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Assessment of the role of the renin-angiotensin system in cardiac contractility utilizing the renin inhibitor remikiren

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1 The role of the renin-angiotensin system in the regulation of myocardial contractility is still debated. In order to investigate whether renin inhibition affects myocardial contractility and whether this action depends on intracardiac rather than circulating angiotensin II, the regional myocardial effects of systemic (i.v.) and intracoronary (i.c.) infusions of the renin inhibitor remikiren, were compared and related to the effects on systemic haemodynamics and circulating angiotensin II in open-chest anaesthetized pigs (25–30 kg). The specificity of the remikiren-induced effects was tested (1) by studying its i.c. effects after administration of the AT_1-receptor antagonist L-158,809 and (2) by measuring its effects on contractile force of porcine isolated cardiac trabeculae.

2 Consecutive 10 min i.v. infusions of remikiren were given at 2, 5, 10 and 20 mg min^{-1}. Mean arterial pressure (MAP), cardiac output (CO), heart rate (HR), sytemic vascular resistance (SVR), myocardial oxygen consumption (MVO_2) and left ventricular (LV) dP/dt_{max} were not affected by remikiren at 2 and 5 mg min^{-1}, and were lowered at higher doses. At the highest dose, MAP decreased by 48%, CO by 13%, HR by 14%, SVR by 40%, MVO_2 by 28% and LV dp/dt_{max} by 52% (mean values; $P<0.05$ for difference from baseline, $n=5$). The decrease in MVO_2 was accompanied by a decrease in myocardial work (MAP × CO), but the larger decline in work (55% *vs.* 28%; $P<0.05$) implies a reduced myocardial efficiency ((MAP × CO)/MVO_2).

3 Consecutive 10 min i.c. infusions of remikiren were given at 0.2, 0.5, 1, 2, 5 and 10 mg min^{-1}. MAP, CO, MVO_2 and LV dP/dt_{max} were not affected by remikiren at 0.2, 0.5 and 1 mg min^{-1}, and were reduced at higher doses. At the highest dose, MAP decreased by 31%, CO by 26%, MVO_2 by 46% and LV dP/dt_{max} by 43% (mean values; $P<0.05$ for difference from baseline, $n=6$). HR and SVR did not change at any dose.

4 Thirty minutes after a 10 min i.v. infusion of the AT_1 receptor antagonist, L-158,809 at 1 mg min^{-1}, consecutive 10 min i.c. infusions ($n=5$) of remikiren at 2, 5 and 10 mg min^{-1} no longer affected CO and MVO_2, and decreased LV dP/dt_{max} by maximally 27% ($P<0.05$) and MAP by 14% ($P<0.05$), which was less than without AT_1-receptor blockade ($P<0.05$). HR and SVR remained unaffected.

5 Plasma renin activity and angiotensin I and II were reduced to levels at or below the detection limit at doses of remikiren that were not high enough to affect systemic haemodynamics or regional myocardial function, both after i.v. and i.c. infusion.

6 Remikiren (10^{-10} to 10^{-4} M) did not affect contractile force of porcine isolated cardiac trabeculae precontracted with noradrenaline. In trabeculae that were not precontracted no decrease in baseline contractility was observed with remikiren in concentrations up to 10^{-5} M, whereas at 10^{-4} M baseline contractility decreased by 19% ($P<0.05$).

7 Results show that with remikiren i.v., at the doses we used, blood pressure was lowered primarily by vasodilatation and with remikiren i.c. by cardiac depression. The blood levels of remikiren required for its vasodilator action are lower than the levels affecting cardiac contractile function. A decrease in circulating angiotensin II does not appear to be the sole explanation for these haemodynamic responses. Data support the contention that myocardial contractility is increased by renin-dependent angiotensin II formation in the heart.

Keywords: Renin-angiotensin system; renin inhibition; remikiren; cardiac contractility; vasodilatation; angiotension II; AT_1-receptor antagonism; L-158,809

Introduction

The renin-angiotensin system (RAS) plays an important role in the regulation of blood pressure and body fluid volumes. An early and rate-limiting step in the synthesis of angiotensin II (AII), the active end-product of the RAS, is the cleavage of angiotensinogen by renin. This results in the formation of the inactive decapeptide angiotensin I (AI). Angiotensin-converting enzyme (ACE) catalyzes the conversion of AI to AII. It is now generally believed that these reactions occur both in the circulating blood and at tissue sites (Campbell, 1987; Admiraal *et al.*, 1990; Danser *et al.*, 1992b).

Blockers of the formation of AII, the ACE inhibitors in particular, are at present widely used for the treatment of hypertension and congestive heart failure. Renin inhibitors are also effective blood pressure lowering agents both in man (Jeunemaitre *et al.*, 1989; van den Meiracker *et al.*, 1990; 1993; Weber *et al.*, 1990; Kiowski *et al.*, 1994) and in experimental

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animals (Tree *et al.*, 1989; Wood *et al.*, 1989;1990; Schaffer *et al.*, 1990; Fischli *et al.*, 1991;1994; Kleinert *et al.*, 1992; Clozel & Fischli, 1993; Wessale *et al.*, 1993a,b; . However, due to the low oral bioavailability of the renin inhibitors studied so far, these agents are not widely used.

ACE inhibitors lower arterial blood pressure through a reduction in peripheral vascular resistance, most probably mediated by inhibition of AII synthesis. The favourable effects of ACE inhibition in heart failure are not yet fully understood. It has been suggested that they are unrelated, at least in part, to the reduction in systemic vascular resistance (Linz *et al.*, 1992). A local cardiac RAS has been postulated (Lindpaintner *et al.*, 1987; Danser *et al.*, 1994), and ACE inhibitors therefore may exert their effect through interference with cardiac AII production. AII is known to have chronotropic and inotropic effects (Kobayashi *et al.*, 1978) and may have effects on myocardial hypertrophy and remodelling (Schelling *et al.*, 1991).

ACE also acts on substrates other than AI, including bradykinin and substance P (Erdös, 1990). On the basis of experiments with the bradykinin antagonist, Hoe 140, it has been claimed that some of the beneficial effects of ACE inhibitors in heart failure are mediated throughout bradykinin accumulation (Hartman *et al.*, 1993).

Little information is available on the cardiac effects of renin inhibitors. In early studies on the vasodilator activity of peptidic renin inhibitors, both increases and decreases in cardiac output were observed (Zusman *et al.*, 1983; Haber *et al.*, 1985; Schaffer *et al.*, 1990; Mangiapane *et al.*, 1990). There is, however, some doubt as to whether these effects were caused by RAS blockade. More recent studies using non-peptidic renin inhibitors have focussed mainly on their ability to lower blood pressure and do not address cardiac effects. The blood pressure lowering effects of these inhibitors appear to be caused by inhibition of the RAS (Tree *et al.*, 1989; Wood *et al.*, 1989; 1990; Fischli *et al.*, 1991; Clozel & Fischli, 1993; Fisher *et al.*, 1994). It is still a matter of debate whether this involves RAS inhibition in the circulation or at tissue sites (van den Meiracker *et al.*, 1990; Fischli *et al.*, 1991; 1994).

The aim of the present study was to investigate whether renin inhibition affects myocardial contractility and whether this action depends on intracardiac rather than circulating AII. We therefore compared the myocardial effects of systemic and intracoronary infusions of remikiren, and related these regional effects to systemic haemodynamic responses and the effects on the circulating RAS. In order to determine whether the effect of remikiren on cardiac contractility is indeed mediated via AII, the intracoronary effects of the inhibitor were also evaluated after systemic administration of the highly potent and selective AT_1-receptor antagonist, L-158,809 (Chang *et al.*, 1992; Siegl *et al.*, 1992). The haemodynamic responses to AII are known to be AT_1-receptor-dependent (Siegl *et al.*, 1992). All studies were performed in open-chest anaesthetized pigs; remikiren is a potent inhibitor of human renin and also inhibits porcine renin (Danser *et al.*, 1992a).

Methods

General

All experiments were performed in accordance with the 'Guiding principles in the care and use of animals' as approved by the American Physiological Society and under the regulations of the Animal Care Committee of the Erasmus University Rotterdam, Rotterdam, The Netherlands.

Cross-bred Landrace × Yorkshire pigs (Hedelse Varkens Combinatie, Hedel, The Netherlands; weight 25–30 kg) of either sex were used. The effects of incremental intravenous (i.v.) and intracoronary (i.c.) doses of remikiren (Ro 42,5892) were studied in 5 and 6 animals, respectively. The effects of incremental i.c. doses of remikiren after AT_1-receptor blockade with L-158,809 were studied in 5 animals.

Instrumentation

Animals were sedated with an intramuscular injection of 20 mg kg^{-1} ketamine (AUV, Cuijk, The Netherlands), and anaesthetized with 20 mg kg^{-1} sodium pentobarbitone (Apharma, Arnhem, The Netherlands) administered via a dorsal ear vein. They were intubated and connected to a ventilator for intermittent positive pressure ventilation with a mixture of oxygen and nitrogen (1:2). Respiratory rate and tidal volume were adjusted to keep arterial blood gases (ABL3, Radiometer, Copenhagen, Denmark) within the physiological range: $7.35 < pH < 7.45$; $35 \text{ mmHg} < P_{CO_2} < 45 \text{ mmHg}$ and $100 \text{ mmHg} < P_{O_2} < 160 \text{ mmHg}$.

A 7 French (Fr) catheter was placed in the superior caval vein for infusion of 10–15 mg kg^{-1} h^{-1} sodium pentobarbitone to maintain a constant depth of anaesthesia. Catheters were also placed in the superior caval vein for administration of haemaccel (Behringwerke A.G., Marburg, Germany) to replace blood withdrawn during sampling and for intravenous infusion of remikiren, L-158,809 or AII. The femoral arteries were cannulated with 8Fr catheters, which were advanced into the descending aorta to measure central aortic blood pressure and to withdraw blood samples for blood gas analysis and measurements of renin and angiotensins. A 7Fr Sensodyn micromanometer-tipped catheter (B. Braun Medical B.V., Uden, The Netherlands), inserted via the left carotid artery, was used to measure left ventricular pressure and its first derivative (LV dP/dt). Rectal temperature was monitored throughout the experiment and maintained between 37°C and 38°C with external heating pads and appropriate coverage of the animal with blankets.

After administration of 4 mg pancuronium bromide (Organon Teknika B.V., Boxtel, The Netherlands) and a midline thoracotomy, the heart was suspended in a pericardial cradle, while the left mammary vessels were ligated and the second left rib was removed to allow further instrumentation. The adventitia surrounding the aorta was dissected free and an electromagnetic flow probe (Skalar, Delft, The Netherlands) was positioned around the artery for measurement of ascending aortic blood flow (cardiac output, CO). A small segment of the left anterior descending coronary artery (LADCA) was dissected free for positioning of an electromagnetic flow probe. For intracoronary infusion of remikiren or its vehicle a small cannula was placed in the proximal LADCA, just distal of the flow probe. The cardiac vein accompanying the LADCA was cannulated to withdraw blood samples for blood gas analysis.

Regional myocardial segment length changes were measured by sonomicrometry (Triton Technology Inc., San Diego, CA, U.S.A.) using two pairs of ultrasound crystals (Sonotek Corporation, Del Mar, CA, U.S.A.). One pair was positioned in the distribution area of the LADCA, and the other pair in the distribution area of the left circumflex coronary artery (LCXCA).

Infusion experiments

After a 30–45 min stabilization period following completion of the surgical procedures, baseline measurements of systemic haemodynamic variables, myocardial blood flow and regional segment shortening were made, and blood samples were collected for determination of the arterial and coronary venous oxygen content and the arterial levels of plasma renin activity (PRA) and AI and AII. The animals were then subjected to one of the following protocols.

Protocol 1. Intravenous infusion of remikiren (as the methane sulphonate; $n=5$) was started at a rate of 2 mg min^{-1}. At 10 min intervals the infusion rate was increased stepwisely to 5, 10 and 20 mg min^{-1}, respectively. These infusion rates corresponded to a dosage regimen ranging from 74 ± 3 (mean ± s.e. mean) to 744 ± 26 μg kg^{-1} min^{-1}. After the highest dose had been infused for 10 min, the infusion was stopped, and the study was continued for a 60 min recovery period.

Systemic haemodynamic and regional myocardial variables were measured and blood samples were collected for the determination of PRA, AI and AII at the end of each infusion step, when a steady-state situation had been reached, and during the recovery period.

Protocol 2. In this series of i.c. experiments, remikiren (as the methane sulphonate; $n=6$) or vehicle, i.e. equivalent amounts of methane sulphonic acid ($n=4$), were infused via the LADCA. Remikiren was infused in incremental doses of 0.2, 0.5, 1, 2, 5 and 10 mg min^{-1} (10 min). The remikiren infusion rates corresponded to a dosage regimen ranging from 8.0 ± 0.1 to 379 ± 6 $\mu g\ kg^{-1}\ min^{-1}$. After the last dose step, the infusion was stopped and the study was continued for a 120 min recovery period. Recordings of systemic haemodynamic and regional myocardial variables were made and blood samples were collected at the end of each infusion step and during the recovery period.

Protocol 3. In a second series of i.c. experiments, a 10 min i.v. infusion of the AT_1-receptor antagonist, L-158,809, was given at 1 mg min^{-1} prior to remikiren administration ($n=5$). To test AT_1-receptor blockade, 3 i.v. bolus injections of AII (0.1, 0.3 and 1.0 $\mu g\ kg^{-1}$) were given immediately before and after L-158,809 infusion.

Thirty min after L-158,809 administration, remikiren (as the methane sulphonate) was infused in incremental doses of 2, 5 and 10 mg min^{-1} (10 min). The remikiren infusions corresponded to a dosage regimen ranging from 74 ± 4 to 368 ± 20 $\mu g\ kg^{-1}\ min^{-1}$. After the last dose step, the infusion was stopped, and the study was continued for a 60 min recovery period. Then AT_1-receptor blockade was tested once more by i.v. bolus injections of AII. Recordings of systemic haemodynamic and regional myocardial variables were made and blood samples were collected before and after L-158,809 infusion, at the end of each remikiren infusion step, and finally during the recovery period. Recordings of haemodynamic variables were also made during the i.v. AII bolus injections.

The i.v. and i.c. doses of remikiren we used were based on a previous study in human subjects, in whom dose-dependent hypotensive effects of remikiren were observed after 10 min i.v. infusions of 10 and 100 $\mu g\ kg^{-1}\ min^{-1}$. In the present study in pigs we chose to give higher doses because the IC_{50} of remikiren for porcine renin is higher (approximately 80 times) than for human renin (Danser *et al.*, 1992a).

Blood sampling

Blood samples for measurement of AI and AII were rapidly drawn (5–10 s) with a plastic syringe containing the following inhibitors (0.25 ml inhibitor solution in 5 ml blood) 6.25 mM disodium EDTA, 1.25 mM 1,10-phenanthroline and 10^{-5} M remikiren (final concentrations in blood) (Danser *et al.*, 1992a). The blood samples were immediately transferred to polystyrene tubes and centrifuged at 3,000 *g* for 10 min at 4°C. Plasma was stored at −70°C and extracted within 2 days after collection.

Blood samples for measurement of PRA were collected in polystyrene tubes containing disodium citrate (0.1 ml in 5 ml blood; final concentration 13 mM). The samples were centrifuged at 1,000 *g* for 10 min at room temperature and plasma was stored at −70°C.

Measurement of angiotensin I and II

AI, AII and their metabolites were extracted from plasma by reversible adsorption to octadecylsilyl-silica (Sep Pak C18, Waters, Millford, MA, U.S.A.) and separated by high performance liquid chromatography (h.p.l.c.), according to the method described by Nussberger *et al.* (1986), with some modifications (Admiraal *et al.*, 1990). Prior to extraction [^{125}I]-AI was added to 2 ml plasma as internal standard. Separations were performed on a reversed-phase Nucleosil C18 steel column of 250 × 4.6 mm and 10 μm particle size (Alltech, Eke, Belgium). Mobile phase A was 25% methanol (vol/vol) in 0.085% ortho-phosphoric acid, containing 0.02% sodium azide. Mobile phase B was 75% methanol (vol/vol) in 0.085% orthophosphoric acid, containing 0.02% sodium azide. The flow was 1.5 ml min^{-1} and the working temperature was 45°C. Vacuum dried SepPak plasma extracts were dissolved in 100 μl mobile phase A and injected. Elution was performed as follows: 85% A/15% B (vol/vol) from 0 to 5 min followed by a linear gradient to 40% A/60% B (vol/vol) until 16 min. The eluate was collected in 20 s fractions into polystyrene tubes coated with bovine serum albumin. The fractions containing AI and AII were neutralized with 0.17 M sodium hydroxide and vacuum dried.

The concentration of [^{125}I]-AI in the h.p.l.c. fractions was measured in a gamma counter. The concentrations of AI and AII in the vacuum dried h.p.l.c. fractions were measured by radioimmunoassay (Admiraal *et al.*, 1990). The detection limit for AI and AII were 1 and 0.5 fmol ml^{-1} respectively. The AI antiserum crossreacted with A-(2–10) nonapeptide (100%), but not (< 0.1%) with AII, AIII, A-(3–8) hexapeptide, A-(4–8) pentapeptide, A-(1–7) heptapeptide, or A-(1–4) tetrapeptide. The AII antiserum crossreacted with AIII (55%), A-(3–8) hexapeptide (73%) and A-(4–8) pentapeptide (100%), but virtually not (< 0.2%) with AI, A-(2–10) nonapeptide, A-(1–7) heptapeptide, or A-(1–4) tetrapeptide.

Measurement of plasma renin activity

PRA was determined by measuring the rate of AI generation at pH = 7.4 during incubation at 37°C in the presence of excess AI antibody ('antibody trapping assay') according to Poulsen & Jorgensen (1974). The incubation mixture consisted of 500 μl sample, 10 μl AI antiserum (final dilution 1 : 10,000) and 10 μl disodium EDTA solution (final concentration 10 mM). Incubation time was 30 min. The trapped AI was quantitated by radioimmunoassay, after dilution of the samples in cold 0.15 M Tris buffer, pH = 7.4, containing [^{125}I]-AI, to give a final antiserum dilution of 1 : 120,000. The diluted samples were kept for 18 h at 4°C. The detection limit of the antibody trapping assay was 1 fmol AI $ml^{-1}\ min^{-1}$.

Experiments with isolated papillary muscle

Left ventricular cardiac tissue was obtained from four pigs. The pigs had served as controls for a series of acute pharmacological experiments under sodium pentobarbitone anaesthesia (20 mg $kg^{-1}\ h^{-1}$). Tissues pieces of 1 g were placed in ice-chilled Krebs buffer (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 8.3), which was gassed with 95% O_2 and 5% CO_2. Trabeculae of 1 mm thickness were carefully dissected free from surrounding tissue. The trabeculae were mounted in organ baths, kept at 37°C and containing Krebs buffer gassed with 95% O_2 and 5% CO_2, and attached to Harvard isometric transducers. Resting tension was set to approximately 20 mN to provide optimal loading conditions. Trabeculae were paced at 1.5 Hz, using field stimulation (3 ms, voltage 20% above threshold).

After a stabilization period of at least 60 min, baseline contractile force was recorded and a concentration-response curve for noradrenaline (10^{-10} to 10^{-5} M) was obtained to check the viability of the tissue. After washing and restabilization, a dose-response for remikiren (as the methane sulphonate; 10^{-10} to 10^{-4} M or 0.0729 ng ml^{-1} to 72.9 $\mu g\ ml^{-1}$) was constructed either directly, or after the tissues had been precontracted with 10^{-5} M noradrenaline. Dose-response curves with equivalent amounts of methane sulphonic acid were also constructed. Responses are expressed as percentage change from baseline (direct dose-response curve) or as a percentage of the response to 10^{-5} M noradrenaline (dose-response curve after precontraction with noradrenaline).

Chemicals

[Ile5]-A-(1–10) decapeptide (AI), [Ile5]-A-(1–8) octapeptide (AII), and [Ile5]-A-(2–8) heptapeptide (AIII) were obtained from Bachem (Bubendorf, Switzerland). [Ile5]-A-(2–10) nonapeptide (A-(2–10)) was from Senn Chemicals (Dielsdorf, Switzerland). [Ile5]-A-(3–8) hexapeptide (A-(3–8)), [Ile5]-A-(4–8) pentapeptide (A-(4–8)), [Ile5]-A-(1–7) heptapeptide (A-(1–7)) and tyrosine were from Peninsula Laboratories (Belmont, CA, U.S.A.). Methanol, ortho-phosphoric acid (both analytical grade) and 1,10-phenanthroline were purchased from Merck, Darmstadt, Germany. Methane sulphonic acid was obtained from Sigma (St. Louis, MO, U.S.A.). Water for high performance liquid chromatography was prepared with a Milli-Q system from Waters, Millford, MA, U.S.A. The specific renin inhibitor, remikiren (as the methane sulphonate; molecular weight 729) was a gift of Dr W. Fischli (Hoffmann-LaRoche, Basel, Switzerland). Its IC_{50} for human renin is 0.7 nM and for porcine renin 50 nM (Danser *et al.*, 1992a). The AT_1-receptor antagonist, L-158,809 (5,7-dimethyl-2-ethyl-3-[[2'-(1H-tetrazol-5yl)[1,1']-biphenyl-4-yl]-methyl]-3H-imidazo[4,5-b]pyridine) (as the tetrazolic acid monohydrate; molecular weight 427.5) was a gift of Dr R.D. Smith (Du Pont Merck Pharmaceutical Company, Wilmington, DE, U.S.A.). This antagonist is 50–200 times more potent than losartan (IC_{50} = 0.2–0.8 nM; Chang *et al.*, 1992).

Data analysis

Systemic and coronary vascular resistance were calculated as the ratios between mean arterial blood pressure (MAP) and CO and between MAP and coronary blood flow (CBF) respectively, while myocardial work was calculated as the product of MAP and CO. Segment length was measured at end-systole (ESL) and at end-diastole (EDL) in order to calculate systolic segment shortening as SS = (EDL − ESL)/EDL × 100%. Myocardial oxygen consumption (MVO_2) was calculated as the product of CBF and the difference in the arterial and coronary venous O_2 contents (Bien *et al.*, 1991).

All data are presented as mean and s.e. mean. A one way analysis of variance for repeated measurements was performed to determine the statistical significance of the remikiren- or vehicle-induced changes. Statistical significance was accepted for $P<0.05$.

Results

Intravenous infusions

Systemic haemodynamics, myocardial function and oxygen consumption MAP, CO, heart rate (HR), systemic vascular resistance (SVR), LV dP/dt_{max}, CBF and MVO_2 were not affected by remikiren at 2 and 5 mg min^{-1} (Figures 1 and 2), and were reduced at higher doses. At the highest dose, MAP decreased by 48±4% ($P<0.05$) and CO by only 13±2% ($P<0.05$). As a result, SVR decreased by 40 ± 4%, suggesting that the decrease in arterial blood pressure was caused predominantly by systemic vasodilatation. Arterial pulse pressure (i.e., systolic arterial blood pressure − diastolic arterial blood pressure) and left ventricular end-diastolic pressure (LVEDP) were not affected. Stroke volume also did not change, because the decrease in HR (14 ± 4% at the highest dose, $P<0.05$) corresponded very closely with the decrease in CO. LV dP/dt_{max} decreased by maximally 52 ± 6% ($P<0.05$).

CBF and coronary vascular resistance (CVR) decreased by maximally 34 ± 8% and 28 ± 5% respectively (Figure 2). Myocardial oxygen extraction did not change; coronary venous oxygen saturation was 23 ± 2% at baseline and 23 ± 1% after infusion of the highest dose. Consequently, the changes in MVO_2 parallelled the changes in CBF (Figure 2). The decrease in MVO_2 was accompanied by a decrease in myocardial work, but the larger decline in myocardial work (55 ± 4% *vs.* 28 ± 3%; $P<0.05$) implies a reduced myocardial efficiency. EDL, ESL and SS in the distribution areas of the LADCA and the LCXCA were not significantly affected (Table 1).

After the infusions had been stopped all variables started to return to their baseline values. During the 60 min recovery period, systemic vascular resistance increased from 60 ± 4% to 86 ± 3% of baseline ($P<0.05$), which was still lower than baseline ($P<0.05$).

Plasma renin activity and angiotensin I and II levels Baseline PRA, AI and AII levels were in the normal range (Danser *et al.*, 1992b). At doses of 2 mg min^{-1} and higher, remikiren

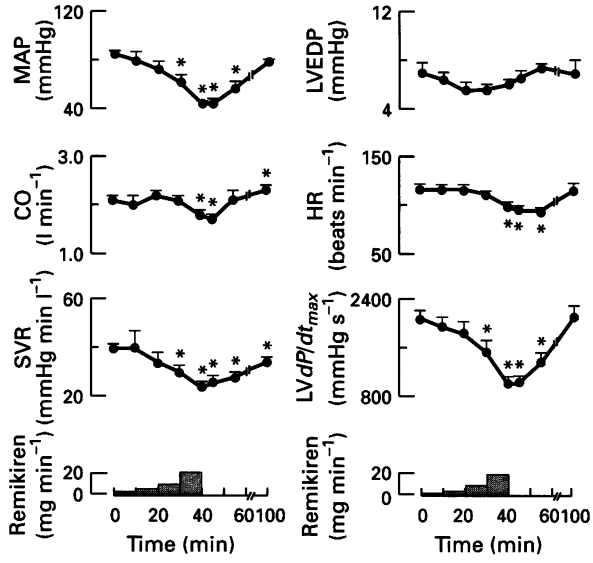

Figure 1 Effects of consecutive 10 min intravenous infusions of remikiren (2, 5, 10 and 20 mg min^{-1}) on mean arterial pressure (MAP), cardiac output (CO), systemic vascular resistance (SVR), left ventricular end-diastolic pressure (LVEDP), heart rate (HR) and LV dP/dt_{max} in open-chest anaesthetized pigs. The recovery of the various parameters at 15 and 60 min after discontinuation of the infusions is also shown. Data are presented as mean and s.e. mean ($n=5$). * $P<0.05$ *vs.* baseline.

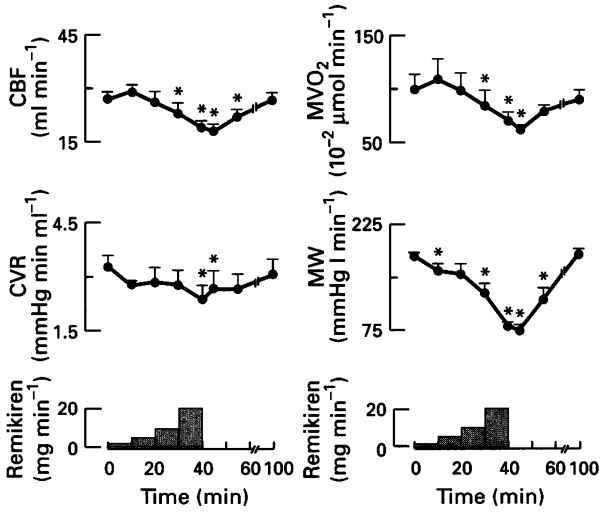

Figure 2 Effects of consecutive 10 min intravenous infusions of remikiren (2, 5, 10 and 20 mg min^{-1}) on coronary blood flow (CBF), coronary vascular resistance (CVR), myocardial oxygen consumption (MVO_2) and myocardial work (MW) in open-chest anaesthetized pigs. The recovery of the various parameters at 15 and 60 min after discontinuation of the infusions is also shown. Data are presented as mean and s.e. mean ($n=5$). * $P<0.05$ *vs.* baseline.

Table 1 Relative segment shortening (%) in distribution areas of LADCA and LCXCA after intravenous or intracoronary administration of remikiren

			Remikiren (mg min^{-1})							
	Baseline	*AT-1 block*	*0.2*	*0.5*	*1.0*	*2.0*	*5.0*	*10.0*	*20.0*	*After recovery*
I.v. experiments										
LADCA	16.7±2.0	–	–	–	–	15.4±2.6	13.9±1.2	15.0±2.1	12.4±2.1	17.0±2.5
LCXCA	9.9±1.2	–	–	–	–	9.2±1.4	9.3±1.2	8.9±1.1	8.6±1.3	9.4±1.4
I.c. experiments without L-158,809										
LADCA	15.5±1.0	–	14.7±1.1	14.9±0.6	14.2±0.9	13.5±1.0*	12.9±1.1*	11.6±1.0*	–	11.6±1.0*
LCXCA	13.3±1.2	–	13.0±1.2	13.8±1.3	13.2±1.2	11.9±1.2	11.3±1.6	11.0±1.3	–	11.9±0.4
I.c. experiments with L-158,809										
LADCA	15.2±2.3	12.8±2.0	–	–	–	14.4±2.5	13.9±2.4	12.2±2.1	–	10.9±2.4
LCXCA	13.9±0.8	12.8±0.5	–	–	–	11.7±0.8	11.8±0.6	11.5±0.7	–	10.9±0.7

Data are expressed as mean±s.e.mean; *$P<0.05$ vs. baseline. LADCA = left anterior descending coronary artery, LCXCA = left circumflex coronary artery.

lowered PRA and plasma AII to levels below the detection limit (Figure 3). Plasma ANG I also fell to low levels, but remained detectable in 4 of the 5 pigs (Figure 3).

After discontinuation of the infusion, PRA remained close to or below the detection limit during the 60 min post-infusion period in 4 of the 5 pigs. In one pig, PRA returned to approximately 30% of its baseline level in the post-infusion period. Plasma AI and AII increased in the post-infusion period, but remained below baseline in most pigs after 60 min (Figure 3).

Intracoronary infusions without AT_1-receptor blockade

Systemic haemodynamics, myocardial function and oxygen consumption Intracoronary infusion of vehicle (methane sulphonic acid) did not cause significant changes in any of the systemic haemodynamic variables or in myocardial function parameters and myocardial oxygen consumption (Figures 4 and 5).

MAP, CO, LV dP/dt_{max} and MVO_2 were not affected by remikiren at 0.2, 0.5 and 1 mg min^{-1} (Figures 4 and 5), and were lowered at higher doses. At the highest dose, MAP decreased by 31 ± 4% ($P<0.05$) and CO by 26 ± 6% ($P<0.05$). The similar decrease in MAP and CO implies that SVR had not changed. Arterial pulse pressure, HR and LVEDP were also unaffected. LV dP/dt_{max} started to decrease during infusion of 2 mg min^{-1} of remikiren, and had falled to 57 ± 6% of the predrug value ($P<0.05$) after the highest dose had been administered.

CBF did not change at any dose of remikiren (Figure 5). This implies that, because of the decrease in MAP, CVR was reduced at the higher doses of remikiren (by maximally 36 ± 7%, $P<0.05$). Myocardial oxygen extraction decreased significantly; coronary venous oxygen saturation was 27 ± 3% at baseline and 63 ± 2% after the highest dose ($P<0.05$) MVO_2 decreased my maximally 46 ± 3% ($P<0.05$) and MW by 48 ± 5% ($P<0.05$). EDL and ESL in the distribution area of the LADCA increased by maximally 5 ± 1% ($P<0.05$) and 10 ± 2% ($P<0.05$), respectively. SS was lowered at the highest dose of remikiren ($P<0.05$; Table 1). EDL, ESL and SS in the distribution area of the LCXCA remained unaltered (Table 1).

During the first 15 min post-infusion, HR and LV dP/dt_{max} decreased further to 81 ± 6% ($P<0.05$) and 47 ± 4% ($P<0.05$) of the predrug value, respectively. After 2 h almost all variables had returned to their predrug values (Figures 4 and 5).

Plasma renin activity and angiotensin I and II levels Baseline PRA, AI and AII were in the normal range (Danser *et al.*, 1992b). Already at a dose of 0.2 mg min^{-1}, remikiren had lowered PRA and plasma AII to levels below the limit of detection in 5 of the 6 pigs (Figure 6). Plasma AI also fell to low levels, but remained detectable in most pigs.

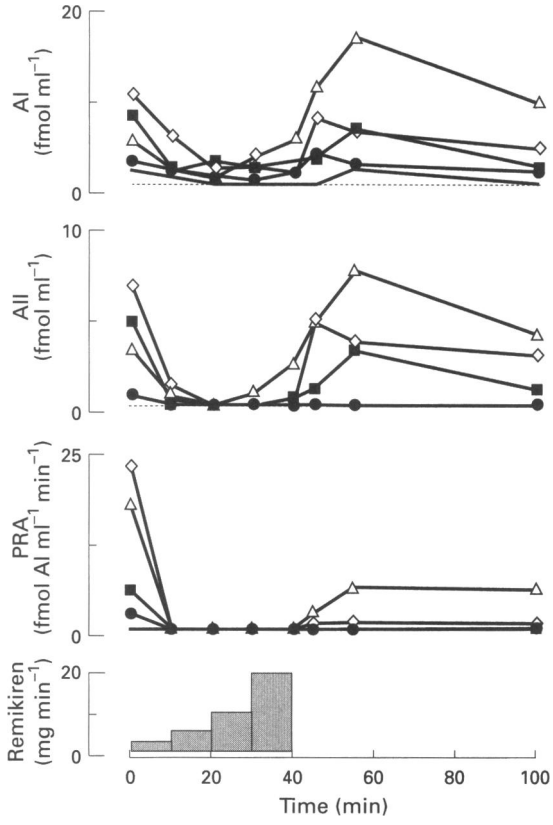

Figure 3 Effects of consecutive 10 min intravenous infusions of remikiren (2, 5, 10 and 20 mg min^{-1}) on plasma angiotensin I (AI), plasma angiotensin II (AII) and plasma renin activity (PRA) in open-chest anaesthetized pigs. The plasma levels of these components at 15 and 60 min after discontinuation of the infusions are also shown. The dotted line represents the limit of detection. Data are presented as individual data points.

In the 2-h post-infusion period, PRA remained below the detection limit in 5 of the 6 pigs; it increased to approximately 50% of baseline in one pig. Plasma AII remained below the detection limit during the entire post-infusion period in 4 pigs, whereas in two pigs it had returned to baseline after 60 min. Plasma AI rose to baseline levels within 60 min (Figure 6).

Vehicle (methane sulphonic acid) did not affect PRA, AI or AII (data not shown).

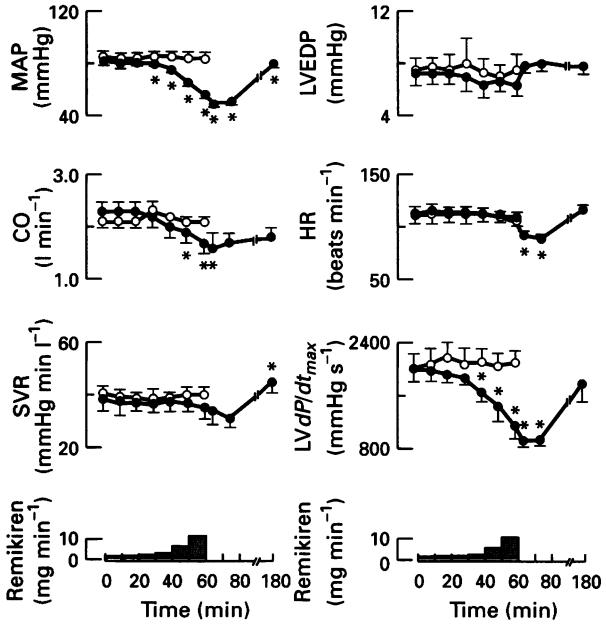

Figure 4 Effects of consecutive 10 min intracoronary infusions of remikiren (0.2, 0.5, 1, 2, 5, and 10 mg min^{-1}; ●) or its vehicle (methane sulphonic acid in equivalent amounts; ○) on mean arterial pressure (MAP), cardiac output (CO), systemic vascular resistance (SVR), left ventricular end-diastolic pressure (LVEDP), heart rate (HR) and LV dP/dt_{max} in open-chest anaesthetized pigs. The recovery of the various parameters at 15 and 120 min after discontinuation of the infusions is also shown. Data are presented as mean and s.e. mean ($n=6$). * $P<0.05$ *vs.* baseline.

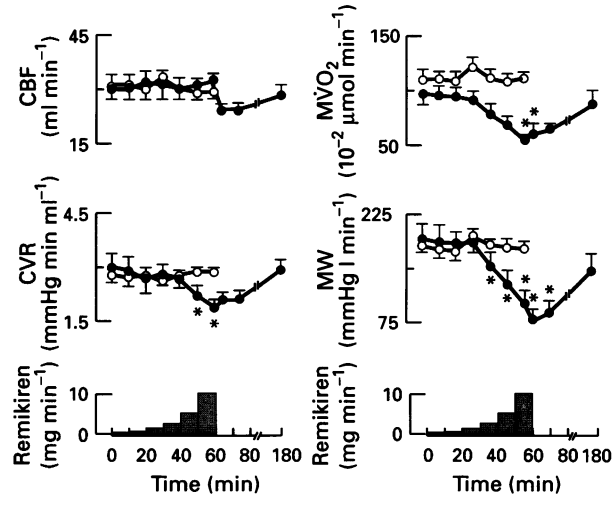

Figure 5 Effects of consecutive 10 min intracoronary infusions of remikiren (0.2, 0.5, 1, 2, 5 and 10 mg min^{-1}; ●) or its vehicle (methane sulphonic acid in equivalent amounts; ○) on coronary blood flow (CBF), coronary vascular resistance (CVR), myocardial oxygen consumption (MVO_2) and myocardial work (MW) in open-chest anaesthetized pigs. The recovery of the various parameters at 15 and 120 min after discontinuation of the infusions is also shown. Data are presented as mean and s.e. mean ($n=6$). * $P<0.05$ *vs.* baseline.

Intracoronary infusions with AT_1-receptor blockade

Systemic haemodynamics, myocardial function and oxygen consumption Before administration of the AT_1-receptor antagonist, L-158,809, a series of i.v. bolus injections of AII (0.1, 0.3 and 1.0 µg kg^{-1}) increased MAP, CO, HR and LV dP/dt_{max} by maximally 64 ± 10, 42 ± 13, 23 ± 8 and 99 ± 12% ($P<0.05$), respectively. Values returned to baseline within 5 min after each bolus injection. After i.v. infusion of 10 mg L-158,809 over a 10 min period, the haemodynamic responses to the same AII challenge were completely abolished (CO, HR and LV dP/dt_{max}) or suppressed by at least 95% (MAP). At the end of the experiment, after a 60 min washout period, the haemodynamic responses to AII were still absent (CO, HR and LV dP/dt_{max}) or suppressed by at least 70% (MAP). These data demonstrate that effective AT_1-receptor blockade was maintained throughout the experimental protocol.

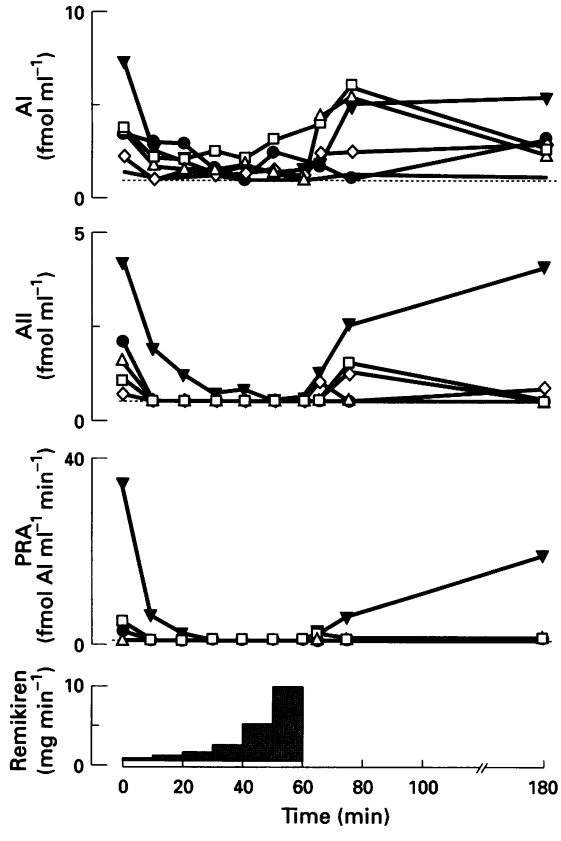

Figure 6 Effects of consecutive 10 min intracoronary infusions of remikiren (0.2, 0.5, 1, 2, 5 and 10 mg min^{-1}; closed symbols) on plasma angiotensin I (AI), plasma angiotensin II (AII) and plasma renin activity (PRA) in open-chest anaesthetized pigs. The plasma levels of these components at 15 and 120 min after discontinuation of the infusions are also shown. The dotted line represents the limit of detection. Data are represented as individual data points.

After administration of L-158,809, HR decreased by 3 ± 1% ($P<0.05$). None of the other haemodynamic parameters was affected by the AT_1-receptor antagonist (Figures 7 and 8). Subsequent i.c. infusion of remikiren at increasing doses (2, 5 and 10 mg min^{-1}) did not affect CO, SVR, LVEDP or HR (Figure 7). Relative to its value after L-158,809 administration, MAP decreased by 14 ± 4% ($P<0.05$) at the highest dose of remikiren (10 mg min^{-1}). Arterial pulse pressure did not change. LV dP/dt_{max} already decreased at the lowest dose of remikiren. At the highest dose it had fallen to 73 ± 5% ($P<0.05$) of its value before administration of the renin inhibitor (Figure 7).

CBF increased by 82 ± 16% ($P<0.05$) at the highest dose of remikiren (Figure 8). Since MAP was only modestly lowered at the highest dose, it follows that CVR was reduced at all three doses of remikiren (by maximally 52 ± 3%, $P<0.05$). Myocardial oxygen extraction decreased significantly; coronary venous oxygen saturation was 24 ± 3% before remikiren and 49 ± 7% after the highest dose ($P<0.05$).

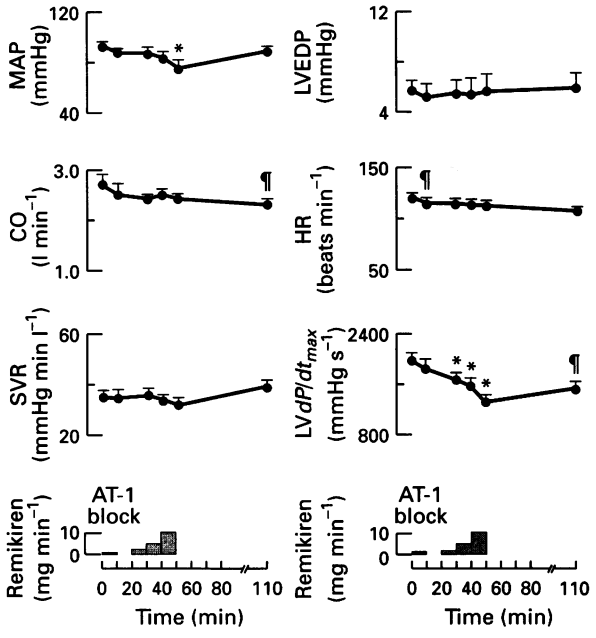

Figure 7 Effects of consecutive 10 min intracoronary infusions of remikiren (2, 5 and 10 mg min^{-1}) after AT_1-receptor blockade with L-158,809 (10 mg i.v.) on mean arterial pressure (MAP), cardiac output (CO), systemic vascular resistance (SVR), left ventricular end-diastolic pressure (LVEDP), heart rate (HR) and LV dP/dt_{max} in open-chest anaesthetized pigs. The recovery of the various parameters at 60 min after discontinuation of the infusions is also shown. Data are presented as mean and s.e. mean ($n=5$). * $P<0.05$ *vs.* baseline after L-158,809; ¶ $P<0.05$ *vs.* baseline before L-158,809.

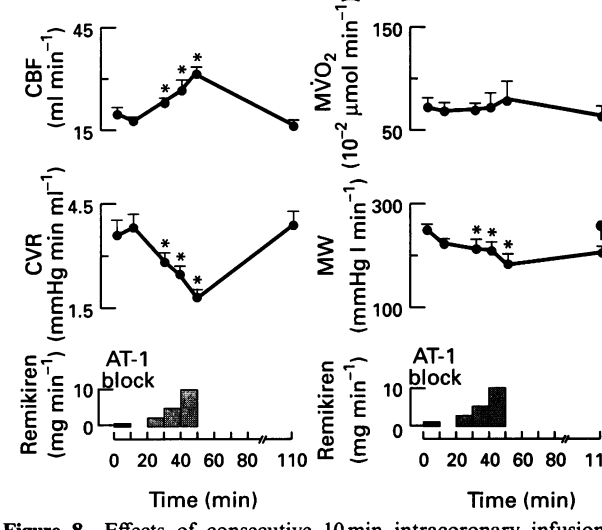

Figure 8 Effects of consecutive 10 min intracoronary infusions of remikiren (2, 5 and 10 mg min^{-1}) after AT_1-receptor blockade with L-158,809 (10 mg i.v.) on coronary blood flow (CBF), coronary vascular resistance (CVR), myocardial oxygen consumption (MVO_2) and myocardial work (MW) in open-chest anaesthetized pigs. The recovery of the various parameters at 60 min after discontinuation of the infusions is also shown. Data are represented as mean and s.e. mean ($n=5$). * $P<0.05$ *vs.* baseline after L-158,809; ¶ $P<0.05$ *vs.* baseline before L-158,809.

However, due to the concomitant increase in CBF, MVO_2 remained unaffected (Figure 8). MW fell to 80 ± 4% ($P<0.05$) of its value prior to remikiren administration.

During remikiren administration under AT_1-receptor blockade, EDL and ESL in the distribution area of LADCA increased in parallel by maximally 9 ± 4% and 11 ± 3% ($P<0.05$). Consequently, SS in that area did not change (Table 1). EDL, ESL and SS in the distribution area of the LCXCA remained unaltered (Table 1).

After 60 min of recovery almost all parameters had returned to predrug values (Figures 7 and 8).

Plasma renin activity and angiotensin I and II levels Baseline PRA, AI and AII were not different from the baseline values found in the previous i.v. and i.c. experiments. No significant changes were observed within the 30 min period after i.v. administration of L-158,809. Already at the lowest dose of remikiren used in these experiments, PRA, AI and AII reached levels at or below the limit of detection (data not shown).

Experiments with isolated papillary muscle

Neither vehicle (methane sulphonic acid) nor remikiren had any effect on the contractile force of left ventricular tissue precontracted with noradrenaline (Figure 9). In tissues that were not precontracted, a slight decrease in baseline contractile force was observed with remikiren at its highest concentration (10^{-4} M), whereas with vehicle a small dose-dependent increase was seen (Figure 9).

Discussion

The role of the RAS in cardiac contractility is still uncertain. ACE inhibitors have been reported to reduce cardiac contractility when administered intracoronary, both *in vivo* (Foult *et al.*, 1988) and *in vitro* (Raddino *et al.*, 1991). Chronic treatment with the ACE inhibitor, cilazapril, depresses left ventricular contractile function in spontaneously hypertensive rats (Christe *et al.*, 1994). However, ACE inhibitors not only lower AII but may also increase bradykinin. Part of their cardiac effects may therefore be attributed to increased bradykinin levels. Especially in isolated hearts, where AI is no longer present (Lindpaintner *et al.*, 1990; Danser *et al.*, 1994), increased bradykinin might contribute to the negative inotropic effects observed after ACE inhibition. In the present study we therefore examined the cardiac effects of the potent and specific renin inhibitor, remikiren, in the intact anaesthetized pig, and compared these regional responses with the systemic haemodynamic effects of remikiren. To exclude a non-specific, non-AII-dependent effect of the renin inhibitor, experiments were performed with and without AII-receptor blockade, by use of the selective AT_1-receptor antagonist, L-158,809.

Intravenous infusion of remikiren lowered arterial blood pressure by peripheral vasodilatation. The absence of a reflex-mediated increase in heart rate, as observed in the present study, is in agreement with previous reports (van den Meiracker *et al.*, 1990; Fischli *et al.*, 1991; Kiowski *et al.*, 1994). Heart rate even appeared to decrease at the highest dose of remikiren.

An important finding in the present study is the large decrease in LV dP/dt_{max} induced by remikiren. This parameter is a widely accepted index of global myocardial contractility. However, LV dP/dt_{max} is also sensitive to changes in heart rate and pre- and afterload. Because heart rate as well as left ventricular end-diastolic pressure did not change, the decrease in LV dP/dt_{max} must have been caused by a decrease in myocardial contractility and/or a decrease in diastolic arterial blood pressure. The reduced myocardial efficiency observed after intravenous administration of remikiren supports the former possibility. Further support comes from earlier studies, using the same model, of the second generation dihydropyridines, nisoldipine and elgodipine (Duncker *et al.*, 1988; Sassen *et al.*, 1990b), and the potassium channel activator, EMD 52692 (Sassen *et al.*, 1990a). These agents caused similar decreases in mean arterial blood pressure to those observed in the present study, without affecting heart rate and left ventricular end-diastolic blood pressure, and had no significant effect on

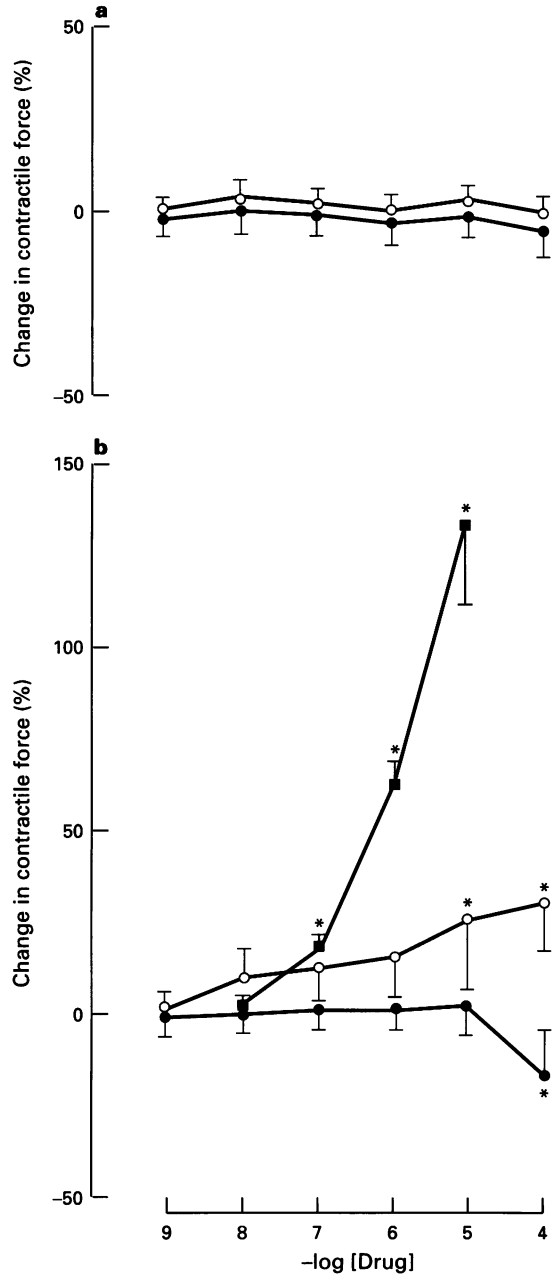

Figure 9 (a) Effect of increasing concentrations of remikiren (●) or its vehicle (methane sulphonic acid; ○) on the contractile force of porcine isolated left ventricular trabeculae precontracted with 10^{-5} M noradrenaline. Data are presented as mean and s.e. mean ($n=4$). (b) Effect of increasing concentrations of remikiren (●), its vehicle (methane sulphonic acid; ○) or noradrenaline (■) on non-precontracted porcine isolated left ventricular trabeculae. Data are presented as mean and s.e. mean ($n=4$). * $P<0.05$ *vs.* zero drug concentration.

LV dP/dt_{max}. It is therefore most likely that remikiren, in addition to its vasodilator action, depresses myocardial contractility at doses higher than 10 mg min^{-1} (or 370 μg $kg^{-1}min^{-1}$).

Our observations during intracoronary infusion of remikiren provide further evidence for a decrease in myocardial contractility. Intracoronary infused remikiren, at doses of 2 mg min^{-1} and higher, caused a decrease in LV dP/dt_{max} comparable to what was observed after intravenous remikiren at doses higher than 10 mg min^{-1}. In contrast with remikiren i.v. however, remikiren i.c. lowered cardiac output in parallel with arterial pressure, so that systemic vascular resistance did not change. Thus, the decrease in blood pressure after intracoronary infusion was mainly due to the cardiac effects of remikiren. In addition, intracoronary infused remikiren decreased regional systolic segment shortening in the distribution area of the vessel used for infusion, whereas no such effect was observed during intravenous infusion. It seems therefore that remikiren lowers blood pressure both through peripheral mechanisms (vasodilatation) and cardiac mechanisms (decrease in LV dP/dt_{max} and cardiac output). The remikiren blood levels required for cardiodepression appear to be higher than the levels causing peripheral vasodilatation. As coronary blood flow is about 4–5% of cardiac output, the cardiodepressant levels are probably at least one order of magnitude higher.

AII receptors are known to be present in cardiac tissue (Urata *et al.*, 1989) and AII has been reported to exert positive inotropic and chronotropic effects, both through actions on the heart itself (Kobayashi *et al.*, 1978; Lindpaintner & Ganten, 1991) and via facilitation of noradrenergic neurotransmission (Gironacci *et al.*, 1994). Furthermore, there is growing evidence for renin-dependent AII formation in the heart (Danser *et al.*, 1994), and for the contention that locally generated AII, in addition to systemic AII, exerts an inotropic effect on cardiac muscle (Lindpaintner & Ganten, 1991). The cardiopressive action of renin inhibition we observed is therefore not too surprising.

In patients with heart failure, remikiren has been given intravenously as a 0.3 mg kg^{-1} bolus followed by infusion at a rate of 0.1 mg kg^{-1} h^{-1} (Kiowski *et al.*, 1994). No effect on cardiac output was observed at these doses. The doses of remikiren, however, at which the cardiac effects were most prominent in the present study, were 4–8 times higher than the highest doses used in previous studies to lower blood pressure (van der Meiracker *et al.*, 1990; Kiowski *et al.*, 1994). Because remikiren was given as its methane sulphonate salt, one could argue that, at the high doses we used, methane sulphonate was given in quantities sufficient to affect cardiac contractility. However, intracoronary infusions of methane sulphonic acid alone did not alter blood pressure or cardiac contractility (Figure 4), nor did methane sulphonic acid reduce contractile force of isolated cardiac tissue (Figure 9).

The maximal concentrations of remikiren reached in the coronary vascular bed during the infusions (approximately 10^{-4}–10^{-5} M) might be too high to exert only renin-specific effects. We used these high doses because remikiren is approximately 80 times less potent towards porcine renin than towards human renin; to obtain nearly 100% inhibition of porcine renin, concentrations of 10^{-5} M and higher are required (Danser *et al.*, 1992a). After administration of the AT_1-receptor blocker, L-158,809 at a dose (10 mg i.v.) that fully blocked the haemodynamic responses to systemically infused AII but had no significant haemodynamic effects by itself, the systemic haemodynamic and myocardial effects of subsequent i.c. infusion of remikiren were either abolished or significantly reduced (Figures 10 and 11). The small effects of remikiren on blood pressure and LV dP/dt_{max} (Figure 10) that were still seen in the presence of L-158,809 are probably due to a small number of receptors not being blocked by L-158,809, either because the dose was not high enough or because these receptors are located at tissue sites which could not be reached by L-158,809. Remikiren is highly lipophilic and may penetrate more easily into the tissues. Coronary blood flow after i.c. remikiren did not change without AT_1-receptor blockade, and increased during AT_1-receptor blockade. This increase in coronary blood flow might be related to the fact that cardiac contractility was maintained under AT_1-receptor blockade. Because of the increased coronary flow, myocardial oxygen delivery could also be maintained.

Our experiments with isolated cardiac tissue also argue against a non-specific cardiodepressive action of the renin inhibitor (Figure 9). There is probably little or no AII formation in such tissue after it has been separated from the circulation (Danser *et al.*, 1994). In support of our *in vitro* findings, other renin inhibitors, with a structure resembling that of remikiren, did not elicit negative inotropic responses in rat isolated Langendorff hearts (Kleinert, 1989).

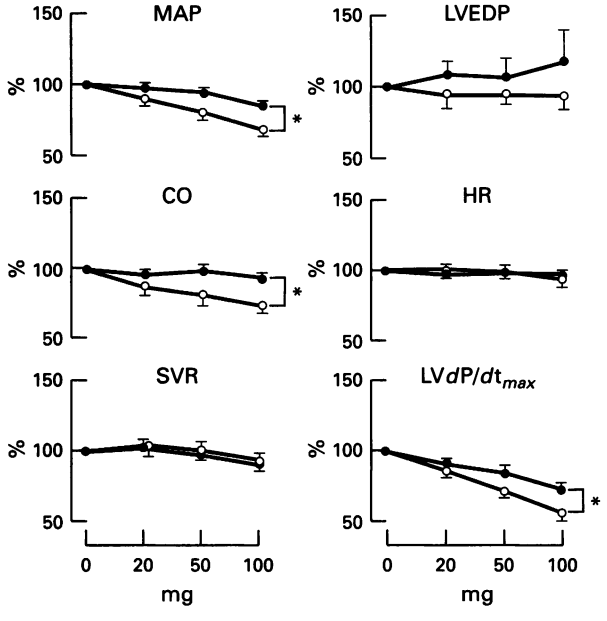

Figure 10 Comparison of the effects of consecutive 10 min intracoronary infusions of remikiren (2, 5 and 10 mg min^{-1}) in the absence (○, $n=5$) or presence (●, $n=5$) of the AT_1-receptor antagonist, L-158,809, on mean arterial pressure (MAP), cardiac output (CO), systemic vascular resistance (SVR), left ventricular end-diastolic pressure (LVEDP), heart rate (HR) and LV dP/dt_{max}. Data are presented as mean and s.e. mean, and are expressed as a percentage of the baseline level measured immediately before remikiren administration. * $P<0.05$ without AT_1-receptor blockade *vs.* with AT_1-receptor blockade.

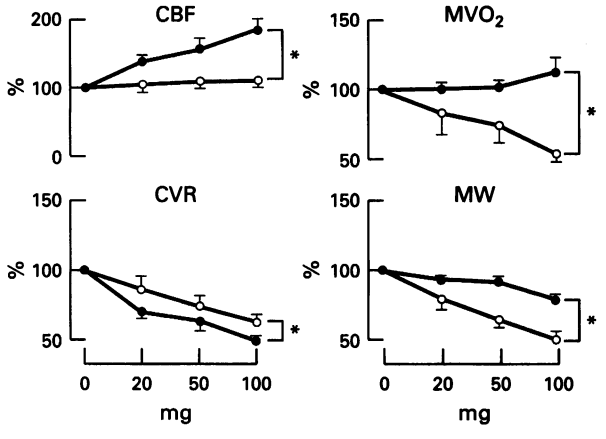

Figure 11 Comparison of the effects of consecutive 10 min intracoronary infusions of remikiren (2, 5 and 10 mg min^{-1}) in the absence (○, $n=5$) or presence (●, $n=5$) of the AT_1-receptor antagonist, L-158,809 on coronary blood flow (CBF), coronary vascular resistance (CVR), myocardial oxygen consumption (MVO_2) and myocardial work (MW). Data are presented as mean and s.e. mean, and are expressed as a percentage of the baseline level measured immediately before remikiren administration. * $P<0.05$ without AT_1-receptor blockade *vs.* with AT_1-receptor blockade.

Are the observed systemic haemodynamic and cardiac effects of remikiren due to the reduction of tissue or plasma AII? PRA and AI and AII were reduced to levels at or below the detection limit at doses of remikiren that were not high enough to affect systemic haemodynamics or myocardial function, both after i.v. and i.c. infusion. This suggests that these responses are related to the inhibition of non-circulating renin, i.e. renin at tissue sites that are more difficult to reach (Fischli *et al.*, 1991; 1994). In addition, after the remikiren infusion had been stopped, the haemodynamic and cardiac parameters returned to baseline more rapidly than PRA and the plasma levels of AI and AII. This again suggests that high concentrations of remikiren are required to inhibit tissue renin. Most likely remikiren is washed away from the tissues via the circulation.

The effect of remikiren on PRA appeared to be more pronounced and to last longer than the effect on the angiotensin levels. This phenomenon has also been observed in previous studies with renin inhibitors (De Gasparo *et al.*, 1989; Delabays *et al.*, 1989; Derkx *et al.*, 1991). The angiotensinase inhibitors that are routinely used in PRA assays cause displacement of protein-bound remikiren, so that the free concentration of remikiren is increased (Derkx *et al.*, 1991). To avoid this *in vitro* artefact we measured PRA with an antibody trapping assay without angiotensinase inhibitors. In this assay the *in vitro* generated AI is trapped by an excess of AI antibody and thereby protected from destruction by angiotensinases. Still, PRA was more suppressed than plasma AI and AII. It is possible therefore that some *in vitro* displacement of the renin inhibitor may occur also in the antibody trapping assay. Decreased AI and AII levels are probably a better measure of renin inhibition.

In conclusion, our results support the contention that renin-dependent intracardiac angiotensin formation has a positive effect on contractility. While our observations on the renin inhibitor, remikiren, point towards an important local function of the renin-angiotensin system, they do not necessarily argue against the use of this inhibitor in hypertension and heart failure, because the inhibitor concentrations causing a reduction in cardiac contractility are higher than the concentrations required for peripheral vasodilatation.

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