 8
Captopril	4	87 ± 10				12 ± 3				−12 ± 3
SQ 28603	8	75 ± 10				104 ± 47				49 ± 21[a]
Cap + SQ	4	54 ± 10				66 ± 24				70 ± 17[ab]
				Δ PRA pmol A I/ml/hr						
Vehicle	5	3.5 ± 1.1				0.1 ± 1.4				0.7 ± 1.6
Captopril	4	1.6 ± 0.3				1.8 ± 1.1				2.7 ± 2.2
SQ 28603	8	2.7 ± 0.2				−1.3 ± 0.2[b]				−0.5 ± 0.2
Cap + SQ	4	2.0 ± 0.3				7.8 ± 1.7[abc]				9.1 ± 3.8[abc]

dogs paced for 3 weeks were 13 ± 8, 38 ± 11, 32 ± 17 and 32 ± 8 mEq, respectively. Although each inhibitor treatment tended to increase sodium excretion, there were no significant differences among the treatments due to the variability of the natriuretic responses. Because the depressor activity of captopril given alone or in combination with SQ 28,603 may have influenced renal excretory function, the relationship between MAP and sodium excretion was examined during the first 90 min after treatment, the times at which MAP was most severely affected. As shown in fig. 7, sodium excretion was positively related to MAP in dogs treated with vehicle ($P < .01$) or captopril ($P < .02$) even though the correlation coefficients were small ($r^2 = 0.192$ and 0.188, respectively). In contrast, the correlation ($r^2 = 0.630$) between sodium excretion and MAP in dogs receiving SQ 28,603 and captopril was defined by a straight line having a steeper slope ($P < .0001$), indicating that the combination of NEP and ACE inhibitors produced a greater natriuresis at a given perfusion pressure than either SQ 28,603 or captopril alone. The natriuretic response to SQ 28,603 in the paced dogs was not significantly correlated with renal perfusion pressure ($P = .67$; $r^2 = 0.005$, data not shown), but was negatively correlated with PRA (fig. 7). Because PRA does not accurately reflect angiotensin II levels after ACE inhibition, the relationships between the renin-angiotensin system and natriuresis were not clear in the animals receiving captopril.

In the dogs paced for 1 week, the effect of 100 μmol/kg of captopril on fractional sodium excretion were not significantly different from the effects of vehicle. In contrast, fractional sodium excretion increased significantly from 0.35 ± 0.06% to 2.83 ± 0.58% ($P < .05$ *vs.* vehicle and captopril) after administration of SQ 28,603. In the presence of captopril, SQ 28,603 increased fractional sodium excretion to a similar extent (0.32 ± 0.12%–2.75 ± 0.44%; $P < .05$ *vs.* vehicle). After 3 weeks of pacing, the peak increases in fractional sodium excretion produced by 10 μmol/kg of captopril, SQ 28,603 and the combination of inhibitors (+2.4 ± 1.2%, +1.7 ± 0.6% and +2.8 ± 0.4%, respectively) were not significantly different from the effects of vehicle (+0.4 ± 0.2%).

Urine volume increased significantly ($P < .05$ *vs.* vehicle) in the dogs paced for 1 week and treated with 100 μmol/kg of captopril (from 0.25 ± 0.03 to 1.04 ± 0.34 ml/min), 10 μmol/kg of SQ 28,603 (from 0.32 ± 0.05 to 1.16 ± 0.08 ml/min) and the combination of ACE and NEP inhibitors (from 0.16 ± 0.03 to 1.28 ± 0.28 ml/min). There were no significant differences among the responses to captopril, SQ 28,603 or the coadministration of NEP and ACE inhibitors at any time after treatment. After 3 weeks of pacing, the diuretic responses to 10 μmol/kg of captopril (+0.67 ± 0.20 ml/min), SQ 28,603 (+0.68 ± 0.35 ml/min) and the combination of captopril + SQ 28,603 (+0.61 ± 0.10 ml/min) were significantly different from the effects of vehicle (−0.03 ± 0.05 ml/min) at only one time point (150 min after SQ 28,603).

Captopril (100 μmol/kg) significantly increased potassium excretion (+12 ± 3 μEq/min) 90 min after treatment of the dogs paced for 1 week. A similar response was obtained by the combination of captopril and SQ 28,603 (+12.2 ± 5.2 μEq/min) whereas SQ 28,603 alone had no significant kaliuretic activity. The changes in potassium excretion produced by each enzyme

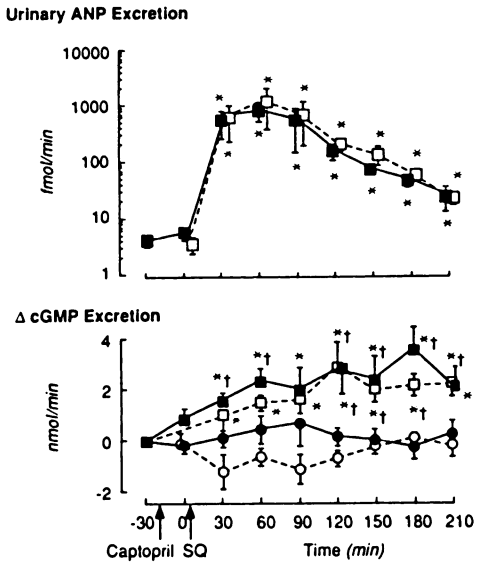

Fig. 3. Urinary excretion of ANP and cGMP responses to NEP and ACE inhibitors in dogs paced for 1 week. Urinary ANP excretion (top graph) was determined in eight dogs treated with 10 μmol/kg i.v. of SQ 28,603 (unfilled squares) and four dogs receiving both inhibitors (filled squares); base-line ANP excretory rates were 3.38 ± 1.07 and 3.78 ± 0.98 fmol/min, respectively. As indicated by the arrows at the bottom of the graph, captopril was given 30 min before SQ 28,603. *P < .05 *vs.* base-line measurements made in each group. The changes in urinary cGMP excretion (bottom graph) were assessed in dogs treated with the 0.85% $NaHCO_3$ vehicle (n = 6, unfilled circles), 100 μmol/kg i.v. of captopril (n = 5, filled circles), 10 μmol/kg i.v. of SQ 28,603 (n = 8, unfilled squares) or both inhibitors (n = 4, filled squares). Base-line urinary cGMP excretory rates were 3.28 ± 0.51, 2.61 ± 0.23, 2.12 ± 0.25 and 0.75 ± 0.05 nmol/min in dogs treated with vehicle, captopril, SQ 28,603 and captopril + SQ 28,603, respectively. P < .05 *vs.* *vehicle, †captopril or §SQ 28,603.

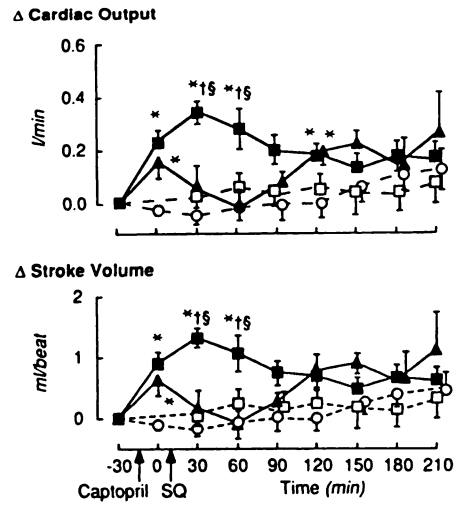

Fig. 4. Hemodynamic responses to NEP and ACE inhibitors in dogs paced for 3 weeks. The changes in CO (top graph) and SV (bottom graph) were determined in dogs treated with the 0.85% $NaHCO_3$ vehicle (n = 5, unfilled circles), 10 μmol/kg i.v. of captopril (n = 4, filled triangles), 10 μmol/kg i.v. of SQ 28,603 (n = 5, unfilled squares) or both inhibitors (n = 8, filled squares). As indicated by the arrows at the bottom of the graph, captopril was given 30 min before SQ 28,603. Base-line CO were 0.91 ± 0.23, 0.79 ± 0.19, 0.63 ± 0.05 and 0.74 ± 0.11 l/min and base-line SV were 3.5 ± 0.8, 3.2 ± 0.8, 3.5 ± 0.8 and 2.8 ± 0.4 ml/beat in dogs receiving vehicle, captopril, SQ 28,603 and captopril + SQ 28,603, respectively. P < .05 *vs.* *vehicle, †captopril or §SQ 28,603.

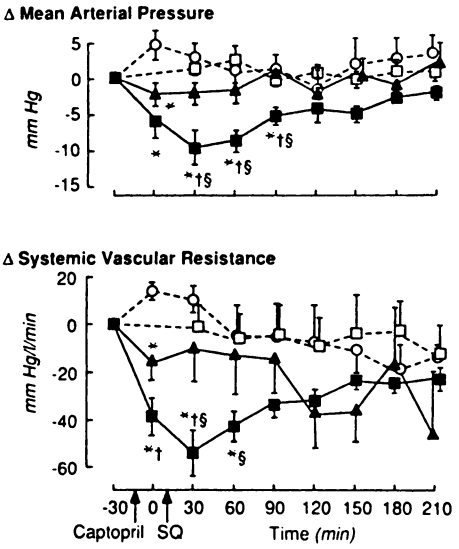

Fig. 5. Hemodynamic responses to NEP and ACE inhibitors in dogs paced for 3 weeks. The changes in MAP (top graph) and SVR (bottom graph) were determined in dogs treated with the 0.85% $NaHCO_3$ vehicle (n = 5, unfilled circles), 10 μmol/kg i.v. of captopril (n = 4, filled triangles), 10 μmol/kg i.v. of SQ 28,603 (n = 5, unfilled squares) or both inhibitors (n = 8, filled squares). As indicated by the arrows at the bottom of the graph, captopril was given 30 min before SQ 28,603. Base-line MAP were 95 ± 3, 104 ± 4, 84 ± 2 and 88 ± 5 mm Hg and base-line SVR were 118 ± 28, 149 ± 36, 127 ± 10 and 121 ± 14 mm Hg/l/min in dogs receiving vehicle, captopril, SQ 28,603 and captopril + SQ 28,603, respectively. P < .05 *vs.* *vehicle, †captopril or §SQ 28,603.

inhibitor given alone or in combination after 3 weeks of pacing were variable and were not significantly different from the effects of vehicle at any time after treatment.

Discussion

The simultaneous inhibition of NEP and ACE markedly improved systemic hemodynamics and renal function in conscious dogs with pacing-induced heart failure. The profile of hemodynamic responses included increases in CO, SV, RBF and GFR with reductions in MAP and SVR. Sodium excretion and urine volume were maintained during coadministration of captopril and SQ 28,603 despite the decrease in renal perfusion pressure caused by addition of the ACE inhibitor. Therefore, the combination of NEP and ACE inhibitors produced a favorable pattern of hemodynamic and renal responses that was not attainable with either inhibitor alone.

SQ 28,603 and captopril, selective inhibitors of NEP and ACE, respectively, produce distinct profiles of hemodynamic and renal actions in congestive heart failure. Previous studies in conscious (Seymour *et al.*, 1993a) or anesthetized (Cavero *et al.*, 1990) dogs paced for 1 week demonstrated that doses of SQ 28,603 ranging from 10 to 200 μmol/kg i.v. stimulated dose-related natriuresis that was associated with increases in urinary excretion of cGMP and ANP. In both previous studies, the natriuresis occurred at doses that increased urinary ANP excretion without significantly affecting plasma ANP concentrations, suggesting greater local protection of ANP from renal degradation. This conclusion was consistent with the observation that SQ 28,603 had not altered systemic hemodynamics except for modest reductions in RAP. The present results confirmed our previous hemodynamic, hormonal and renal findings in conscious paced dogs (Seymour *et al.*, 1993a) and

TABLE 3

Effects of ACE and NEP inhibitors after 3 weeks of rapid ventricular pacing in conscious dogs

$P < .05$ *vs.* [a] vehicle, [b] captopril or [c] SQ 28,603. Data are expressed as mean ± S.E.M. Where $n = 2$ for the vehicle treatment, those data were excluded from the statistical analysis; the averages of the two dogs are given for reference only. Abbreviations used are: SQ, SQ 28603; Cap, captopril.

Inhibitor	*n*	Base Line	Captopril	Time after SQ 28,603 (*min*)						
				30	60	90	120	150	180	210
				Δ RBF *ml/min*						
Vehicle	2	63	2	2	3	2	3	4	3	2
Captopril	3	55 ± 3	10 ± 2	12 ± 3	12 ± 2	14 ± 2	15 ± 2	19 ± 3	18 ± 1	20 ± 1
SQ 28603	5	62 ± 14		1 ± 1	6 ± 2	7 ± 2	10 ± 3	12 ± 4	16 ± 6	17 ± 7
Cap + SQ	7	55 ± 7	7 ± 2	23 ± 4[c]	23 ± 2[b,c]	21 ± 4[c]	22 ± 4	20 ± 3	18 ± 3	19 ± 2
				Δ RVR *mm Hg/ml/min*						
Vehicle	2	1.23 ± 0.03	0.07	−0.01	−0.05	−0.02	−0.06	−0.07	−0.02	0.23
Captopril	3	1.72 ± 0.17	−0.32 ± 0.12	−0.33 ± 0.03	−0.34 ± 0.04	−0.32 ± 0.02	−0.39 ± 0.03	−0.40 ± 0.04	−0.43 ± 0.08	−0.43 ± 0.10
SQ 28603	5	1.36 ± 0.24		0.02 ± 0.04	−0.06 ± 0.05	−0.18 ± 0.05	−0.21 ± 0.06	−0.26 ± 0.09	−0.29 ± 0.08	−0.30 ± 0.07
Cap + SQ	7	1.44 ± 0.18	−0.32 ± 0.10	−0.60 ± 0.14[c]	−0.58 ± 0.11[c]	−0.48 ± 0.09[c]	−0.46 ± 0.09	−0.47 ± 0.08	−0.42 ± 0.07	−0.40 ± 0.07
				Δ GFR *ml/min*						
Vehicle	5	56 ± 8	−6 ± 8	−2 ± 5	−8 ± 4	5 ± 4	2 ± 5	−3 ± 5	2 ± 4	−2 ± 6
Captopril	4	46 ± 6	10 ± 4	6 ± 2	11 ± 3	7 ± 3	10 ± 3	2 ± 6	8 ± 5	1 ± 8
SQ 28603	5	40 ± 7		1 ± 2	0 ± 2	1 ± 4	11 ± 5	2 ± 2	0 ± 3	8 ± 8
Cap + SQ	8	34 ± 3	4 ± 4	11 ± 3[a,c]	21 ± 7[a,c]	14 ± 5	18 ± 3[a]	14 ± 6[a,b,c]	14 ± 2[a,c]	13 ± 6
				Δ Plasma ANP concentration *fmol/ml*						
Vehicle	4	88 ± 17				−3 ± 7				2 ± 7
Captopril	4	94 ± 20				−12 ± 11				−8 ± 13
SQ 28,603	5	72 ± 8				161 ± 38[a,b]				98 ± 9[a,b]
Cap + SQ	8	87 ± 24				110 ± 30[a,b]				55 ± 15[a,b,c]
				Δ PRA *pmol A I/ml/hr*						
Vehicle	4	5.1 ± 2.2				−1.5 ± 0.8				−0.5 ± 0.3
Captopril	4	1.6 ± 0.1				1.6 ± 1.0				2.8 ± 1.2[a,c]
SQ 28,603	5	4.3 ± 2.6				−1.5 ± 0.7				−1.5 ± 0.8
Cap + SQ	8	2.9 ± 0.9				2.3 ± 1.2[a,c]				1.9 ± 0.8[a,c]

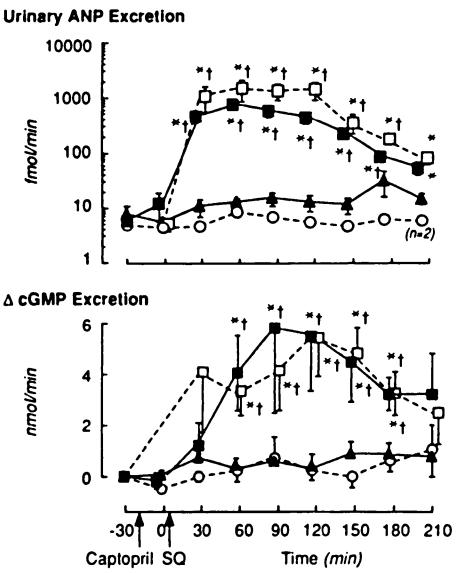

Fig. 6. Urinary excretion of ANP and cGMP responses to NEP and ACE inhibitors in dogs paced for 1 week. Urinary ANP excretion (top graph) was determined in dogs treated with the 0.85% $NaHCO_3$ vehicle (n = 2, unfilled circles), 10 μmol/kg i.v. of captopril (n = 4, filled triangles), 10 μmol/kg i.v. of SQ 28,603 (n = 5, unfilled squares) or both inhibitors (n = 8, filled squares). As indicated by the arrows at the bottom of the graph, captopril was given 30 min before SQ 28,603. Base-line ANP excretory rates were 4.36, 6.90 ± 2.57, 4.45 ± 1.13 and 5.11 ± 0.66 fmol/min in the dogs receiving vehicle, captopril, SQ 28,603 and captopril + SQ 28,603, respectively. $P < .05$ *vs.* *base-line measurements made in each group, †$P < .05$ *vs.* captopril treatment. The vehicle group was excluded from the statistical analysis due to the small number of observations and is included for reference purposes only. The changes in urinary cGMP excretion (bottom graph) were assessed in dogs treated with the 0.85% $NaHCO_3$ vehicle (n = 5, unfilled circles), 10 μmol/kg i.v. of captopril (n = 4, filled circles), 10 μmol/kg i.v. of SQ 28,603 (n = 5, unfilled squares) or both inhibitors (n = 8, filled squares). Base-line cGMP excretory rates were 1.40 ± 0.39, 1.72 ± 0.52, 1.99 ± 0.44 and 2.12 ± 0.47 nmol/min in dogs receiving vehicle, captopril, SQ 28,603 and captopril + SQ 28,603, respectively. $P < .05$ *vs.* *vehicle, †captopril or ‡SQ 28,603.

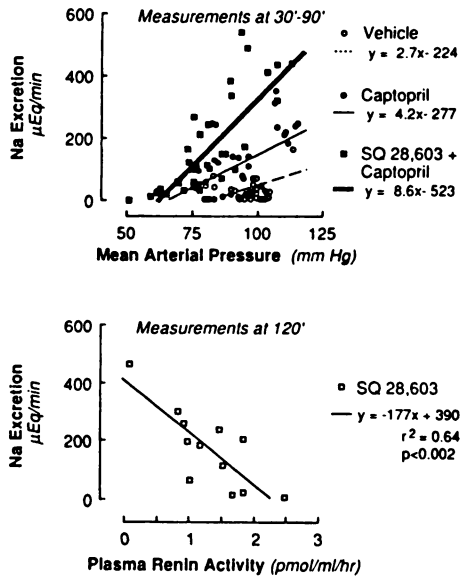

Fig. 7. Factors determining the natriuretic activities of the NEP and ACE inhibitors in paced dogs. The relationships between MAP and sodium excretion were measured during the first 90 min after inhibitor treatment in dogs paced for 1 or 3 weeks (top graph). The linear equations were determined by regression analysis for the dogs treated with vehicle (unfilled circles and dashed line), captopril (filled circles and single line) and the combination of SQ 28,603 and captopril (filled squares and double line). The slopes of the relationships were significant for vehicle ($P < .01$; $r^2 = 0.192$), captopril ($P < .02$; $r^2 = 0.188$) and the combination of NEP and ACE inhibitors ($P < .0001$; $r^2 = 0.630$). There was no significant relationship between blood pressure and sodium excretion in the dogs treated with SQ 28,603 (data not shown). However, PRA and sodium excretion measured 120 min after SQ 28,603 were inversely related in the dogs paced for 1 or 3 weeks (bottom graph).

demonstrated that the low dose of 10 μmol/kg i.v. of SQ 28,603 increased urinary ANP excretion equally after 1 or 3 weeks of tachycardia. A similar pattern of natriuresis and diuresis with minimal hemodynamic activity was also produced by SQ 28,603 in conscious rats with healed myocardial infarctions (Trippodo *et al.*, 1991), by thiorphan (a related thiol NEP inhibitor) in rats with arteriovenous fistula (Wilkins *et al.*, 1990) and by the carboxyalkyl NEP inhibitor UK 69,578 in dogs with atrioventricular block (Northridge *et al.*, 1989). UK 69,578 also reduced atrial pressures and stimulated natriuresis and diuresis in patients with mild heart failure (Northridge *et al.*, 1990). Therefore, acute administration of a variety of chemically distinct NEP inhibitors produced natriuresis, diuresis and small decreases in atrial pressures in clinical heart failure and in diverse experimental models. These *in vivo* actions of NEP inhibitors are characteristic of all chemical classes and are presumably mediated by protection of endogenous ANP.

The renal and hemodynamic effects of ACE inhibitors have been well documented in experimental heart failure as well as in clinical trials. Captopril, the selective ACE inhibitor used in the present study, reduced afterload and preload in patients with chronic congestive heart failure, as indicated by decreases in SVR and left ventricular filling pressure. The reduction in the hydraulic load on the heart increased cardiac index, SV (Davis *et al.*, 1979; Dzau *et al.*, 1980) and ejection fraction (Dzau *et al.*, 1980). In a similar manner, oral administration of captopril decreased peripheral vascular resistance and increased CO in dogs with established pacing-induced heart failure (Riegger, 1985). Captopril administered orally also depressed plasma concentrations of angiotensin II (Dzau *et al.*, 1980; Riegger, 1985) and aldosterone (Dzau *et al.*, 1980; Riegger, 1985) in heart failure patients (Dzau *et al.*, 1980) and in dogs (Riegger, 1985) undergoing sustained tachycardia. Therefore, the effects of oral captopril in the pacing-induced heart failure model are similar to the clinical results and are mediated largely by the suppression of angiotensin II generation.

The hemodynamic responses to acute intravenous administration of captopril in our paced dogs and in paced sheep (Fitzpatrick *et al.*, 1992) were consistent with the activities previously reported after oral administration in patients (Davis *et al.*, 1979; Dzau *et al.*, 1980) or in dogs with pacing-induced heart failure (Riegger, 1985). Specifically, infusion of captopril decreased MAP and LAP in sheep paced at 225 beats/min for 5 days (Fitzpatrick *et al.*, 1992). In the current investigation, bolus administration of intravenous captopril (100 μmol/kg) transiently reduced MAP, SVR and LAP in dogs paced for 1 week. During the third week of pacing, the reductions in MAP and LAP were achieved with a lower dose of the ACE inhibitor (10 μmol/kg) and were accompanied by significant increases in CO and SV. The reason for the enhanced sensitivity to captopril with prolonged pacing was not immediately apparent as PRA remained within the normal range. Interestingly, similar results have been obtained during ACE inhibitor treatment of heart failure patients with normal levels of PRA and have been

attributed to blockade of the tissue renin-angiotensin systems (reviewed by Hirsch *et al.*, 1990). In summary, intravenous captopril improved cardiac performance by reductions in peripheral vascular resistance, and perhaps by the decreases in LAP, in the canine model of pacing-induced heart failure, presumably by suppression of circulating and tissue angiotensin II generation.

Surprisingly, SQ 28,603 exaggerated and prolonged the decreases in MAP and SVR produced by captopril in our conscious dogs subjected to sustained tachycardia. In a previous study of anesthetized dogs undergoing rapid ventricular pacing (Margulies *et al.*, 1991b), SQ 28,603 slightly reduced MAP and RAP without affecting CO. In dogs receiving oral captopril for 6 days before anesthesia and SQ 28,603 administration, the NEP inhibitor actually caused a slight decrease in CO and a corresponding increase in SVR. The weak hemodynamic activity of the combined NEP and ACE inhibition in the previous study differed markedly from our present findings in conscious dogs and may result from the differences in experimental conditions, such as the use of anesthesia or the differences in the route, duration and timing of captopril administration.

In the present study, the concurrent increases in CO and SV may be attributed primarily to the additional reduction in SVR produced by SQ 28,603 after captopril pretreatment. However, the mechanism by which coadministration of captopril and SQ 28,603 elicited greater systemic vasodilation was not apparent from the present experiment. Circulating ANP was an unlikely mediator because the increases in plasma ANP induced by SQ 28,603 (≤100 fmol/ml) were less than those required to produce hemodynamic responses in paced dogs (~800 fmol/ml) (Riegger *et al.*, 1988) or in heart failure patients (~700 fmol/ml) (Cody *et al.*, 1986). In addition, captopril did not significantly alter the effects of SQ 28,603 on plasma ANP or its second messenger cGMP in the present investigation. Therefore, the greater hemodynamic activity of the combination of NEP and ACE inhibitors cannot be explained by additional protection of circulating ANP from enzymatic degradation.

It is also unlikely that the NEP inhibitor enhanced the activity of captopril by further suppression of angiotensin II levels. Because NEP degrades both angiotensin I and II *in vitro* (Shimamori *et al.*, 1986), NEP inhibition would theoretically increase rather than decrease angiotensin levels. Indeed, SCH 39,370, a carboxyalkyl NEP inhibitor, enhanced plasma angiotensin II levels before and after injection of angiotensin I in rats (Yamamoto *et al.*, 1992). Nevertheless, NEP appears to serve only as a secondary degradative pathway for angiotensins as a NEP inhibitor did not alter the *in vivo* pressor activity of either angiotensin I or angiotensin II in conscious rats (Seymour *et al.*, 1989b). The modest hemodynamic and hormonal responses to SQ 28,603 alone in the present experiment also failed to support a primary role for NEP in the metabolism of angiotensin in the paced dogs. However, the expected increase in PRA due to interruption of the angiotensin feedback loop was greater after coadministration of SQ 28,603 and captopril than with either inhibitor alone. Inhibition of NEP by SQ 28,603 may have protected the angiotensin I generated in the assay of PRA from enzymatic degradation thereby increasing the apparent renin activity. Alternatively, the hypotensive response to the combined inhibitors may have stimulated a true increase in renin secretion. The latter possibility is consistent with the observation that PRA was highest in the dogs paced for 1 week where the depressor response was most pronounced.

Finally, the combination of ACE and NEP inhibitors may have potentiated other vasoactive peptides that are substrates for both enzymes. BNP is structurally related to ANP with the highest degree of homology found within the 17-member ring formed by the disulfide bond. Integrity of the rings of both ANP and BNP appears to be essential for recognition of each peptide by the guanylate cyclase-linked receptor that mediates their biological activities. Furthermore, BNP is susceptible to endocyclic hydrolysis by NEP *in vitro* (Norman *et al.*, 1991) and is potentiated *in vivo* by SQ 28,603 (Seymour *et al.*, 1992). Whereas ANP is not a substrate for ACE, the C-terminus of pig BNP was cleaved by the purified enzyme *in vitro* (Vanneste *et al.*, 1990). In addition, the plasma half-life of labeled pig BNP increased from 1.2 to 7.0 min in rats treated with captopril and to 15.3 min in rats receiving both captopril and the NEP inhibitor phosphoramidon (Vanneste *et al.*, 1990). Although the evidence suggests that both ACE and NEP degrade pig BNP *in vivo*, the effects of ACE inhibitors on the biological activity of BNP must be assessed before one can conclude that the enzymatic cleavage by ACE also inactivates the peptide. Furthermore, the exocyclic residues of BNP are poorly conserved among species so that additional studies would be needed to determine whether captopril protects canine BNP or potentiates its activity in dogs. In the present study, the increases in cGMP levels produced by SQ 28,603 were not affected by coadministration of captopril. Because the biological activity of BNP is mediated by cGMP, further protection of bioactive forms of BNP by the ACE inhibitor appeared unlikely. However, we did not measure BNP concentrations in the present study so that the effects of the combination of ACE and NEP inhibitors on endogenous BNP in the paced dogs remain unsettled.

In addition, several other vasoactive peptides are known substrates for both ACE and NEP, including bradykinin, substance P and enkephalins (Skidgel *et al.*, 1987). Previous studies had demonstrated that selective NEP inhibitors did not affect the depressor activity of bradykinin (Seymour *et al.*, 1989b) or substance P (A. A. Seymour, unpublished observation). However, recent studies in this laboratory (Sheldon *et al.*, 1993) indicate that the combination of SQ 28,603 and captopril enhanced both the renal vasodilatory and excretory responses to intrarenal bradykinin in a manner that is not anticipated from the activities of the individual inhibitors. Therefore, we cannot exclude the possibility that the hemodynamic responses to the combination of SQ 28,603 and captopril in the paced dogs were mediated by bradykinin or some endogenous peptide other than ANP or angiotensin.

In the dogs paced for 1 week in the present study, the combination of the NEP and ACE inhibitors tended to increase sodium excretion, but because of the variability of the responses, these changes were not significantly different from the effects of the other treatments. The variability appears to result from the initial decreasing of blood pressure to less than 75 mm Hg in half of the animals treated with the combination of captopril and SQ 28,603. This level of renal perfusion is known to abolish the natriuretic activity of exogenous ANP in anesthetized dogs (Seymour *et al.*, 1987) and may have attenuated the renal activity of the paced dogs in the present study. Inspection of the data from individual dogs confirmed that sodium excretion was highest when MAP remained above 75 mm Hg and increased as blood pressure recovered from the administration of captopril and SQ 28,603. Further analysis of

the relationship between renal perfusion pressure and sodium excretion in our conscious paced dogs demonstrated that sodium excretion was directly related to MAP in animals receiving vehicle, captopril or the combination of the NEP and ACE inhibitors. Although depressor response to the combination of inhibitors attenuated sodium excretion in the paced dogs, the natriuresis obtained with SQ 28,603 and captopril at any given renal perfusion pressure was greater than that stimulated by the ACE inhibitor alone. These data suggested that the NEP inhibitor lessened the detrimental renal effects of equidepressor doses of captopril in the conscious paced dogs.

The present results differed from the enhanced natriuresis reported by Margulies *et al.* (1991b) in anesthetized paced dogs given SQ 28,603 after 3 days of oral captopril treatment. Unlike the depressor response measured in our conscious paced dogs, neither SQ 28,603 nor the combination of inhibitors altered blood pressure in the anesthetized animals. The differences in the hemodynamic responses in the two studies may account for the differences in the natriuretic responses.

The present study in conscious dogs confirmed the observation in anesthetized dogs (Margulies *et al.*, 1991b) that the combination of SQ 28,603 and captopril increased glomerular filtration rate. However, the increases in glomerular filtration rates produced by the combination of the NEP and ACE inhibitors in the present investigation were not significantly different from the increases stimulated by captopril alone. Coadministration of SQ 28,603 and captopril in our conscious dogs also increased RBF and decreased RVR, responses that were not observed in anesthetized paced dogs (Margulies *et al.*, 1991b) or in anesthetized rats with healed myocardial infarctions (Lee *et al.*, 1991). This renal vasodilation in the present study may have helped to sustain sodium excretion despite the reduction in renal perfusion pressure by dilution of the medullary concentration gradient or by changing the intrarenal hemodynamic state to one less favorable for sodium reabsorption.

The renal activity of SQ 28,603 in the present study was associated with significant increases in urinary excretion of ANP and cGMP, indicating that the natriuretic response was mediated at least in part by the protection of ANP from intrarenal degradation. The addition of captopril did not change urinary excretion of either cGMP or ANP, suggesting that the unique renal hemodynamic and excretory responses to coadministration of SQ 28,603 and captopril were not dependent upon greater salvage of intrarenal ANP. In recent studies, chronic administration of ACE inhibitors partially restored ANP-induced natriuresis in rats with healed myocardial infarction (Lee *et al.*, 1991) and fully re-established the natriuretic activity of exogenous ANP in rats (Abassi *et al.*, 1990) or dogs (Villarreal *et al.*, 1992) with high output heart failure due to an arteriovenous fistula. In each model, the beneficial effects of the ACE inhibitor on the renal excretory responses to ANP were attributed to suppression of angiotensin II generation even when circulating renin activity was normal. Reducing angiotensin II would diminish its vasoconstrictor and antinatriuretic activities and allow greater expression of the vasodilatory and natriuretic effects of ANP. This conclusion was supported by the observations that intrarenal infusions of pathophysiological doses of angiotensin II blunted the natriuretic response to SQ 28,603 in the anesthetized paced dogs (Margulies *et al.*, 1991b) and attenuated the renal activity of ANP in normal animals (Mizelle *et al.*, 1989; Showalter *et al.*, 1988). In addition, the natriuretic activity of SQ 28,603 in the present study was lessened as PRA increased. Therefore, the improvements in renal hemodynamics and the maintenance of sodium excretion in the conscious paced dogs treated with the combination of NEP and ACE inhibitors may result from reductions in intrarenal angiotensin II levels. As discussed above, endogenous peptides other than ANP or angiotensin may mediate the renal as well as the vascular responses obtained during coadministration of SQ 28,603 and captopril in the conscious dogs with pacing-induced heart failure.

In summary, intravenous SQ 28,603 increased sodium excretion without affecting systemic hemodynamics in conscious dogs undergoing sustained tachycardia. Intravenous captopril transiently reduced MAP and LAP, indicating reductions in afterload and preload. The renal responses to the ACE inhibitor were highly variable and were dependent upon the prevailing renal perfusion pressure in the canine model of heart failure. The NEP and ACE inhibitors given together in the conscious paced dogs diminished MAP, SVR and LAP thereby increasing CO and SV. These responses were not predicted by the responses to the individual inhibitors. SQ 28,603 and captopril coadministration also increased the slope of the pressure-natriuresis curve in the paced dogs suggesting that the detrimental renal effects of hypotensive doses of captopril were lessened by the NEP inhibitor. In conclusion, the combination of NEP and ACE inhibitors produced beneficial changes in systemic hemodynamics and renal function in conscious dogs with pacing-induced heart failure.

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