British Journal of Pharmacology (1996) 119, 943–948

Renal effects of concurrent E-24.11 and ACE inhibition in the aorto–venocaval fistula rat

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1 The present studies compare the early renal response to (a) an endopeptidase-24.11 (E-24.11) inhibitor (candoxatrilat) (b) an angiotensin-converting enzyme (ACE) inhibitor (lisinopril) and (c) the combination of endopeptidase-24.11 and ACE inhibition in the rat A–V fistula model of chronic volume overload.

2 Candoxatrilat (3 and 10 mg kg^{-1}) i.v. produced a prompt 3 fold increase in urinary sodium and cyclic GMP excretion without affecting significantly blood pressure or glomerular filtration rate (GFR).

3 Lisinopril (0.03 mg kg^{-1}) alone inhibited the pressor response to angiotensin I but had no significant effect on urinary sodium excretion or blood pressure.

4 Lisinopril (0.03 mg kg^{-1}) attenuated significantly the early natriuretic response to candoxatrilat (3 mg kg^{-1}) and the associated rise in urinary cyclic GMP, but sodium excretion eventually reached levels associated with acute E-24.11 inhibition.

5 Doses of the dual E-24.11/ACE inhibitor, sampatrilat, that inhibited the pressor response to angiotensin I reduced mean arterial blood pressure and produced a delayed natriuresis and rise in urinary cyclic GMP excretion when compared to candoxatrilat alone.

6 Concurrent administration of an ACE inhibitor reduces the early renal response to E-24.11 inhibition in the A–V fistula rat, an effect attributable to the hypotensive action of this combination.

Keywords: Neutral endopeptidase; ACE inhibitor; heart failure; renal effects

Introduction

Endopeptidase (E-24.11) inhibitors reduce the metabolic clearance of atrial and brain natriuretic peptides (ANP and BNP) (Stephenson & Kenny, 1987; Danilewicz *et al.*, 1989; Kenny *et al.*, 1993) and enhance their renal and hypotensive actions *in vivo* (Trapani *et al.*, 1989; Seymour *et al.*, 1989; 1992; Kirk & Wilkins, 1993). These properties suggest that E-24.11 inhibitors may be useful for the treatment of cardiac failure. Given alone, however, the effects of these compounds in patients with heart failure are modest and variable (Northridge *et al.*, 1989; Munzel *et al.*, 1992; Good *et al.*, 1995). The initial response to E-24.11 inhibitors is a 2 to 7 fold increase in urinary sodium excretion; chronic dosing leads to a small, sustained reduction in right and left atrial pressure but little or no reduction in systemic blood pressure (Northridge *et al.*, 1989; Munzel *et al.*, 1992).

The renin-angiotensin system counter-regulates the renal and vascular actions of the natriuretic peptides and may constrain the effects of E-24.11 inhibitors. Chronic inhibition of angiotensin-converting enzyme (ACE), to reduce angiotensin II production, has been shown to improve the natriuretic response to ANP in animal models of cardiac failure (Raya *et al.*, 1989; Abassi *et al.*, 1990; Villarreal *et al.*, 1992). This has prompted interest in the effect of ACE inhibitors on the response to E-24.11 inhibition and the possibility of synthesizing dual inhibitors i.e. molecules with both ACE and E-24.11 inhibitory activity, has given further impetus to studies of this interaction.

Chronic inhibition of ACE has been shown to increase the early renal response to subsequent E-24.11 inhibition in dogs with heart failure secondary to rapid ventricular pacing (Margulies *et al.*, 1991). However, if dual E-24.11/ACE inhibitors are to be developed then it is also important to examine the renal response when ACE and E-24.11 inhibitors are started together. Under these circumstances, the combination of E-24.11 and ACE inhibitors did not increase urinary sodium excretion significantly over that produced by either E-24.11 or ACE inhibitor alone, in rapidly paced dogs (Seymour *et al.*, 1993), cardiomyopathic hamsters (Trippodo *et al.*, 1993) or rats with low-output cardiac failure secondary to coronary artery ligation (Helin, 1993).

The balance of E-24.11 and ACE inhibition may be critical in determining the acute renal response, as marked falls in systemic blood pressure would be expected to offset any gain in natriuresis. The present studies examine the renal effects of combined inhibition of E-24.11 and ACE in an animal model of chronically elevated endogenous ANP levels (the aorto-venocaval fistula rat) and address the possibility that low-dose ACE inhibition may augment the natriuretic effects of E-24.11 inhibitors by avoiding marked falls in systemic blood pressure.

Methods

A–V fistula rats

A–V fistula surgery was performed on male Wistar rats (280–300 g) under Hypnorm (fentanyl/flunasone) anaesthesia as described previously (Wilkins *et al.*, 1992). Briefly, the fistula (1–1.5 mm long) was made through a side to side anastomosis between the aorta and inferior vena cava approximately 10 mm distal to the renal arteries. Sham-operations were performed by exposing and temporarily clamping (5 min) the aorta and vena cava without cutting and suturing the vessels. After surgery, the animals were returned to their cages and allowed free access to water and a standard rat diet. The rats were studied 7 days post-surgery.

To determine the degree of activation of the renin-angiotensin system achieved in this model, randomly selected A–V fistula rats and sham-operated animals were used for blood sampling only. Cannulae (Portex tubing 0.58 mm) were placed in a femoral artery and vein of each rat under Hypnorm (fentanyl/flunasone) anaesthesia and the animals allowed to regain consciousness in individual restraining cages. After 3 h

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blood (5 ml) was collected from the arterial line into potassium EDTA (2 mg ml^{-1}) tubes on ice to determine plasma renin activity.

Angiotensin I pressor response test

Inhibition of the pressor response to angiotensin I was used to demonstrate ACE inhibition and was measured in conscious, cannulated A–V fistula rats prepared and restrained as described above. When mean arterial blood pressure was stable (2.5–3 h after cannulation surgery), bolus injections of angiotensin I 300 ng kg^{-1} (representing the plateau of the dose-pressor response curve; data not shown) were given via the femoral line on 3 occasions before and at 15 min intervals after i.v. administration of ACE inhibitor. The peak rise in mean blood pressure from baseline was measured after each bolus injection via the femoral artery (MacLab instruments). Animals were randomized to receive: (i) lisinopril 0.3 mg kg^{-1}, 0.03 mg kg^{-1} and 0.01 mg kg^{-1}; $n=5$ each group and (ii) sampatrilat (dual E-24.11/ACE inhibitor) 0.3 mg kg^{-1}, 0.1 mg kg^{-1} and 0.03 mg kg^{-1}; $n=5$ each group

% inhibition of pressor response was calculated as =
[(mean Δ blood pressure before ACE inhibitor − Δ blood pressure after ACE inhibitor) ÷ mean Δ blood pressure before ACE inhibitor] × 100

where Δ blood pressure = peak mean blood pressure after angiotensin I − baseline pressure.

Effect of E-24.11 inhibition and ACE inhibition on renal function and blood pressure

These studies were conducted in conscious, restrained, cannulated A–V fistula rats. Cannulae (Portex tubing 0.58 mm) were placed in a femoral artery, a femoral vein and bladder under Hypnorm (fentanyl/flunasone) anaesthesia. The animals were allowed to regain consciousness in individual restraining cages. An infusion of sodium chloride 150 mmol l^{-1} (2.38 ml min^{-1}) containing [^{3}H]-inulin (Amersham) 2 μCi ml^{-1} was given via the femoral vein, which was also used to administer drugs, and continued for the duration of the study. Observations began 3 h after the infusion commenced. Mean arterial blood pressure was measured via the femoral artery every 15 min. Urine was collected at 15 min intervals into preweighed tubes for determination of urine volume, sodium, guanosine 3′:5′-cyclic monophosphate (cyclic GMP) and [^{3}H]-inulin concentrations. Urinary cyclic GMP was measured as described previously (Wilkins *et al.*, 1990). Arterial blood samples (200 ml) were taken at 30 and 90 min from the start of observations for [^{3}H]-inulin determination. Glomerular filtration rate (GFR) was calculated using the formula:

GFR (ml min^{-1}) =
[concentration [^{3}H] – inulin in urine (c.p.m. ml^{-1}) × urine flow (ml min^{-1})] ÷ concentration [^{3}H] – inulin in plasma (c.p.m. ml^{-1})

The animals were randomized to receive: (i) candoxatrilat (specific E-24.11 inhibitor; K_i 10 nM), 3 and 10 mg kg^{-1}; $n=11$ and 6 respectively; (ii) lisinopril (specific ACE inhibitor; K_i 0.1 nM), 0.01–0.1 mg kg^{-1}; $n=5$ each group; (iii) candoxatrilat 3 mg kg^{-1} and lisinopril 0.03 mg kg^{-1}; $n=5$ each group and (iv) sampatrilat (dual E-24.11/ACE inhibitor; K_i 8 and 1.2 nM respectively), 0.1–1 mg kg^{-1}; $n=5$ each group. Control groups of A–V fistula rats received vehicle (150 mmol l^{-1} saline) alone. The drug or vehicle was given as a bolus injection in 100 ml after 3 baseline urine collections and observations were continued for a further 5 collections (75 min).

Drugs

Candoxatrilat and sampatrilat were provided by Pfizer Central Research (UK) Ltd. Lisinopril was purchased from Sigma, Poole, Dorset, UK.

Assays

Urine volumes were determined gravimetrically. Urinary sodium concentrations were determined by flame photometry (Corning 480). [^{3}H]-inulin levels in urine and plasma were determined by liquid scintillation counting in Insta-gel (Packard) (Wilkins *et al.*, 1992). Urinary cyclic GMP concentrtion was measured by radioimmunoassay on appropriately diluted samples as described previously (Wilkins *et al.*, 1990). Plasma renin activity was determined by quantitation of generated angiotensin I using a commercially available radioimmunoassay kit for [^{125}I]-angiotensin I (DuPont Co. (UK) Ltd).

Statistics

Data are presented as mean ± s.e.mean. Mean arterial blood pressure and renal responses were examined by analysis of variance with respect to treatment and time (urine collection). Statistical significance was assessed by Scheffes' test. Statistical significance was assumed when the P value was <0.05 or less. Differences in heart weight and plasma renin activity were assessed by Student's unpaired t test. All calculations were made with Complete Statistical System (StatSoft) software.

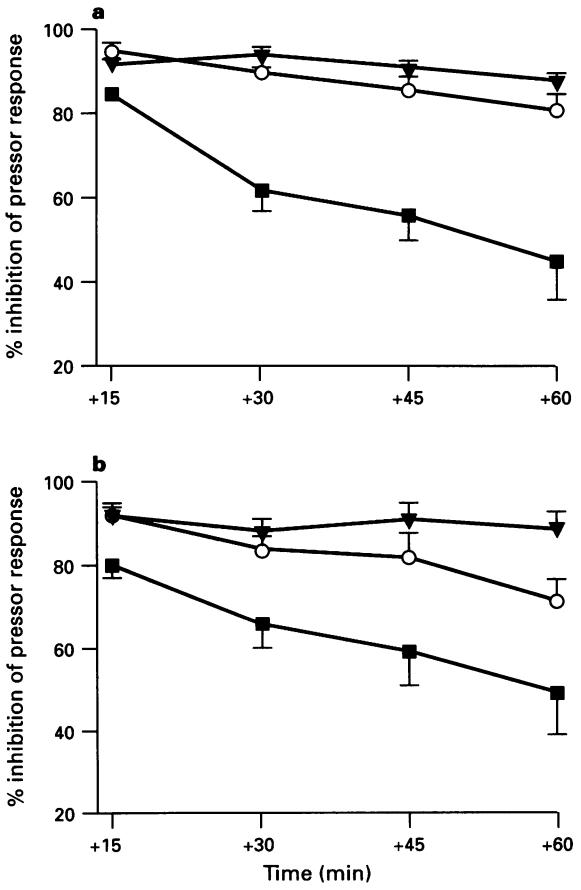

Figure 1 Effect of ACE inhibitors on the pressor to angiotensin I: (a) lisinopril 0.01 mg kg^{-1} (■); 0.03 mg kg^{-1} (○) and 0.3 mg kg^{-1} (▼); (b) sampatrilat 0.03 mg kg^{-1} (■); 0.1 mg kg^{-1} (○) and 0.3 mg kg^{-1} (▼).

Results

A–V fistula rats

The perioperative mortality rate for the rats used in this study was less than 10%. The rats used had 'compensated' for the A–V shunt; those animals that fail to 'compensate' die within the first 7 days after surgery (Winaver *et al.*, 1988). Mean plasma renin activity was 10.3 ± 2.2 ng angiotensin I ml^{-1} h^{-1} in A–V fistula rats ($n=7$) and 7.4 ± 1.5 ng angiotensin I ml^{-1} h^{-1} in sham-operated controls ($n=9$; NS).

Angiotensin I pressor-responses

Lisinopril 0.01–0.3 mg kg^{-1} and sampatrilat 0.03–0.3 mg kg^{-1} i.v. produced dose-dependent inhibition of the pressor response to angiotensin I; lisinopril 0.03 mg kg^{-1} and sampatrilat 0.1 mg kg^{-1} produced >80% inhibition for up to 60 min post-administration, indicating significant inhibition of ACE (Figure 1).

Effect of E-24.11 inhibition and ACE inhibition on renal function and blood pressure

A gradual <2 fold increase in urinary sodium excretion followed administration of vehicle (Figure 2a; Table 1). Candoxatrilat 3 mg kg^{-1} produced a prompt and sustained 3 fold increase in urinary sodium excretion in the first 15 min urine collection and this was sustained for the period of observation. Increasing the dose of candoxatrilat to 10 mg kg^{-1} produced no further increase in natriuresis. Administration of lisinopril 0.03–0.1 mg kg^{-1} alone was followed by a gradual rise in sodium excretion similar to that seen with vehicle. However, low-dose lisinopril (0.03 mg kg^{-1}) blunted the early natriuretic response to candoxatrilat 3 mg kg^{-1} ($P<0.05$ at 60 min); thereafter sodium excretion increased to reach levels seen with the specific E-24.11 inhibitor alone. Similarly, the lowest dose of the dual E-24.11/ACE inhibitor, sampatrilat (0.1 mg kg^{-1}), required to produce 80% inhibition of the pressor response to angiotensin I for the period of study induced a steady but delayed increase in sodium excretion compared to candoxatrilat alone (Figure 3a; Table 1). Higher doses of this compound demonstrated a dose-dependent reduction in natriuresis after administration.

The changes in urinary sodium excretion were associated with a similar pattern of urinary cyclic GMP excretion. Given alone, candoxatrilat 3 mg kg^{-1} provoked a marked rise in cyclic GMP excretion; lisinopril 0.03 mg kg^{-1} alone produced only a small increase in cyclic GMP excretion compared to vehicle (Figure 2b). Concurrent administration of lisinopril and candoxatrilat was associated with a delayed rise in cyclic GMP excretion compared to candoxatrilat alone and levels remained below those produced by the E-24.11 inhibitor alone. In concert with the progressive increase in urinary sodium output, sampatrilat 0.1 mg kg^{-1} produced a steady rise in urinary cyclic GMP excretion but the response was significantly reduced compared to candoxatrilat (Figure 3b).

Candoxatrilat alone (3 and 10 mg kg^{-1}) had no effect on blood pressure when compared to vehicle-treated rats (Table 2). Lisinopril reduced blood pressure in a dose-dependent manner over the range 0.03–0.1 mg kg^{-1}. Lisinopril 0.03 mg kg^{-1}

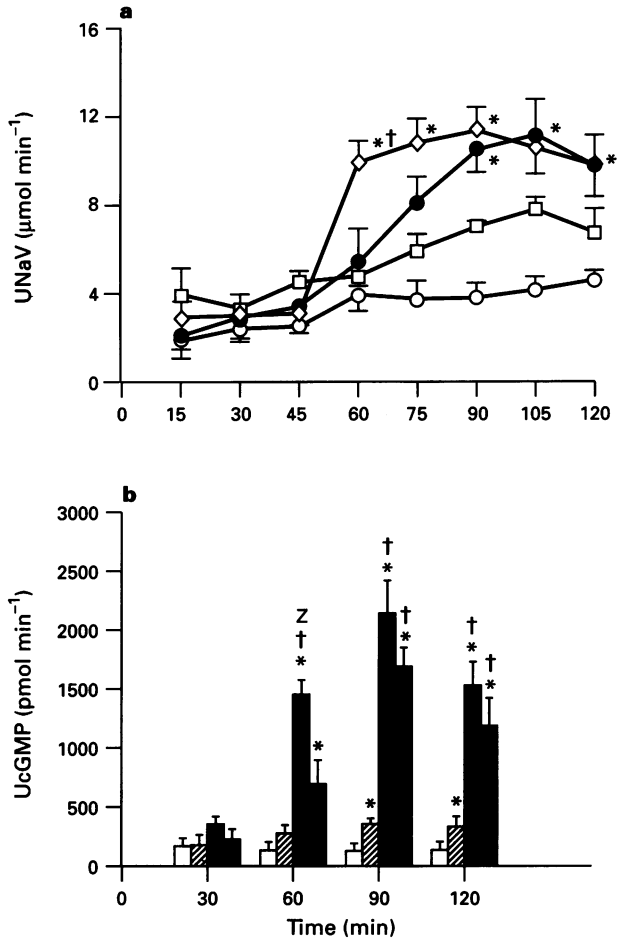

Figure 2 (a) Urinary sodium excretion (UNaV) in response to candoxatrilat 3 mg kg^{-1} (◇; $n=11$), lisinopril 0.03 mg kg^{-1} alone (□; $n=5$), the combination of lisinopril 0.03 mg kg^{-1} and candoxatrilat 3 mg kg^{-1} (●; $n=5$) and vehicle (○; $n=9$) in A–V fistula animals. All compounds were given after the 45 min collection. Data are expressed as mean±s.e.mean. *$P<0.05$ compared to lisinopril 0.03 mg kg^{-1} alone and †$P<0.05$ compared to lisinopril and candoxatrilat. (b) Urinary cyclic GMP (UcGMP) excretion in response to vehicle (□; $n=9$), lisinopril 0.03 mg kg^{-1} (▨; $n=5$), candoxatrilat 3 mg kg^{-1} (■; $n=11$) and the addition of lisinopril 0.03 mg kg^{-1} to candoxatrilat 3 mg kg^{-1} (▩; $n=5$). All compounds were given after the 45 min collection. Data are expressed as mean±s.e.mean. *$P<0.05$ compared to vehicle; †$P<0.05$ compared to lisinopril 0.03 mg kg^{-1} alone; and $^{z}P<0.05$ compared to lisinopril and candoxatrilat.

Table 1 Effect of E-24.11 and ACE inhibition on sodium excretion in the aorta-venocaval fistula rat

	Time (min)							
UNaV (μmol min^{-1})	*15*	*30*	*45*	*60*	*75*	*90*	*105*	*120*
Vehicle	1.9±0.4	2.4±0.4	2.6±0.3	3.9±0.7	3.8±0.8	3.8±0.7	4.2±0.6	4.6±0.5
Can 3 mg kg^{-1}	2.9±0.8	3.0±0.6	3.1±0.5	9.9±1.0*	10.8±1.1*	11.4±1.0*	10.6±1.2*	9.8±1.4*
Can 10 mg kg^{-1}	2.5±0.8	2.8±5	2.9±0.6	7.9±6*	9.3±2.1*	10.7±1.6*	10.4±1.4*	11.7±2.2*
Lisinopril 0.03 mg kg^{-1}	3.9±1.2*	3.3±0.6	4.5±0.5*	4.9±0.5^{+}	6.0±0.7*†	7.1±0.2*†	7.8±0.6*	6.8±1.1*
Lisinopril 0.1 mg kg^{-1}	2.2±1.6	2.9±1.2	3.1±0.6	3.1±1.1†	2.6±1.0†	4.3±1.6^{+}	5.9±2.6†	6.6±2.1†
Can±Lis 0.03 mg kg^{-1}	2.1±1.0	2.9±1.0	3.5±1.2	5.5±1.5	8.2±1.1*	10.6±1.1*	11.1±1.6*	9.8±1.3*
Sampatrilat 0.1 mg kg^{-1}	2.3±0.3	2.9±0.5	3.5±0.7	5.2±1.1†	7.5±0.9*†	6.9±1.3*†	9.6±0.9*	8.2±0.6*
Sampatrilat 0.3 mg kg^{-1}	2.4±1.3	2.5±1.2	2.3±0.7	4.7±1.6^{+}	5.7±1.1†	6.9±0.9*†	6.6±0.8*†	5.5±0.5^{+}
Sampatrilat 1 mg kg^{-1}	1.0±0.9	1.1±0.4	1.7±0.5	2.4±1.0^{+}	2.1±0.8†	3.6±0.5†	5.4±0.5†	5.3±0.6^{+}

Data are mean±s.e.mean. Compounds were administered after 45 min. *$P<0.05$ compared to vehicle, †$P<0.05$ compared to candoxatrilat 3 mg kg^{-1} alone.

alone had no significant effect on blood pressure compared to candoxatrilat although a small fall was observed at the end of the study period compared to vehicle. However, the combination of lisinopril 0.03 mg kg^{-1} and candoxatrilat 3 mg kg^{-1} reduced blood pressure below that recorded with candoxatrilat alone, an effect most pronounced in the first observation period after giving the compounds. Sampatrilat in doses that significantly inhibited the pressor response to angiotensin I reduced blood pressure in this rat model below that recorded with candoxatrilat alone.

There was a small and transient rise in GFR after giving candoxatrilat ($P=0.056$ versus vehicle; Table 3). A small fall in GFR was seen immediately following treatment with lisinopril and no rise in GFR was seen when lisinopril 0.03 mg kg^{-1} was combined with candoxatrilat. No changes in GFR were seen with sampatrilat.

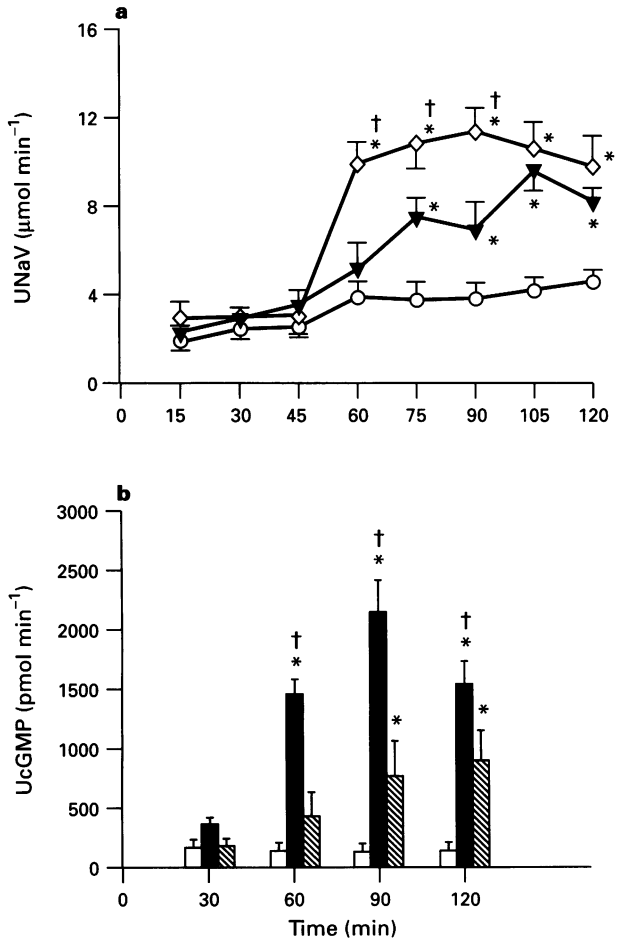

Figure 3 (a) Urinary sodium excretion (UNaV) in response to candoxatrilat, 3 mg kg^{-1} (◇; $n=11$) and sampatrilat, 0.1 mg kg^{-1} (▼; $n=5$) and vehicle (○; $n=9$) in A–V fistula animals. All compounds were given after the 45 min collection. Data are expressed as mean±s.e.mean. *$P<0.05$ compared to vehicle; †$P<0.05$ compared to sampatrilat. (b) Urinary cyclic GMP (UcGMP) excretion in response to vehicle (□; $n=9$), candoxatrilat, 3 mg kg^{-1} (■; $n=11$) and sampatrilat, 0.1 mg kg^{-1} (▨; $n=5$). All compounds were given after the 45 min collection. Data are expressed as mean±s.e.mean. *$P<0.05$ compared to vehicle, †$P<0.05$ compared to sampatrilat.

Discussion

The natriuretic-diuretic response to E-24.11 inhibitors is increased when circulating ANP and BNP levels are raised. Introduction of an A–V shunt (1–1.5 mm) in the rat leads to a chronic increase in venous return and stimulates cardiac ANP synthesis and secretion (Lattion *et al.*, 1986; Wilkins *et al.*, 1990; Brown *et al.*, 1993). A small proportion of rats fail to compensate for the shunt and die in the first week post-surgery with sodium retention, manifest as pulmonary oedema and ascites (Winaver *et al.*, 1988). The majority of animals compensate well and live an active existence with a sustained 5–8 fold elevation of circulating ANP levels (Wilkins *et al.*, 1990; 1992). Consistent with our previous observations in these animals (Wilkins *et al.*, 1992; Kirk & Wilkins, 1993) and with effects mediated by natriuretic peptides, candoxatrilat given as a single bolus injection produced a prompt natriuresis accompanied by a rise in urinary cyclic GMP excretion (the second messenger for this peptide family).

Concurrent administration of an ACE inhibitor might be expected to improve the renal response to E-24.11 inhibition for several reasons. First, given that angiotensin II and aldosterone are potent physiological antagonists of ANP and BNP, any level of activity of the renin-angiotensin system may significantly oppose the renal actions of ANP and BNP. Second, inhibition of E-24.11 alone actually increases angiotensin II levels, by reducing conversion of angiotensin I to angiotensin 1–7 (thereby making more angiotensin I available for angiotensin II production) and by inhibiting angiotensin II degradation (Yamamoto *et al.*, 1992; Ward *et al.*, 1995). Third, bradykinin is a substrate for both E-24.11 and ACE and co-inhibition of these enzymes has been shown to potentiate synergistically the natriuretic effects of bradykinin in anaesthetized dogs (Seymour *et al.*, 1994). However, instead of increasing the natriuretic response, significant ACE inhibition blunted the acute natriuretic effect of E-24.11 inhibition in the A–V fistula rat.

Recently, several groups have reported their experience of combined ACE and E-24.11 inhibition in animal models of heart failure. Seymour *et al.* (1993) studied conscious dogs after 1 and 3 weeks of rapid ventricular pacing and found that concurrent administration of an E-24.11 (SQ 28,603) and an

Table 2 Effect of E-24.11 and ACE inhibition on blood pressure in the aorta-venocaval fistula rat

	Time (min)							
BP (mmHg)	*15*	*30*	*45*	*60*	*75*	*90*	*105*	*120*
Vehicle	116±3	117±4	116±4	115±4	115±4	116±4	118±4	119±4
Can 3 mg kg^{-1}	116±3	116±2	117±2	116±2	116±2	116±2	114±2	113±2
Can 10 mg kg^{-1}	114±3	112±3	112±2	111±4	111±2	112±1	116±1	119±1
Lisinopril 0.03 mg kg^{-1}	117±3	120±3	116±4	113±5	112±5	114±5	109±5	109±5
Lisinopril 0.1 mg kg^{-1}	113±4	110±5	111±5	96±4*†	96±6*†	98±4*†	101±3*†	103±3*†
Can±Lis 0.03 mg kg^{-1}	117±5	118±4	117±5	107±5†	109±4	108±4	110±3	110±4
Sampatrilat 0.1 mg kg^{-1}	115±3	116±3	116±4	106±2†	107±2†	108±2	111±3	116±4
Sampatrilat 0.3 mg kg^{-1}	116±5	117±1	116±4	106±4	108±4	111±5	110±5	111±3
Sampatrilat 1 mg kg^{-1}	118±5	119±6	121±2	102±7*	103±6	105±5	104±4	108±2

Data are mean±s.e.mean. Compounds were administered after 45 min,. *$P<0.05$ compared to vehicle, †$P<0.05$ compared to candoxatrilat 3 mg kg^{-1} alone.

Table 3 Effect of E-24.11 and ACE inhibition on glomerular filtration rate in the aorta-venocaval fistula rat

	Time (min)							
GFR (ml min^{-1})	*15*	*30*	*45*	*60*	*75*	*90*	*105*	*120*
Vehicle	2.7±0.1	2.9±0.3	2.7±0.1	2.8±0.2	2.6±0.2	2.5±0.1	2.8±0.1	2.6±0.2
Can 3 mg kg^{-1}	2.9±0.2	2.9±0.3	2.5±0.2	3.5±0.3	2.9±0.1	2.9±0.2	2.8±0.2	2.7±0.2
Can 10 mg kg^{-1}	2.5±0.2	2.6±0.1	2.1±0.2	3.2±0.3	2.3±0.2	2.7±0.3	2.4±0.1	2.5±0.2
Lisinopril 0.03 mg kg^{-1}	2.6±0.1	2.1±0.4*	2.6±0.2	2.1±0.2*†	2.3±0.2	2.7±0.3	2.6±0.2	2.3±0.3
Lisinopril 0.1 mg kg^{-1}	2.6±0.4	3.3±0.3	2.6±0.2	1.9±0.5†	1.9±0.2†	2.7±0.3	2.7±0.3	2.9±0.3
Can±Lis 0.03 mg kg^{-1}	2.7±0.4	3.2±0.6	3.2±0.4	3.0±0.3	3.5±0.3*	3.6±0.4*	3.2±0.4	3.2±0.3
Sampatrilat 0.1 mg kg^{-1}	2.7±0.3	2.4±0.2	2.7±0.2	2.5±0.4	2.6±0.2	2.1±0.2	3.1±0.5	2.8±0.4
Sampatrilat 0.3 mg kg^{-1}	2.9±0.5	2.9±0.3	2.7±0.3	2.2±0.4	2.1±0.6	2.4±0.5	2.8±0.2	2.0±0.6
Sampatrilat 1 mg kg^{-1}	2.9±0.2	2.7±0.2	3.2±0.3	2.9±0.1	2.7±0.2	3.3±0.3*	3.3±0.4	3.0±0.4

Data are mean±s.e.mean. Compounds were administered after 45 min. *$P<0.05$ compared to vehicle, †$P<0.05$ compared to candoxatrilat 3 mg kg^{-1} alone.

ACE (captopril) inhibitor improved systemic haemodynamics but did not increase cumulative urinary sodium excretion in the 90 min following administration of the compounds when compared to E-24.11 inhibition alone. Trippodo *et al.* (1993) observed that co-treatment with ACE (enalaprilat) and E-24.11 (SQ 28,603) inhibitors decreased left ventricular end-diastolic pressure in the cardiomyopathic hamster (which was not seen with either compound alone) but, as in the studies of Seymour and colleagues, the combination did not significantly affect 90 min cumulative sodium excretion. Helin (1993) observed an increased natriuretic response to combined inhibition with E-24.11 (SCH 24826) and ACE (captopril) inhibitors in normal animals (compared to E-24.11 inhibition alone) in a 36 h period following dosing but not in coronary artery ligated rats.

These findings contrast with the effect of pretreatment with an ACE inhibitor on the response to ANP or E-24.11 inhibition in animal studies i.e. giving the ACE inhibitor in advance of inhibiting E-24.11. Pretreatment with an ACE inhibitor has been shown to improve renal responsiveness to subsequent treatment with ANP or E-24.11 inhibitor (Margulies *et al.*, 1991). The most likely explanation for this difference is the acute fall in blood pressure that accompanies initiation of E-24.11 and ACE inhibition together, which has not been observed when E-24.11 inhibitors have been introduced after ACE inhibition has been established (Margulies *et al.*, 1991). Considerable effort was taken in our studies to find the lowest dose of lisinopril sufficient to inhibit ACE effectively but with minimal hypotensive effects. Nonetheless, a fall in blood pressure was observed during concurrent treatment with an E-24.11 inhibitor. Others have also noted that acute dosing with dual ACE and E-24.11 inhibitors produce haemodynamic effects (such as an increase in cardiac output or fall in systemic blood pressure) not observed when the same dose of either inhibitor was given alone (Seymour *et al.*, 1993; Trippodo *et al.*, 1993). It is noteworthy that it was not possible to find a dose of the dual E-24.11/ACE inhibitor, sampatrilat, that inhibited ACE for the duration of the study but did not lower blood pressure. One candidate for the greater hypotensive effect of the combined treatment is bradykinin; bradykinin levels might be expected to increase to a greater degree when both enzymes are inhibited acutely rather than either alone.

One consequence of blood pressure reduction is a fall in renal perfusion pressure. It is well recognised that the renal response to ANP is dependent on renal perfusion pressure. Seymour *et al.* (1993) examined the pressure-natriuresis relationship in their study in dogs with pacing-induced heart failure and found that for any given blood pressure the natriuresis was greater with co-inhibition of ACE and E-24.11 than with either inhibitor alone. Nonetheless the reduction in blood pressure due to concomitant E-24.11 and ACE inhibition prevented an overall increase in sodium excretion above that produced by E-24.11 inhibition alone in that study.

In addition to reducing renal perfusion, the reduction in blood pressure in some studies may have led to a significant decrease in cardiac work and so a decline in ANP and BNP secretion, the principle mediators of E-24.11 induced natriuresis. In support of this, the rise in urinary cyclic GMP during combined treatment with lisinopril and candoxatrilat was less than that with candoxatrilat alone; a similar observation has been made by others (Trippodo *et al.*, 1993). However, captopril had no effect on the rise in plasma or urinary ANP and cyclic GMP levels following E-24.11 inhibition in ventricle-paced dogs (Seymour *et al.*, 1993).

In summary, concurrent administration of an ACE inhibitor significantly blunts the early natriuretic response to acute E-24.11 inhibition in the A–V fistula rat. This outcome is most likely due to the accompanying fall in systemic blood pressure, an effect observed even with low-dose ACE inhibition. Our data, taken together with others, indicates that the acute benefits of combining the 2 compounds on renal function are more apparent if ACE inhibition precedes the introduction of an E-24.11 inhibitor.

The authors are grateful to Dr P Barclay of Pfizer Central Research for the gift of sampatrilat.

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(Received April 2, 1996
Revised July 11, 1996
Accepted August 7, 1996)