ELSEVIER

European Journal of Pharmacology 294 (1995) 17–27

ejp

# Effects of angiotensin II and angiotensin-converting enzyme inhibitors on human myocardium

Olaf Lenz [a], Bernhard Schmid [a], Heiko Kilter [a], Karl La Rosée [a], Markus Flesch [a], Ferdinand Kuhn-Regnier [b], Michael Südkamp [b], Michael Böhm [a,*]

[a] *Klinik III für Innere Medizin der Universität zu Köln, Cologne, Germany*
[b] *Klinik und Poliklinik für Kardiochirurgie der Universität zu Köln, Cologne, Germany*

Received 9 March 1995; revised 7 August 1995; accepted 11 August 1995

**Abstract**

Human myocardial angiotensin II receptors and the angiotensin $AT_1$ and $AT_2$ receptor subtypes were characterised using the partial angiotensin II receptor agonist [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II and the selective antagonists losartan (2-n-butyl-4-chloro-5-hydroxymethyl-1-[2'((1*H*-tetrazol-5-yl)biphenyl-4-yl)-methyl]imidazole) and PD 123177 (1-[(4-amino-3-methylphenyl)methyl]-5-(diphenyl-acetyl)-4,5,6,7-tetrahydro-1*H*-imidazol[4,5-*c*]pyridine-6-carboxylic acid). The density of angiotensin II receptors was higher in atrial than in ventricular myocardium. Angiotensin $AT_2$ receptors were predominant in atria and ventricles (80–85% of total angiotensin II receptors). Only in isolated, electrically driven atrial trabeculae but not in ventricular preparations, angiotensin II did produce a concentration-dependent positive inotropic effect, which was antagonized exclusively by the angiotensin $AT_1$ receptor antagonist losartan and which amounted to about 20% of the positive inotropic effect of milrinone and isoprenaline. The application of the angiotensin-converting enzyme inhibitors captopril, enalaprilat and ramiprilat had no inotropic effect in either tissue. It is concluded that angiotensin $AT_1$ receptors exclusively mediate direct positive inotropic effects in atrial myocardium. Since angiotensin-converting enzyme inhibitors do not produce any inotropic effect, tonic regulation of basal force of contraction by angiotensin II does not occur.

*Keywords:* Heart failure; Renin-angiotensin-aldosterone system; Angiotensin II; Angiotensin II receptor; Angiotensin-converting enzyme inhibitor

## 1. Introduction

Angiotensin II has been reported to be a positive inotropic and chronotropic agent in cardiovascular tissue of various species including dogs (Kobayashi et al., 1978), cats (Dempsey et al., 1971), rabbits (Freer et al., 1976) and bovines (Rogers, 1984). In vitro experiments on human myocardial tissue also showed a positive inotropic response (Moravec et al., 1990; Holubarsch et al., 1993; Zerkowski et al., 1993). The inotropic action of angiotensin II in the guinea pig atrium is mediated by $AT_1$ stimulation (Feolde et al., 1993a). In the human myocardium, the functional significance and the receptor subtypes mediating potential angiotensin II effects still have to be elucidated.

A blockade of angiotensin II generation by angiotensin-converting enzyme inhibitors would reduce direct and indirect angiotensin II effects, which could lead to important effects during therapy of chronic heart failure. In patients with severe chronic heart failure, oral treatment with the angiotensin-converting enzyme inhibitor enalapril in addition to conventional therapy reduced mortality and improved symptoms (The CONSENSUS Trial Study Group, 1987). Due to the fact that intravenous application of enalaprilat (Swedberg et al., 1992 CONSENSUS II) failed to produce beneficial effects but rather precipitated hypotension and heart failure in certain patients, the direct effects of the angiotensin-converting enzyme inhibitors on force of contraction warrant attention. In recent

* Corresponding author. Klinik III für Innere Medizin der Universität zu Köln, Joseph Stelzmann-Strasse 9, 50924 Köln, Germany. Tel.: 0049 221 478 6207; fax: 0049 221 478 6550.


*SSDI* 0014-2999(95)00514-5

studies, an anti-adrenergic effect of enalaprilat in isolated electrically driven rat papillary muscle strips (Llambi et al., 1990) has been observed. Fosinoprilat prolongs the action potential in guinea pig myocytes (Räcke et al., 1994) as does captopril in hypertrophied rat ventricle (Ribout et al., 1994). Enalaprilat produced negative inotropic effects in vivo in humans following intracoronary infusion (Foult et al., 1988). These effects could lead to a significant depression of contractility in situations in which neurohumoral activation occurs, such as early after myocardial infarction. Nevertheless, it is still unknown if these potential effects are due to a reduction of angiotensin II generation or direct angiotensin-converting enzyme inhibitor effects and whether these agents act directly on the human heart.

The present study was designed to characterize human myocardial angiotensin II receptors and to get more information about their functional coupling. Therefore, the direct inotropic effects of angiotensin II and of the angiotensin-converting enzyme inhibitors captopril, enalaprilat and ramiprilat were studied in atrial and ventricular preparations and compared with the cAMP-dependent inotropic effects of the cAMP-phosphodiesterase inhibitor milrinone and the $\beta$-adrenoceptor agonist isoprenaline. Experiments on force of contraction with angiotensin II were performed in the presence of the specific angiotensin $AT_1$ and $AT_2$ receptor antagonists losartan (2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[2′((1*H*-tetrazol-5-yl)biphenyl-4-yl)-methyl]imidazole) and PD 123177 (1-[(4-amino-3-methylphenyl)methyl]-5-(diphenyl-acetyl)-4,5,6,7-tetrahydro-1*H*-imidazol[4,5-*c*]pyridine-6-carboxylic acid), respectively. In order to exclude indirect inotropic actions of angiotensin II elicited by presynaptic norepinephrine release, postsynaptic $\beta_1$- and $\beta_2$-adrenoceptors were blocked with the specific antagonists CGP 207.12A (1-[2-((3-carbamoyl-4-hydroxy)-phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy]-propan-2-ol) and ICI 118.551 (*erythro*-1-(7-methylindan-4-yloxy)-3-(isopropylamine)-butan-2-ol). Radioligand experiments were performed using the partial angiotensin II receptor agonist [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II and the selective angiotensin $AT_1$ and $AT_2$ receptor antagonists losartan and PD 123177.

## 2. Materials and methods

### 2.1. Myocardial tissue

Experiments were performed on isolated electrically stimulated human ventricular papillary muscle strips and right auricular trabeculae or on membrane preparations from human left ventricular and right atrial myocardium. Tissue was obtained during aortocoronary bypass operations (without heart failure) ($n$ = 7, 4 female, 3 male; age: mean 61.4 years, range 41–71) or cardiac transplantation ($n$ = 10, 4 female, 6 male; age: mean 45.3 years, range 31–64). Human nonfailing myocardium was obtained from five organ donors (4 men, 1 woman; age: mean 39 years, range 18–56) who died from injury to the brain. For technical reasons the nonfailing hearts had not been suitable for transplantation. All patients gave written informed consent before surgery. Medical therapy consisted of diuretics, nitrates, angiotensin-converting enzyme inhibitors and cardiac glycosides. Patients receiving catecholamines, $\beta$-adrenoceptor or $Ca^{2+}$ channel antagonists were not eligible for the study. Cardiac surgery was performed with cardioplegic arrest during hypothermia. The cardioplegic solution was a modified Bretschneider solution containing (mmol/l): NaCl 15, KCl 10, $MgCl_2$ 4, histidine 180, tryptophan 2, mannitol 30 and potassium dihydrogen oxoglutarate 1.

### 2.2. Contraction experiments

Immediately after excision, the papillary muscles and atrial trabeculae were placed in ice-cold preaerated modified Tyrode's solution (composition see below). The experiments were performed on isolated electrically driven (1 Hz) muscle preparations. Muscle strips were dissected under microscopic control in aerated bathing solution at room temperature. Connective tissue, if visibly present, was trimmed away carefully. The preparations were attached to a bipolar platinum stimulating electrode and suspended individually in 75 ml glass tissue chambers for recording of isometric contractions. The bathing solution used was a modified Tyrode's solution containing in mmol/l: NaCl 119.8, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaH_2PO_4$ 0.42, $NaHCO_3$ 22.6, $Na_2EDTA$ 0.05, ascorbic acid 0.28, glucose 5.0. It was continuously gassed with 95% $O_2$ and 5% $CO_2$ and maintained at 37°C; its pH was 7.4. Isometric force of contraction was measured with an inductive force transducer (W. Fleck, Mainz, Germany) attached to a Gould recorder (Brush 2400, Gould, Cleveland, OH, USA). Each muscle was stretched to the length at which force was maximal. The preparations were paced electrically at 1 Hz with rectangular pulses of 5 ms duration (Grass stimulator SD 9), the voltage was 20% above threshold. All preparations were allowed to equilibrate at least 90 min in a drug-free bathing solution until complete mechanical stabilization. After 45 min, the solution was changed. Concentration-dependent mechanical effects were obtained. Control strips kept in Tyrode's solution with identical composition as original experiments revealed maximally 10% reduction of baseline isometric tension over the period necessary to complete pharmacological

testing. Agents were applied cumulatively to the organ bath. Each muscle was used only once to record a concentration-response curve.

### 2.3. Human myocardial membrane preparation

Left ventricular myocardium was chilled in 30 ml ice-cold homogenization buffer (20 mmol/l Tris/HCl, 1 mmol/l EDTA, 1 mmol/l dithiothreitol, pH 8.0). Connective tissue was trimmed away and myocardial tissue was minced with scissors, disrupted with an Ultraturrax (Janke and Kunkel, Staufenbreisgau, Germany) and homogenized with a motor-driven glass teflon potter for 1 min. The homogenate was spun at $480 \times g$ for 10 min (JA 20, Beckman, Palo Alto, USA). The supernatant was further used and the pellet was discarded. This homogenate was diluted with an equal volume of ice-cold 1 mol/l KCl and stored on ice for 10 min. The supernatant was centrifuged at $100000 \times g$ for 45 min. The pellet was resuspended in 50 volumes of homogenization buffer and recentrifuged at $100000 \times g$ for 45 min. The final pellet was resuspended in incubation buffer (50 mmol/l Tris/HCl, 50 mmol/l $NaH_2PO_4$, 10 mmol/l $MgCl_2$, 0.2% bovine serum albumin and proteinase inhibitors: trypsin inhibitor 0.2 mg/ml, pepstatin A 0.25 mg/ml and leupeptin 0.25 mg/ml, pH 7.1). Angiotensin II receptors in cardiac tissue were investigated in saturation experiments using [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II as radiolabelled ligand. 1 $\mu$mol angiotensin II was used to determine nonspecific binding. The assay was performed in a total volume of 250 $\mu$l incubation buffer. The incubation was carried out at 24°C for 60 min. These conditions allowed a complete equilibration of the receptor with the radioligand. The reaction was terminated by rapid vacuum filtration through Whatmann GF/C filters (Whatman, Clifton, NJ); the filters were washed immediately 3 times with 5 ml of ice-cold incubation buffer. All experiments were performed in triplicate. Filters were dried at 90°C and placed in 10 ml scintillation fluid (Quickszint 501, Zinsser analytics, Frankfurt, Germany) and radioactivity was determined in a liquid scintillation counter. The maximal density ($B_{max}$) and apparent affinity ($K_d$) of binding sites were obtained in individual experiments from Scatchard plots determined by linear regression analysis. In a second series of experiments, angiotensin $AT_1$ and $AT_2$ receptor subtypes were determined in competition experiments using the $AT_1$ selective antagonist losartan and the $AT_2$ selective antagonist PD 123177. The ratio angiotensin $AT_1$ to $AT_2$ receptors was determined from competition of [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II binding (approximate $K_d$ value) by 10 nmol/l losartan and 30 $\mu$mol/l PD123177. These concentrations of the selective antagonists completely antagonized binding to the angiotensin $AT_1$ and $AT_2$ receptor subtypes, as judged from complete competition curves analyzed according to the method of De Lean et al. (1982). The densities of angiotensin $AT_1$ and $AT_2$ receptors were calculated from competition of saturating concentrations of the respective antagonists and from the $B_{max}$ values obtained in saturation experiments of the same samples.

Protein concentrations were determined according to Lowry et al. (1951).

### 2.4. Materials

Angiotensin II, isoprenaline and the proteinase inhibitors: trypsin inhibitor, pepstatin A and leupeptin were obtained from Sigma Chemical Co. (Deideshofen, Germany), milrinone was provided by Sterling-Whinthrop AG (Munich, Germany). Losartan and enalaprilat were provided by Merck Sharp and Dohme (Munich, Germany). PD123177 was from Parke Davis (Berlin, Germany). ICI 118.551 was purchased from ICI GmbH (Heidelberg, Germany) and CGP 207.12A came from Ciba Geigy AG (Basel, Switzerland). The ligand [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II was obtained from Amersham-Buchler (Braunschweig, Germany). Other substances used were ramiprilat donated by Hoechst AG (Frankfurt, Germany). Captopril was kindly donated by Schwarz Pharma AG (Monheim, Germany). All other compounds used were of analytical or best grade commercially available. Only deionized and twice destilled water was used throughout.

### 2.5. Statistics

All data are shown as means ± S.E.M.. Statistical significance was estimated using Student's $t$-test for paired and unpaired observations. A $P$ value of less than 0.05 was considered significant.

## 3. Results

### 3.1. Effects of angiotensin II on force of contraction in atrial and ventricular myocardium

Original recordings of force of contraction in Fig. 1 demonstrate the effects of angiotensin II in isolated electrically stimulated atrial and ventricular preparations. In right atrial trabeculae, angiotensin II at 30 $\mu$mol/l produced a positive inotropic effect which was maximal within 1 min. In contrast, in preparations from the left ventricle (Fig. 1, lower panel), 30 $\mu$mol/l angiotensin II had no effect on force of contraction. In Fig. 2, the contractile response to angiotensin II in atrial preparations is shown in comparison to the concentration-response curves for the $\beta$-adrenoceptor agonist isoprenaline and the cAMP phosphodiesterase inhibitor milrinone. Angiotensin II induced a concen-

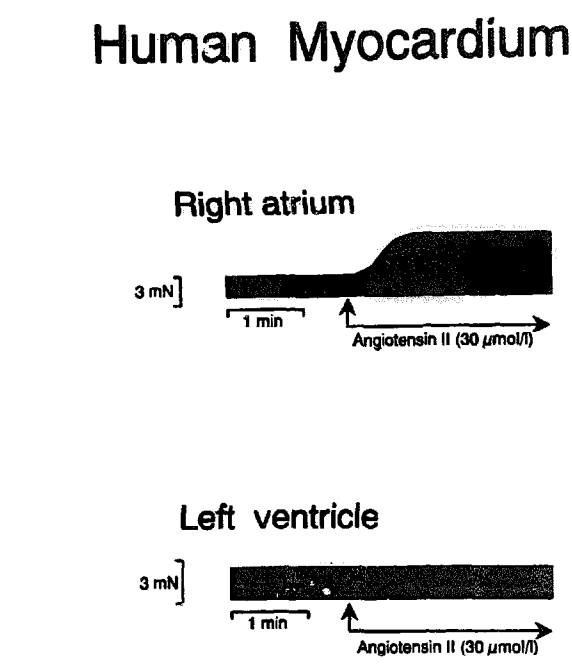


Fig. 1. Original recordings of isometric force of contraction in an isolated electrically driven human trabeculae from right atrium (upper panel) and a papillary muscle strip from the left ventricle (lower panel) following application of 30 μmol/l angiotensin II. Note that angiotensin II produced a positive inotropic response in the atrial but not in the ventricular myocardium.

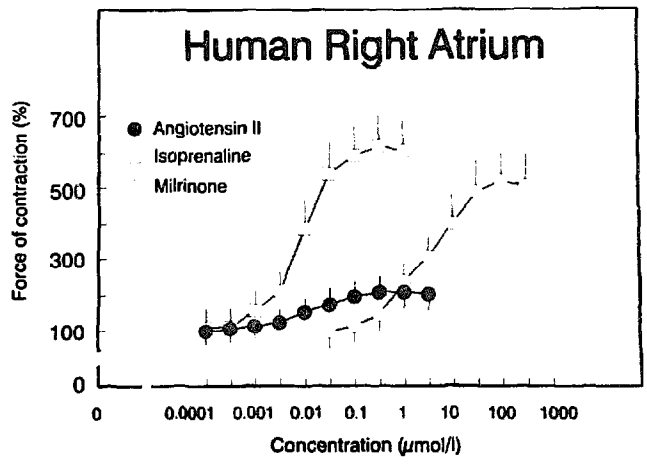


Fig. 2. Concentration-response curves for the effects of angiotensin II (0.0001–3 μmol/l), isoprenaline (0.0001–1 μmol/l) and milrinone (0.03–300 μmol/l) on force of contraction in isolated electrically driven atrial trabeculae. Ordinate: force of contraction in % of predrug value. Abscissa: Concentration of drug in μmol/l.

tration-dependent increase in force of contraction. The positive inotropic effect was maximal at 0.3 μmol/l angiotensin II. This concentration of the peptide increased the force of contraction by 92%. The efficacy of isoprenaline and milrinone was 4–5 times higher than that of angiotensin II. The absolute values of the maximal effects and the $EC_{50}$ values are listed in Table 1.

Fig. 3 shows the cumulative concentration-response curves for angiotensin II, isoprenaline and milrinone in electrically driven ventricular preparations. While isoprenaline and milrinone produced concentration-dependent positive inotropic effects, angiotensin II had no effect on force of contraction in ventricular preparations at 0.001–10 μmol/l. However, angiotensin II receptors can rapidly desensitize during the application of increasing agonist concentrations, which could mask an inotropic response. In order to address this issue, the effect of 30 μmol/l angiotensin II administered to

Table 1
Force of contraction in human papillary muscle strips and auricular trabeculae

| | *n* | Basal mN | Max. PIE mN | $EC_{50}$ μmol/l |
|---|---|---|---|---|
| *Human auricular trabeculae* | | | | |
| Isoprenaline | 9 | 1.9 ± 0.5 | +6.1 ± 0.7 [a] | 0.01 (0.006–0.042) |
| Milrinone | 6 | 1.5 ± 0.5 | +5.2 ± 0.8 [a] | 12.7 (4.4–49.3) |
| Angiotensin II alone | 6 | 2.5 ± 0.9 | +2.3 ± 0.7 [a] | 0.018 (0.006–0.056) |
| In the presence of: | | | | |
| Losartan (0.03 μmol/l) | 6 | 2.3 ± 0.3 | +1.3 ± 0.4 [a] | 1.75 (0.95–3.22) |
| PD123177 (3 μmol/l) | 5 | 3.6 ± 0.5 | +4.3 ± 0.7 [a] | 0.013 (0.005–0.031) |
| CGP 207.12A (0.3 μmol/l) | 5 | 3.6 ± 0.7 | +4.7 ± 0.9 [a] | 0.009 (0.003–0.026) |
| ICI 118.551 (50 nmol/l) | 5 | 3.4 ± 0.6 | +2.9 ± 0.5 [a] | 0.016 (0.006–0.045) |
| *Human papillary muscle strips* | | | | |
| Isoprenaline | 9 | 1.7 ± 0.3 | +2.4 ± 0.3 [a] | 0.06 (0.04–0.084) |
| Milrinone | 5 | 1.7 ± 0.5 | +2.1 ± 0.5 [a] | 45.3 (33.3–61.6) |
| Angiotensin II | 7 | 2.7 ± 0.5 | – | – |
| $Ca^{2+}$ alone | 8 | 1.9 ± 0.2 | +4.2 ± 0.2 [a] | – |
| In the presence of | | | | |
| Ramiprilat (100 μmol/l) | 8 | 2.0 ± 0.3 | +5.1 ± 0.7 [a] | – |
| Enalaprilat (100 μmol/l) | 6 | 2.6 ± 0.5 | +6.1 ± 1.4 [a] | – |
| Captopril (100 μmol/l) | 6 | 2.6 ± 0.8 | +5.8 ± 1.5 [a] | – |

[a] $P < 0.05$ vs. predrug value. $EC_{50}$ values are quoted as geometric means (with 95% confidence limits). Abbreviation: PIE, change in force of contraction in mN.

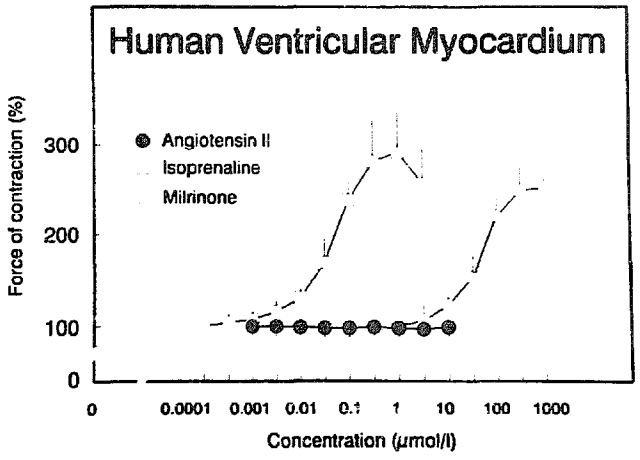


Fig. 3. Concentration-response curves for the effects of angiotensin II (0.0001–10 μmol/l), isoprenaline (0.0001–3 μmol/l) and milrinone (1–1000 μmol/l) on force of contraction in isolated electrically driven human papillary muscle strips. Ordinate: force of contraction in % of predrug value. Abscissa: Concentration of drug in μmol/l.

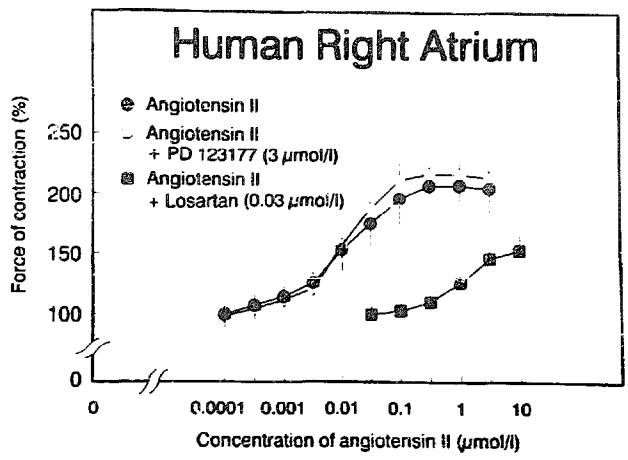


Fig. 5. Concentration-response curves for the effects of angiotensin II (0.0001–10 μmol/l) in the presence of the selective angiotensin $AT_1$ receptor antagonist losartan (0.03 μmol/l) and the selective angiotensin $AT_2$ receptor antagonist PD 123177 (3 μmol/l) on force of contraction in isolated electrically driven human trabeculae from the right atrium. Ordinate: force of contraction in % of predrug value. Abscissa: concentration of angiotensin II in μmol/l.

ventricular preparations was determined, as shown in Fig. 1 (lower panel). Even after application of 30 μmol/l angiotensin II, no positive inotropic effect was observed.

### 3.2. Functional characterization of the positive inotropic effect of angiotensin II in atrial myocardium

Since there is experimental evidence for the existence of postsynaptic angiotensin II receptors as well as for neuronal presynaptic angiotensin II receptors facilitating norepinephrine release from presynaptic stores (Starke, 1970), the positive inotropic effect of angiotensin II on right atrial preparations was investigated in the presence of the selective angiotensin II angiotensin $AT_1$ and $AT_2$ receptor antagonists losartan and PD 123177 as well as in the presence of the $\beta_1$- and $\beta_2$-adrenoceptor antagonists CGP 207.12A and ICI 118.551. There were no direct inotropic effects of losartan or PD 123177 prior to angiotensin II application. Fig. 4 shows original recordings of these experiments. The positive inotropic effect of angiotensin II was antagonized by 10 μmol/l of the angiotensin $AT_1$ receptor antagonist losartan but unchanged in the

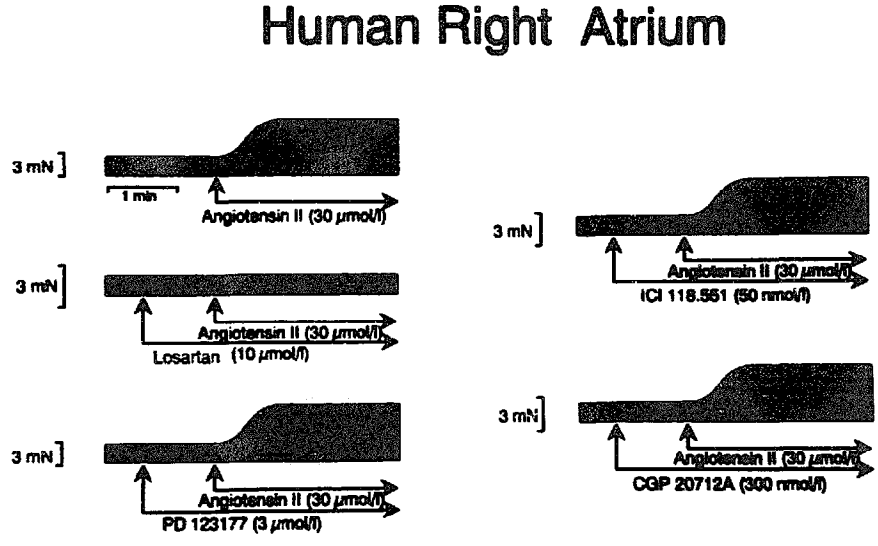


Fig. 4. Original recordings of isometric force of contraction in isolated electrically driven human trabeculae from the right atrium following application of angiotensin II (30 μmol/l) alone or in the presence of the angiotensin $AT_1$ receptor antagonist losartan (10 μmol/l), the angiotensin $AT_2$ receptor antagonist PD 123177 (3 μmol/l), left panel, or the $\beta_2$-adrenoceptor antagonist ICI 118.551 (50 nmol/l) and the $\beta_1$-adrenoceptor antagonist CGP 207.12A (300 nmol/l), right panel.

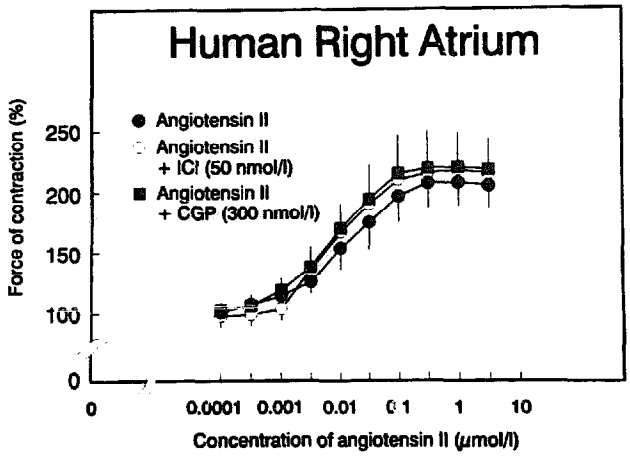


Fig. 6. Concentration-response curves for the effects of angiotensin II (0.0001–3 μmol/) alone and in the presence of the selective $\beta_1$-adrenoceptor antagonist CGP 207.12A (300 nmol/l) and the selective $\beta_2$-adrenoceptor antagonist ICI 118.551 (50 μmol/l) on force of contraction in isolated electrically driven atrial trabeculae. Ordinate: force of contraction in % of predrug value. Abscissa: concentration of angiotensin II in μmol/l.

presence of the angiotensin $AT_2$ receptor antagonist PD 123177 (Fig. 4, left panel). Fig. 5 demonstrates concentration-response curves for angiotensin II in the presence of 0.03 μmol/l losartan and 3 μmol/l PD 123177. Losartan shifted the concentration-response curve to the right as an indication for receptor antagonism while PD 123177 had no effect on the potency or efficacy of the peptide. Thus, these experiments provided evidence for an angiotensin $AT_1$ receptor-mediated positive inotropic effect of angiotensin II in human atrial myocardium.

However, at this stage it still remained open whether postsynaptic angiotensin $AT_1$ receptors mediate the inotropic response or whether activation of presynaptic angiotensin $AT_1$ receptors by angiotensin II facilitates norepinephrine release, possibly activated by the electrical stimulation of the preparations. Angiotensin $AT_1$ receptor-mediated norepinephrine release could stimulate cardiac β-adrenoceptors, which in turn could produce positive inotropic effects. The $\beta_1$- and $\beta_2$-selective adrenoceptor antagonists CGP 207.12A and ICI 118.551 had no effect on the inotropic response of angiotensin II at 30 μmol/l bolus application (Fig. 4, right panel). Fig. 6 shows cumulative concentration-response curves with the $\beta_1$-selective and $\beta_2$-selective antagonists CGP 207.12A and ICI 118.551, respectively. Neither the potency nor the efficacy of the positive inotropic effect of angiotensin II was antagonized when $\beta_1$- and $\beta_2$-adrenoceptors were blocked with either CGP 207.12A or ICI 118.551. Thus, stimulation of presynaptic angiotensin $AT_1$ receptors appears not to be involved in the inotropic response to isoprenaline.

### 3.3. Characterization of angiotensin II receptors in human myocardium

Human angiotensin II receptors were measured with the nonspecific partial agonistic radioligand [$^{125}$I]-[Sar$^1$,Ile$^8$]angiotensin II. The representative saturation experiment in Fig. 7 shows concentration-dependent binding of the radioligand [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II to ventricular membranes of a nonfailing human heart. In saturation experiments, application of higher concentrations of [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II resulted in radioligand binding to a nonspecific binding site, which could not be antagonized by angiotensin II. Therefore, higher radioligand concentrations were not used. In the right atrial membranes, the density of total angiotensin II receptors was 8.6 ± 0.5 fmol/mg protein with a $K_d$ value of 0.218 ± 0.027 nmol/l ($n = 5$). Nonspecific binding at $K_d$ amounted to about 40% of total binding. In ventricular membranes the density of angiotensin II receptors was about 35% lower ($B_{max}$ 5.6 ± 0.3 fmol/mg protein, $K_d$ 0.3 ± 0.1 nmol/l). No significant difference could be observed in the densities of angiotensin II receptors in membranes from patients classified as NYHA IV and nonfailing controls (not shown). In order to address the angiotensin $AT_1$ and $AT_2$ receptor subtype distribution, competition experiments with the selective antagonists losartan and PD 123177 were performed. For these experiments 0.2 nmol/l [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II (atrial mem-

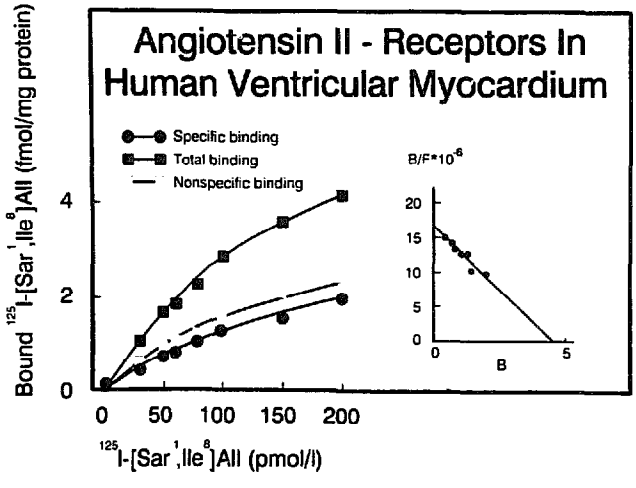


Fig. 7. Representative saturation experiment of the binding of [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II to a ventricular membrane. Inset: linear transformation. Bound [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II (fmol) bound per milligram protein was plotted as a function of the ratio of bound-to-free ($B/F * 10^{-6}$) [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II. The intercept with the abscissa is the maximal number of binding sites ($B_{max}$); the slope is the apparent affinity ($K_d$). Data points are the mean of triplicate observations. Ordinate: bound [$^{125}$I]-[Sar$^1$,Ile$^8$]angiotensin II in fmol/mg protein. Abscissa: concentration of [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II in pmol/l.

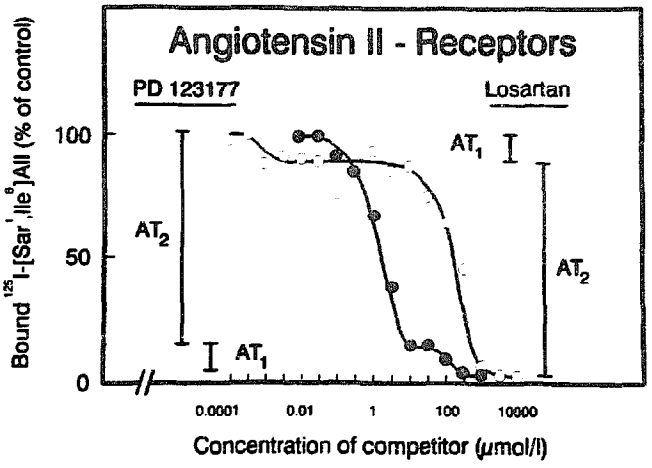


Fig. 8. Representative competition experiments with the selective angiotensin $AT_1$ receptor antagonist losartan (0.0001–10000 μmol/l) and the selective angiotensin $AT_2$ receptor antagonist PD 123177 (0.01–1000 μmol/l) for binding of [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II to left ventricular membranes. Ordinate: bound [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II in % of control. Abscissa: concentration of competitor in μmol/l: ● PD 123177; ○ Losartan.

branes) and 0.3 nmol/l [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II (ventricular membranes) were used (approximately $K_d$). Fig. 8 shows representative competition curves with losartan and PD 123177 for binding of [$^{125}$I]-[Sar$^1$,Ile$^8$]angiotensin II. Losartan and PD 123177 competition was biphasic, indicating an interaction with one high- and one low-affinity binding site. The $K_i$ values for losartan and PD 123177 were $4.3 \pm 0.8$ nmol/l and $0.76 \pm 0.04$ μmol/l, respectively. Losartan 10 nmol/l occupied all angiotensin $AT_1$ and PD 123177 30 μmol/l all angiotensin $AT_2$ receptors. The angiotensin $AT_1$ receptor, which has to be focused on because of its functional importance, represents about 21% of the total angiotensin II receptors in the atrium and about 15% of the total angiotensin II receptors in the ventricle. The densities of angiotensin II receptors and the angiotensin $AT_1$ and $AT_2$ receptor subtypes are summarized in Fig. 9.

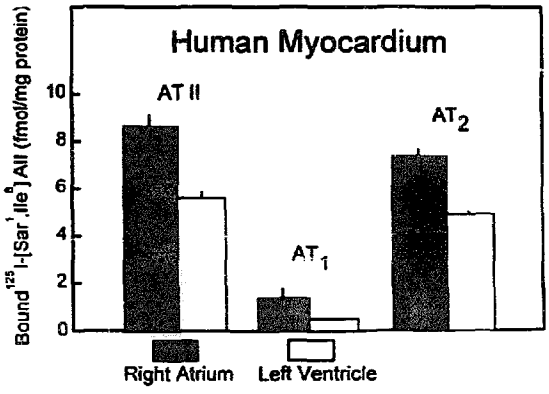


Fig. 9. Density of angiotensin II receptors and angiotensin $AT_1$ and $AT_2$ receptor subtypes in myocardial membranes from right atria and left ventricle of nonfailing hearts ($n = 5$). Ordinate: bound [$^{125}$I][Sar$^1$,Ile$^8$]angiotensin II in (fmol/mg protein).

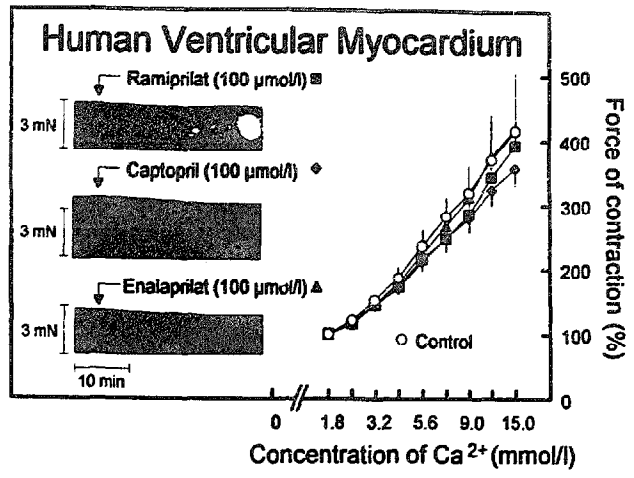


Fig. 10. Left. Original recordings of the isometric force of contraction in isolated electrically driven human papillary muscle strips following application of 100 μmol/l ramiprilat, captopril and enalaprilat. Right. Concentration-response curves for the effects of $Ca^{2+}$ (1.8–15 mmol/l) 30 min after angiotensin-converting enzyme inhibitor application. Ordinate: force of contraction in % of predrug value. Abscissa: concentration of $Ca^{2+}$ in mmol/l.

### 3.4. Effects of ramiprilat, enalaprilat and captopril on myocardial force of contraction

The inotropic effects of the angiotensin-converting enzyme inhibitors ramiprilat, enalaprilat and captopril on electrically driven human papillary muscle strips from terminal failing hearts (NYHA IV) are shown in Fig. 10.

None of the angiotensin-converting enzyme inhibitors applied in a concentration of 100 μmol/l produced a significant inotropic effect on failing human myocardium (left panel). The same results were obtained for cumulative-concentration-response curves. No differences were detected when the curves were compared with the effects of the respective control condition (i.e. drug-free bathing solution or the respective solvent). $Ca^{2+}$ concentration-response curves were made 30 min after application of the angiotensin-converting enzyme inhibitors to confirm that the preparations were able to maximally increase force of contraction. No difference in efficacy or potency could be observed under control conditions or in the presence of the angiotensin-converting enzyme inhibitors (Fig. 10). All data of contraction experiments are summarized in Table 1.

## 4. Discussion

The existence of cardiac angiotensin II receptors has been reported in various mammalian species (Baker et al., 1984; Baker and Singer, 1988) including the human heart (Urata et al., 1989). They have been

subclassified into $AT_1$ and $AT_2$ receptors on the basis of their different affinities for various nonpeptide receptor antagonists, e.g. losartan and PD 123319 (Bumpus et al., 1991; Smith et al., 1992). A positive inotropic response in hearts following receptor stimulation with angiotensin II has been observed in dogs (Kobayashi et al., 1978), cats (Dempsey et al., 1971) rabbits (Freer et al., 1976; Bonnardeaux and Regoli, 1973) and humans (Moravec et al., 1990; Holubarsch et al., 1993; Zerkowski et al., 1993). The two angiotensin II receptor subtypes $AT_1$ and $AT_2$ have been characterized in the rabbit heart by radioligand binding studies with the selective angiotensin II receptor antagonists losartan and PD 121981 (Scott et al., 1992; Rogg et al., 1990). The relationship between selective $AT_1$ stimulation and an increase in force of contraction was studied by Scott et al. (1992) and Ishihata and Endoh (1993) in the rabbit ventricular myocardium. In concert, Feolde et al. (1993a) observed a positive inotropic effect of angiotensin II in guinea pig atria, an effect which was prevented by losartan but not by PD 123319. In contrast to the adult guinea pig atrium, in which the angiotensin $AT_1$ receptor is reported to be dominant, binding experiments in neonatal rat atria showed the predominance of angiotensin $AT_2$ receptor subtypes (Feolde et al., 1993b). However, the distribution of the receptor subtypes as well as the inotropic response to angiotensin II stimulation seems to be species specific and cannot be extrapolated uncritically to the situation in the human myocardium.

The present study shows the existence of angiotensin II receptor subtypes $AT_1$ and $AT_2$ in human atrial and ventricular tissue. Competition binding studies were performed with the selective nonpeptide angiotensin II receptor antagonists losartan and PD 123177. The density of angiotensin $AT_1$ receptors was about 15–20% of the density of angiotensin II receptors in both ventricular and atrial membranes. The absolute angiotensin II receptor density in ventricular preparations was about 35% lower than the density in the atria. Although the receptor densities were low, these findings are in agreement with results of Urata et al. (1989) and Regitz et al. (1995) who reported a low density of angiotensin II receptors in human myocardial preparations ($B_{max}$: ~1.4 fmol/mg, ~11.2 fmol/mg protein, respectively). In order to study whether the different distribution of angiotensin II receptors is functionally relevant, the effects of angiotensin II were studied in isolated electrically driven papillary muscle strips and atrial trabeculae in the presence of the specific angiotensin $AT_1$ receptor antagonist losartan and the angiotensin $AT_2$ receptor antagonist PD 123177. Only in the atrial preparations did angiotensin II produce a positive inotropic response of about 92%. No effect was observed in human papillary muscle strips. This difference could be explained either by the greater density of angiotensin II receptors in the atrium than in the ventricle or by a failure of ventricular angiotensin $AT_1$ receptors to couple to postreceptor events like the phospholipase C or the receptor signal transducing G-protein ($G_{q\alpha}$). Moreover, the inotropic effect of angiotensin II in the atrium could only be blocked with losartan, indicating that the inotropic response is mediated by angiotensin $AT_1$ receptor stimulation although this receptor subtype represents only 15–20% of total angiotensin II receptors. Angiotensin $AT_1$ receptors are reported to be functionally coupled to phospholipase C, as evidenced by an increased formation of inositol phosphate and $[Ca^{2+}]_i$ after exposure of isolated rat cardiac cells to angiotensin II (Baker et al., 1989; Feolde et al., 1993b). In the present study, we observed a positive inotropic effect of angiotensin II in human atrial preparations which was about 4–5 times less effective than stimulation of the same preparation with isoprenaline or milrinone, which both act by an enhancement of intracellular cAMP. These findings indicate that the positive inotropic effect of angiotensin II in human atrial trabeculae, which is probably mediated by phospholipase C stimulation, is much less efficacious than inotropic interventions by the cAMP-system. Whether the inositol phophate pathway is involved in signal transduction of angiotensin II receptors in human myocardium has to be provided. In addition, the concentrations of angiotensin II required to produce positive inotropic effects were higher than those producing vasodilatory properties in vessels. On first glance, this is evidence that angiotensin II could elicit its physiologically relevant effects on the vasculature rather than on atrial myocardium. However, local formation and paracrine release could produce high concentrations of angiotensin II at atrial angiotensin $AT_1$ receptors, indicating that some positive responses are nevertheless possible.

Finally, an 'indirect' positive inotropic effect of angiotensin II as a result of facilitated endogenous noradrenaline release has to be excluded. At the presynaptic sites angiotensin II has been reported to increase the rate of noradrenaline synthesis (Roth, 1972), to enhance noradrenaline release induced by nerve stimulation (Starke et al., 1969; Starke, 1970; Hughes and Roth, 1971; Zimmermann, 1978; Rump and Majewski, 1987; Szabo et al., 1990; Schwieler et al., 1991) and to inhibit the reuptake of the catecholamine (Peach et al., 1969; Khairallah, 1972). In the present study, blockade of postsynaptic $\beta_1$- and $\beta_2$-receptors with the specific receptor antagonists ICI 118.551 and CGP 207.12A did not affect the positive inotropic effect of angiotensin II, indicating its independence from $\beta$-adrenoceptor stimulation.

Patients with congestive heart failure undergo a general neurohumoral activation with high levels of

sympathetic activity and an elevation of the renin-angiotensin-aldosterone system (RAAS) activity (Francis et al., 1984). The activation of the RAAS results in an increase of angiotensin II which exerts direct cardiac effects as well as vasoconstriction of the coronary arteries and other vascular systems (Longnecker et al., 1984; Simon et al., 1984; Dzau and Safar, 1988). Much of the information concerning the role of angiotensin II in this setting, as well as in normal physiology, has been derived from experimental and clinical studies performed with angiotensin-converting enzyme inhibitors. For example, the clinical efficacy of angiotensin-converting enzyme inhibitors in hypertension (Johnson et al., 1984), heart failure (The CONSENSUS Trial Study Group, 1987) and myocardial ischemia (Ertl, 1988; Pfeffer et al., 1988) points to some more indirect angiotensin II effects as well as to some direct effects of the angiotensin-converting enzyme inhibitors.

Foult et al. (1988) demonstrated a reduction of cardiac index, ejection fraction, and end-systolic stress/end-systolic volume ratio after a bilateral intracoronary infusion of enalaprilat. This result points towards direct negative inotropic effects of angiotensin-converting enzyme inhibitors, effects which have to be investigated in human myocardium. However, the systemic application of angiotensin-converting enzyme inhibitors during therapy of chronic congestive heart failure leads to an improvement of ejection fraction, cardiac output and cardiac index of patients treated chronically (Curtiss et al., 1978; Captopril Multicenter Research Group, 1983). In contrast, an immediate intravenous application of enalaprilat after myocardial infarction does not improve survival (Swedberg et al., 1992, CONSENSUS II), but produces heart failure in many patients (132 patients vs. 104 patients in the placebo group). In contrast, oral administration of ramipril between the second and ninth day after infarction (i.e. after complete hemodynamic stabilization) resulted in a reduction of mortality (The Acute Infarction Ramipril Efficacy (AIRE) Study Investigators, 1993). From these clinical data it is tempting to speculate that a negative inotropic effect of intravenously applied angiotensin-converting enzyme inhibitor could contribute to the adverse outcome in several patients. Whether these effects are mediated by direct effects of the angiotensin-converting enzyme inhibitors on human myocardial function or whether they are caused by an inhibition of endogenous angiotensin II formation, thereby blocking the endogenous effects of angiotensin II on contractility, is unknown.

The second part of the present study therefore aimed at investigating the direct myocardial effects of the angiotensin-converting enzyme inhibitors ramiprilat, enalaprilat and captopril. The application of 100 $\mu$mol/l of the angiotensin-converting enzyme inhibitors had no significant effect on the myocardial force of contraction of electrically stimulated human papillary muscle strips and human atrial trabeculae from terminal failing hearts. $Ca^{2+}$ concentration-response curves made 30 min after angiotensin-converting enzyme inhibitor application showed no reduction of efficacy or potency compared to control preparations. Taken together, it seems unlikely that direct effects of angiotensin-converting enzyme inhibitors on myocardial contractility are responsible for the different observations made in acute or long term studies with different angiotensin-converting enzyme inhibitors. Since there is no negative inotropic effect in failing ventricular muscle (possibly due to a lack of angiotensin II receptors coupled to force of contraction), these data are in favour of the notion that angiotensin-converting enzyme inhibitors can be used safely in heart failure without causing negative inotropic events, regardless of the fact that the local RAAS is activated. In atrial heart muscle, angiotensin II could provide inotropic support to the ventricular filling, when the filling pressures of the failing left ventricle are elevated. In this respect, it is interesting to note that an alternative angiotensin II forming pathway, namely myocardial chymase activity, has been recently identified (Urata et al., 1990). Therefore, it is still possible that atrial contractility is under tonic stimulation by angiotensin II formed by this alternative pathway, which is not targeted by angiotensin-converting enzyme inhibitors. However, potent and effective inhibitors of this enzyme are not available at present.

In summary, the present study shows that the angiotensin II receptor density is lower in the human ventricle than in the atrium. In atria and ventricles, the angiotensin $AT_1$ receptors represent about 15–20% of total angiotensin II receptors. This could be one reason among others for the lack of an inotropic response to angiotensin II stimulation in the ventricle. Furthermore, $AT_1$ but not angiotensin $AT_2$ receptors have been shown to mediate the positive inotropic response of angiotensin II in human atrial trabeculae, whereas a facilitation of noradrenaline release is of no further importance. The angiotensin-converting enzyme inhibitors ramiprilat, enalaprilat and captopril had no effect on myocardial force of contraction in this study, indicating that they can safely be used in situations of depressed contractility.

## Acknowledgements

Experimental work was supported by a grant of the Deutsche Forschungs-Gemeinschaft (DFG). M.B. is a recipient of the Gerhard-Hess and Heisenberg programs of the Deutsche Forschungsgemeinschaft. This work contains parts of the doctoral thesis of B.S.

(University of Munich, in preparation) and of the doctoral thesis of O.L. (University of Cologne, in preparation).

## References


Baker, K.M. and H.A. Singer, 1988, Identification and characterization of the guinea pig angiotensin II ventricular and atrial receptors: coupling to inositol phosphate production, Circ. Res. 62, 896.

Baker, K.M., C.P. Campanile, G.J. Trachte and M.J. Peach, 1984, Identification and characterization of the rabbit angiotensin II myocardial receptor, Circ. Res. 54, 286.

Baker, K.M., H.A. Singer and J.F. Aceto, 1989, Angiotensin II receptor-mediated stimulation of cytosolic-free calcium and inositol phosphates in chick myocytes, J. Pharmacol. Exp. Ther. 251, 578.

Bonnardeaux, J.L. and D. Regoli, 1973, Action of angiotensin and analogues on the heart, Can. J. Physiol. Pharmacol. 52, 50.

Bumpus, F.M., K.J. Catt, A.T. Chiu, M. DeGasparo, T. Goodfriend, A. Husain, M.J. Peach, D.G. Taylor Jr., and P.B.M.W.M. Timmermans, 1991, Nomenclature for angiotensin receptors, Hypertension 17, 720.

Captopril Multicenter Research Group, 1983, A placebo controlled trial of captopril in refractory chronic congestive heart failure, J. Am. Coll. Cardiol. 2, 755.

Curtiss, C., J.N. Cohn, T. Vrobel and J.A. Francioza, 1978, The role of the renin-angiotensin system in the systemic vasoconstriction of chronic congestive heart failure, Circulation 58, 763.

De Lean, A., A.A. Hancock and R.J Lefkowitz, 1982, Validation and statistical analysis of a computer modeling method for quantitative analysis of radioligand binding data for mixtures of pharmalogical receptor subtypes, Mol. Pharmacol. 21, 5.

Dempsey, P.J., Z.T. McCallum, K.M. Kent and T. Cooper, 1971, Direct myocardial effects of angiotensin II, Am. J. Physiol. 220, 477.

Dzau, V.J. and M.I. Safar, 1988, Large conduit arteries in hypertension; role of the vascular renin angiotensin system, Circulation 77, 947.

Ertl, G., 1988, Angiotensin converting enzyme inhibitors and ischaemic heart disease, Eur. Heart J. 9, 719.

Feolde, E., P. Vigne and C. Frelin, 1993a, Angiotensin AT1 receptors mediate a positive inotropic effect of angiotensin II in guinea pig atria, Eur. J. Pharmacol. 245, 63.

Feolde, E., P. Vigne and C. Frelin, 1993b, Angiotensin II receptor subtypes and biological responses in the rat heart, J. Moll. Cell Cardiol. 25, 1359.

Foult, J.-M., O. Tavolaro, I. Antony and A. Nitenberg, 1988, Direct myocardial and coronary effects of enalaprilat in patients with dilated cardiomyopathy: assessment by a bilateral intracoronary infusion technique, Circulation 77 (2), 337.

Francis, G.S., S.R. Goldsmith, T.B. Levine, M.T. Olivari and J.N. Cohn, 1984, The neurohumoral axis in congestive heart failure, Ann. Intern. Med. 101, 370.

Freer, R., A. Pappano, M. Peach, K. Bing, M. McLean, S. Vogel and N. Sperelakis, 1976, Mechanism for the positive inotropic effect of angiotensin II on isolated cardiac muscle, Circ. Res. 39, 178.

Holubarsch, C., G. Hasenfuss, S. Schmidt-Schweda, A. Knorr, B. Pieske, T. Ruf, R. Fasol and H. Just, 1993, Angiotensin I and II exert inotrtopic effects in atrial but not in ventricular human myocardium, Circulation 88, 1228.

Hughes, J. and R.H. Roth, 1971, Evidence that angiotensin enhances transmitter release during sympathtic nerve stimulation, Br. J. Pharmacol. 41, 239.

Ishihata, A. and M. Endoh, 1993, Pharmacological characteristics of the positive inotropic effect of angiotensin II in the rabbit ventricular myocardium, Br. J. Pharmacol. 108, 999.

Johnson, C.I., L. Arnold and M. Hiwatari, 1984, Angiotensin-converting enzyme inhibitors in the treatment of hypertension, Drugs 27, 271.

Khairallah, P.A., 1972, Action of angiotensin on adrenergic nerve endings: inhibition of norepinephrine uptake, Fed. Proc. 31, 1351.

Kobayashi, M., Y. Furukawa and S. Chiba, 1978, Positive chronotropic and inotropic effects of angiotensin II in the dog heart, Eur. J. Pharmacol. 501, 17.

Llambi, H.G., C.M. Taquini, A. Mazzadi, A. Gallo, M. Fontan and A.C. Taquini, 1990, Effect of enalaprilic acid on cardiac contractile response to $\beta$ adrenergic stimulation, Hypertension 15 (Suppl. I), I-51.

Longnecker, D.I., M.I. Durcus, K.R. Donovan, E.D. Miller and M.J. Peach, 1984, Saralasin dilates arterioles in SHR but not WKY rats, Hypertension 1 (Suppl. 1), 106.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951, Protein measurements with the folin phenol reagent, J. Biol. Chem. 193, 265.

Moravec, C.S., M.D. Schluchter, L. Paranandi, B. Czerska, R.W. Stewart, E. Rosenkranz and M. Bond, 1990, Inotropic effects of angiotensin II on human cardiac muscle in vitro, Circulation 82, 1973.

Peach, M.J., F.M. Bumpus and P.A. Khairallah, 1969, Inhibition of norepinephrine uptake in hearts by angiotensin II and analogs, J. Pharmacol. Exp. Ther. 167, 291.

Pfeffer, M.A., G.A. Lamas, D.E. Vaughn and E. Braunwald, 1988, Effect of captopril on progressive ventricular dilatation after myocardial infarction, New Engl. J. Med. 319, 80.

Räcke, H.-F., D. Koppers, P. Lemke, H. Casaretto and O. Hauswirth, 1994, Fosinoprilate prolongs the action potential: reduction of $K_i$ and enhancement of the L-type calcium current in guinea pig ventricular myocytes, Cardiovasc. Res. 28, 201.

Regitz, V., N. Friedel, A. Heymann, P. Bauer, M. Neuß, A. Rolfs, C. Steffen, A. Hildebrandt, R. Lotzer and E. Fleck, 1995, Regulation, chamber localization, and subtype distribution of angiotensin II receptors in human hearts, Circulation 91, 1461.

Ribout, C., R. Cardinal, L. Gouin, P. Moreau, D. Godin, M. Vermeulen, J. de Champlain, L. Rochette and R. Nadeau, 1994, Prolongation by captopril of action potential duration in the normal and hypertrophied rat ventricle: direct action or inhibition of the local angiotensin converting enzyme, Cardiovasc. Res. 28, 221.

Rogers, T.B., 1984, High affinity angiotensin II receptors in myocardial sarcolemmal membranes, J. Biol. Chem. 259, 8106.

Rogg, H., A. Schmid and M. DeGasparo, 1990, Identification and characterization of angiotensin II receptor subtypes in rabbit ventricular myocardium, Biochem. Biophys. Res. Commun. 173, 416.

Roth, R.H., 1972, Action of angiotensin on adrenergic nerve endings: enhancement of norepinephrine biosynthesis, Fed. Proc. 31, 1358.

Rump, L.C. and H. Majewski, 1987, Beta adrenoceptor facilitation of norepinephrine release is not dependent on local angiotensin II formation in the rat isolated kidney, J. Pharmacol. Exp. Ther. 243, 1107.

Schwieler, J.H., T. Kahan, J. Nussberger and P. Hjemdahl, 1991, Influence of the renin-angiotensin system on sympathetic neurotransmission in canine skeletal muscle in vivo, Naunyn-Schmied. Arch. Pharmacol. 343, 166.

Scott, A.L., R.S.L. Chang, V.J. Lotti and P.K.S. Siegl, 1992, Cardiac angiotensin receptors: effects of selective angiotensin II receptor antagonists, Losartan and PD 121981, in rabbit heart, J. Pharmacol. Exp. Ther. 261, 931.

Simon, A.C.H., J.A. Levenson, J.I. Bouther and M.E. Safar, 1984, Comparison of oral MK 421 and propanolol in mild to moderate hypertension and their effects on arterial and venous vessels of the forearm, Am. J. Cardiol. 53, 781.

Smith, K.D., A.T. Chiu, P.C. Wong, W.F. Herblin and P.B.M.W.M. Timmermans, 1992, Pharmacology of nonpeptide angiotensin II receptor antagonists, Annu. Rev. Pharmacol. Toxicol. 32, 135.

Starke, K., 1970, Interactions of angiotensin and cocaine on output of noradrenaline from isolated rabbit hearts, Naunyn-Schmied. Arch. Pharmacol. 265, 170.

Starke, K., U. Werner and H.J. Schümann, 1969, Wirkung von Angiotensin auf Funktion und Noradrenalinabgabe isolierter Kaninchenherzen in Ruhe und bei Sympathicusreizung, Naunyn-Schmied. Arch. Pharmacol. 265, 170.

Swedberg, K., P. Held, J. Kjekshus, K. Rasmussen, L. Rydén and H. Wedel on behalf of the CONSENSUS II study group, 1992, Effects of the early administration of enalapril on mortality in patients with acute myocardial infarction, New Engl. J. Med. 327, 678.

Szabo, B., L. Hedler, C. Schurr and K. Starke, 1990, Peripheral presynaptic facilitatory effect of angiotensin II on noradrenaline release in anesthetized rabbits, J. Cardiovasc. Pharmacol. 15, 968.

The Acute Infarction Ramipril Efficacy (AIRE) Study Investigators, 1993, Effect of ramipril on mortality of survivors of acute myocyrdial infarction with clinical evidence of heart failure, Lancet 342, 821.

The CONSENSUS Trial Study Group, 1987, Effects of enalapril on mortality in severe congestive heart failure: results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS), New Engl. J. Med. 316, 1429.

Urata, H., B. Healy, E.W. Stewart, F.M. Bumpus and A. Husain, 1989, Angiotensin II receptors in normal and failing human hearts, J. Clin. Endocrinol. Metab. 69, 54.

Urata, H., A. Kinoshita, K.S. Misono, F.M. Bumpus and A. Husain, 1990, Identification of a highly specific chymase as the major angiotensin II-forming enzyme in the human heart, J. Biol. Chem. 265, 22348.

Zerkowski, H.R., A. Broede, K. Kunde, S. Hillemann, E. Schäfer, M. Vogelsang, M.C. Michel and O.E. Brodde, 1993, Comparison of the positive inotropic effects of serotonin, histamin, angiotensin II, endothelin and isoprenaline in the isolated human right atrium, Naunyn-Schmied. Arch. Pharmacol. 347, 347.

Zimmermann, B.G., 1978, Actions of angiotensin on adrenergic nerve endings, Fed. Proc. 37, 199.