

# Angiotensin II-receptor subtypes in human atria and evidence for alterations in patients with cardiac dysfunction

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Angiotensin II (AII) has been implicated as an important factor in the pathophysiology of heart diseases. Following the recent identification of two subtypes of the AII receptor in cardiac tissue of animals, we investigated the possible occurrence of these, or similar, subtypes in human atrial tissue. In right-atrial tissue from patients undergoing heart surgery, we determined the AII-receptor profile in receptor binding studies, using [ $^{125}$ I]-angiotensin as radioligand and AII as well as two compounds selective for the receptor subtypes to identify and quantify AII-receptor subpopulations. In 35 patients (23 requiring coronary bypasses, 10 valvular surgery and two combined coronary and valvular surgery), the left-ventricular ejection fraction was determined in the preoperative phase, and right- and left-atrial pressure during surgery. In membranes of human right atria, AII receptors are present in high density (median:  $B_{max} = 294 \text{ fmol} \cdot \text{mg}^{-1} \text{ protein}$ , range: 111–2073) and two different subtypes can be distinguished. Type-1 receptors ( $AT_1$ ) accounted for  $33 \pm 10\%$  of the population whereas

type-2 receptors ( $AT_2$ ) made up  $67 \pm 10\%$  of the population. There was no correlation between any of the measured cardiac functions and total AII-receptor density or receptor affinity. However, the percentage of  $AT_1$  receptors was higher in the atria of patients with normal right-atrial pressure; left-ventricular ejection fraction was positively and right-atrial pressure inversely correlated with the percentage of  $AT_1$  receptors ( $r = 0.740$  and  $-0.901$ , respectively;  $P < 0.001$ , for both). Moreover, the percentage of  $AT_2$  receptors was directly correlated with the levels of left-atrial pressure ( $r = 0.853$ ;  $P < 0.001$ ). It is concluded that the ratio of  $AT_1$  to  $AT_2$  receptors correlates well with right-atrial pressure and left-ventricular function. This is a first indication of a possible involvement of AII-receptor subtypes in the pathophysiology of cardiac dysfunctions. (Eur Heart J 1996; 17: 1112–1120)

**Key Words:** Human, angiotensin II, receptor, subtype,  $AT_1$ ,  $AT_2$ , cardiac, atrium, CGP 42 112 A, Losartan.

## Introduction

Angiotensin II (AII) is the principal effector peptide of the renin-angiotensin system, which is the major regulatory mechanism for electrolyte balance and blood pressure. There is also increasing evidence of the existence of an intrinsic renin-angiotensin system in the heart: findings made mainly in animals show the presence in myocardial tissue of mRNAs coding for angiotensinogen and renin<sup>[1–4]</sup>, and of renin, angiotensin-converting enzyme, and AII, as well as its receptor<sup>[5–13]</sup>.

The physiological and pathophysiological roles of this AII-generating pathway and the circulating AII in the heart have not yet been fully elucidated. However, they may include inotropic and chronotropic activities, effects on coronary vascular tone, arrhythmogenesis, and impairment of relaxation, as well as a permissive or regulatory role in modulating cardiac growth and development<sup>[14–18]</sup>. There is indirect evidence tending to implicate the renin-angiotensin system in the initiation of proto-oncogene expression and cell growth in smooth muscle and myocardial cells<sup>[19–25]</sup>, and inhibition has been shown to prevent left ventricular hypertrophy in rats with pressure overload<sup>[26–28]</sup> and to promote the regression of chronic pressure-overload hypertrophy in man<sup>[29–30]</sup>.

Recently, two distinct subtypes of AII receptors have been discovered<sup>[31–34]</sup>. They can be identified

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Table 1 Patient characteristics

Patient	Sex	Age	Intervention	Medication	Systolic BP (mmHg)	Diastolic BP (mmHg)	EF (%)
1	M	67	C	B,C,N	120	70	66
2	M	53	C,V	N,O	120	80	58
3	M	73	C	A,C,B,N	110	70	49
4	M	60	C	C,D,Ao,O	160	80	68
5	F	68	V	D	120	60	62
6	M	47	V	C,D,Ao,G	125	70	34
7	F	33	V	—	105	70	31
8	M	59	C	C,D,N	110	70	67
9	M	63	C	B,N,O	140	80	64
10	M	56	C	B,C,N	130	70	40
11	M	60	V	A	125	56	69
12	F	60	V	G,O	120	70	34
13	M	65	C	C	130	70	51
14	M	67	C	B,C,N	120	65	50
15	M	64	C	B,C,N	170	90	79
16	M	43	V	—	—	—	—
17	M	67	C	A	140	80	36
18	F	65	V	D,G,O	160	100	41
19	M	58	C	B,C,N	100	80	58
20	M	72	C	C,O	130	75	59
21	M	60	C	B,C,N	165	65	50
22	M	58	C	B,C	150	90	46
23	M	63	V	A,B,C,N,G,T	125	70	56
24	M	62	C	C,D	110	70	33
25	M	49	V	A,C,D,Ao,G	110	70	40
26	M	54	C	B,C,N	120	80	56
27	M	53	C	B,C,N	130	80	64
28	M	66	C	B,N,C,O	120	70	59
29	M	62	C	B,C,Ao,N	110	65	52
30	M	71	C	B,C,N	110	80	36
31	M	58	V	—	120	80	35
32	M	53	C	B,C	130	70	48
33	M	59	C	B,Ao	110	75	44
34	M	41	C	B,C,Ao,N	110	80	46
35	M	67	C,V	D, O	110	80	41

C=coronary bypass surgery; V=replacement of valve(s).

A=ACE inhibitor; Ao=anticoagulant; B= $\beta$ -adrenergic antagonist; C=calcium antagonist;

D=diuretic; G=glycoside; N=nitrate; T=other cardiotonics; O=others.

BP=blood pressure. EF=ejection fraction.

unequivocally with the aid of the novel agents Losartan and CGP 42 112 A, which have an inverse affinity for the subtypes and selectivity factors of more than 1000<sup>[31]</sup>. Losartan is a specific non-peptidic AII antagonist having a high affinity for the AT<sub>1</sub> subtype, to which several functions, such as vasoconstriction, can be attributed; the function of the AT<sub>2</sub> subtype, which shows the greatest affinity for CGP 42 112 A remains unclear<sup>[31–35]</sup>. Both subtypes have been demonstrated in rabbit myocardial tissue<sup>[36]</sup> and rat myocardium<sup>[37]</sup>.

The potential functional significance of the two cardiac AII-receptor subtypes is unknown. Up to now, no attempt had been made to differentiate AII-receptor subtypes in human myocardial tissue. This paper reports, for the first time, the existence and the properties of two subtypes in human right atria. If AII-receptor subtypes play a role in cardiac function, their abundance or properties may differ according to the degree of dysfunction. Studies in rats have indicated changes in AII-receptor subtypes with cardiac hypertrophy using

different models<sup>[38,39]</sup>. To test this hypothesis, we investigated the AII-receptor subtypes in right-atrial tissue from patients with varying degrees of cardiac dysfunction. Our results demonstrate that the relative proportions of the AII-receptor subtypes are closely associated with altered diastolic and systolic properties of the heart.

## Methods

### Patients and study design

Atrial-tissue specimens ( $0.6 \pm 0.2$  g; mean  $\pm$  SD) were excised from the right-atrial appendage during cannulation in 35 patients (31 males, four females, aged  $60 \pm 9$  years, weighing  $81 \pm 12$  kg and  $173 \pm 8$  cm in height) undergoing open-heart surgery. The left-ventricular ejection fraction was derived from the levocardiogram recorded during preoperative left-heart catheterization in all but one patient ( $51 \pm 13\%$ ,  $n=34$ ).

Prior to surgery, all but three patients had been receiving medical therapy (Table 1): two patients had been on monotherapy and the remaining 30 on combined medication with two or more drugs of the following categories (calcium-channel blockers in 22; nitrates in 17, beta-blockers in 18; antiarrhythmic agents in eight; glycosides in six; diuretics in eight; converting-enzyme inhibitors in five; antiplatelet drugs in 10; oral anticoagulants in seven). The antiplatelet agents were withdrawn 10 days before the operation and the anticoagulants on the day before surgery.

On the night before surgery, the patients received flunitrazepam (1 mg) orally and 1 h before induction of anaesthesia morphine ( $0.1 \text{ mg} \cdot \text{kg}^{-1}$ ) with hyoscine hydrobromide ( $0.1 \text{ mg} \cdot 25 \text{ kg}^{-1}$ ) intramuscularly. Anaesthesia was induced with either sodium thiopental ( $2\text{--}3 \text{ mg} \cdot \text{kg}^{-1}$ ) or etomidate ( $0.2 \text{ mg} \cdot \text{kg}^{-1}$ ) plus fentanyl citrate ( $3\text{--}5 \mu\text{g} \cdot \text{kg}^{-1}$ ). To maintain anaesthesia, enflurane ( $0.3\text{--}1.0$  volume % in an air/oxygen mixture) and supplementary doses of fentanyl were used. Pancuronium bromide ( $0.1 \text{ mg} \cdot \text{kg}^{-1}$ ) was given as muscle relaxant. Ventilation was controlled to maintain the partial pressure of carbon dioxide between  $4.5$  and  $5.0$  kPa and the inspiratory flow of oxygen was set at  $0.6$ . Right- ( $7 \pm 4$  mmHg) and left-atrial pressures ( $8 \pm 5$  mmHg) were recorded, and the values closest to the time of excision of right-atrial tissue (maximum 20 min) were taken for analysis. In 23 patients, coronary-bypass surgery was performed, in 10 patients replacement of one or two valves (aortic valves in six; four for aortic stenosis, three for aortic regurgitation: mitral valves in four; three for mitral regurgitation and one for mitral stenosis) and two patients received bypass grafts and aortic valve replacements (both for aortic stenosis, with additional regurgitation in one). The clinical and biochemical evaluations were performed separately, and the investigators were blinded for putative dependent variables. The codes were not broken until the two sets of data were ready to be collated.

### Membrane preparation

Intraoperatively excised right-atrial tissue was immediately rinsed in ice-cold cardioplegic solution, frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$ . Atrial membranes were prepared using a slight modification of a published procedure<sup>[36]</sup>. In brief, the tissue was thawed on ice and a homogenate (20% w/v) was prepared in  $0.25 \text{ M}$  sucrose,  $25 \text{ mM}$  Tris pH  $7.5$ , with a Polytron ( $2 \times 30 \text{ s}$ ) at setting 6. The homogenate was sedimented at  $10\,000 \text{ g}$  for 20 min, the pellet discarded and the supernatant centrifuged at  $100\,000 \text{ g}$  for 30 min. The pellet was resuspended in  $5 \text{ ml}$  of  $0.6 \text{ M}$  KCl and  $30 \text{ mM}$  histidine at pH  $7.0$  (per g of tissue) and resedimented at  $100\,000 \text{ g}$  for 30 min. The pellets obtained from the final centrifugations were washed three times and resuspended in  $25 \text{ mM}$  Tris pH  $7.5$  and  $10 \text{ mM}$   $\text{MgCl}_2$  using a tight-fitting Teflon-pestle homogenizer. All steps above were carried out at  $0\text{--}4^\circ\text{C}$ . The membrane preparations

were frozen in liquid nitrogen and held in aliquots at  $-80^\circ\text{C}$  until used. There was no apparent loss of AII-binding activity with time. Protein was assayed by the method of Bradford<sup>[40]</sup> using bovine serum albumin from Sigma as standard.

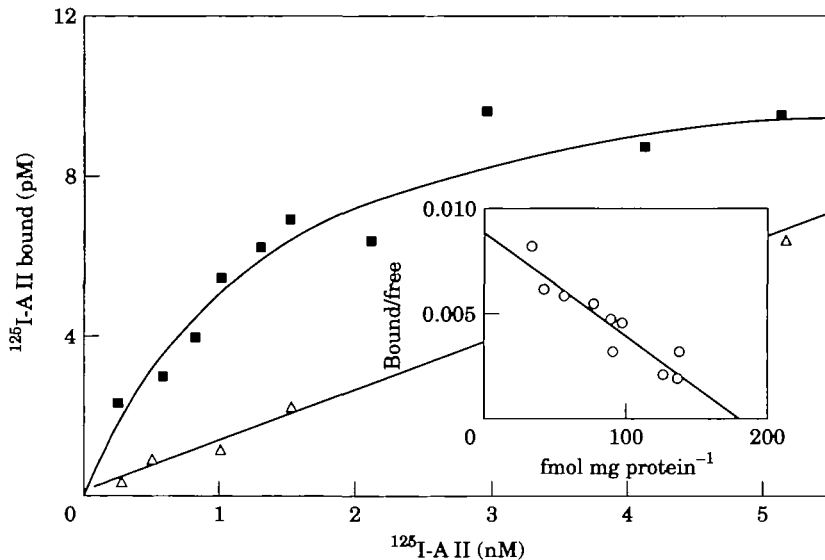
### Binding assay

Equilibrium-binding studies were performed at  $25^\circ\text{C}$  for 50 min in  $0.1 \text{ ml}$  Tris pH  $7.5$  containing  $\text{MgCl}_2$  ( $10 \text{ mM}$ ), bovine serum albumin ( $0.2\%$ ), membranes ( $4.9 \pm 2.8 \mu\text{g}$  protein; mean  $\pm$  SD), [ $^{125}\text{I}$ ]-AII ( $2200 \text{ Ci/mmol}$  purchased from Anawa, Wangen, Switzerland; Concentrations as stated in the figures), and the peptidase inhibitors antipain, phosphoramidon, leupeptin, pepstatin, bestatin, amastatin, each at  $1 \mu\text{g} \cdot \text{ml}^{-1}$  (all obtained from Novabiochem, L aufelfingen, Switzerland), and bacitracin at  $100 \mu\text{g} \cdot \text{ml}^{-1}$ . Control experiments showed that under these conditions equilibrium was reached and the binding was linearly related to the amount of protein used. Bound and free ligand were separated by addition of  $4 \text{ ml}$  of ice-cold Tris pH  $7.5$  and subsequent rapid filtration through Whatman GF/F filters presoaked in  $0.1\%$  bovine serum albumin. This was followed by three additional washes with  $4 \text{ ml}$  of the same buffer, and radioactivity trapped on the filter was then measured in a gamma-counter (Pharmacia LKB, Uppsala, Sweden) at  $80\%$  efficiency. Non-specific binding was determined in the presence of  $10 \mu\text{M}$  unlabelled AII (human sequence; obtained from Bachem, Bubendorf, Switzerland). Typically, values of total bound AII were about  $2000 \text{ cpm}$  and non-specific binding amounted to about  $500 \text{ cpm}$ .

The proportions of the receptor subtypes were determined in inhibition experiments performed in triplicate, using selective concentrations of CGP 42 112 A ( $0.3 \mu\text{M}$ ) and Losartan ( $3 \mu\text{M}$ ), respectively (Losartan and CGP 42 112 A were synthesized by Ciba-Geigy). The integrity of the radioligand before and after incubation was determined by reversed-phase thin-layer chromatography, as described elsewhere<sup>[36]</sup>. These controls showed that under the incubation conditions used as much as  $84 \pm 1\%$  ( $n=35$ ) of the radioligand remained intact.

### Data analysis

The binding data were analysed using the LIGAND curve-fitting program<sup>[41]</sup>. The data were obtained from experiments each performed in duplicate, and the geometric means are given when dose-response curves were averaged. The means of the percentages of  $\text{AT}_1$  and  $\text{AT}_2$  receptors calculated from the two determinations using selective concentrations of CGP 42 112 A and Losartan, respectively, were averaged. All other data were analysed with Student's *t*-test and linear regression. Results are expressed as mean  $\pm$  SD, unless otherwise stated. Differences at  $P < 0.05$  were accepted as statistically significant.

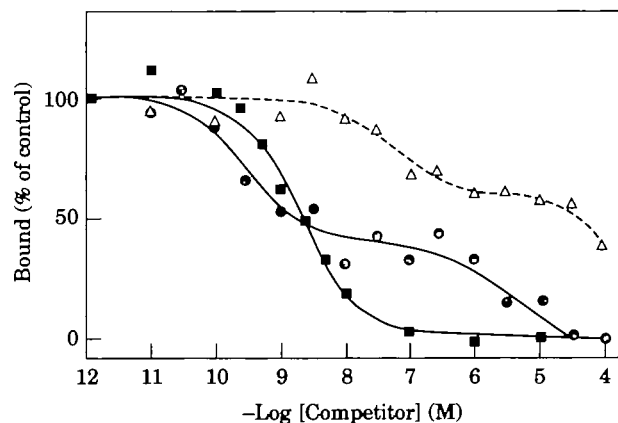


**Figure 1** Saturation binding of [ $^{125}$ I]-AII to human right-atrial membranes. Specific (■) and non-specific binding (△) as a function of increasing concentrations of [ $^{125}$ I]-AII is shown. A Scatchard analysis of the data is given in the inset. The values given are representative of three experiments. The experiment is an example showing the occurrence in human atrial membranes of AII receptors having a high affinity and density.

## Results

### Identification of AII-receptor subtypes in human atrial membranes

Saturation binding of [ $^{125}$ I]-AII to human right-atrial membranes is depicted in Fig. 1. The data demonstrate the presence of specific sites with high affinity for AII. In the 35 specimens investigated, the median receptor density was 294 fmol  $\cdot$  mg $^{-1}$  protein (range 111–2073). Half-saturation of the receptors is reached at 1.3 nM (range 0.6–2.0). To characterize the AII receptors in human atria, we performed separate competition-binding experiments ( $n=35$  for AII;  $n=2$  for each subtype-selective competitor) (Fig. 2). AII displaces the radioligand monophasically. The Hill coefficient is near unity. These data indicate the presence of one homogeneous class of high-affinity receptors. The highly selective ligands, CGP 42 112 A and Losartan, possessing an inverse affinity for AII-receptor subtypes, however, displace the radioligand in a biphasic manner, unmasking the presence of two distinct binding sites. These were further characterized in competition-binding experiments performed in the presence of CGP 42 112 A and Losartan in concentrations sufficient to block their high-affinity sites (Fig. 3). These experiments show that AII possesses the same affinity for the two receptor subtypes. However, Losartan has a 1000 times higher affinity for the AT $_1$  than for the AT $_2$  receptor and, conversely, CGP 42 112 A has a high affinity for the AT $_2$  receptor and only a very weak affinity for the AT $_1$  receptor. This demonstrates the pharmacological difference between these two entities. Furthermore, it was

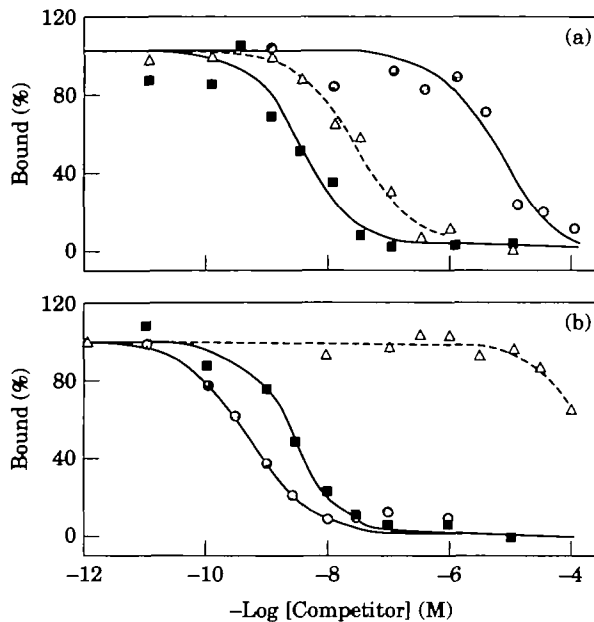


**Figure 2** Competition curves with AII (■), CGP 42 112 A (●) and Losartan (△) in human right-atrial membranes. The experiments were carried out in the presence of 0.5 nM [ $^{125}$ I]-AII. The biphasic displacement curves of the subtype-specific antagonists demonstrate the presence of AT $_1$  and AT $_2$  receptors.

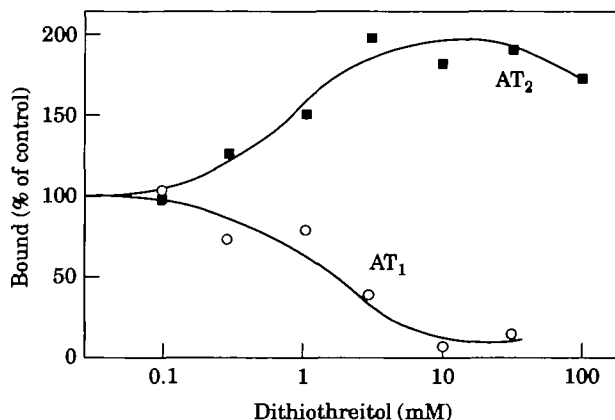
found that they are chemically distinguishable. They are, for instance, affected differently by the disulphide-reducing reagent dithiothreitol: the AT $_1$  receptor is inhibited, whereas the binding of the hormone to the AT $_2$  receptor is stimulated (Fig. 4).

### AII receptors and correlation with cardiac parameters

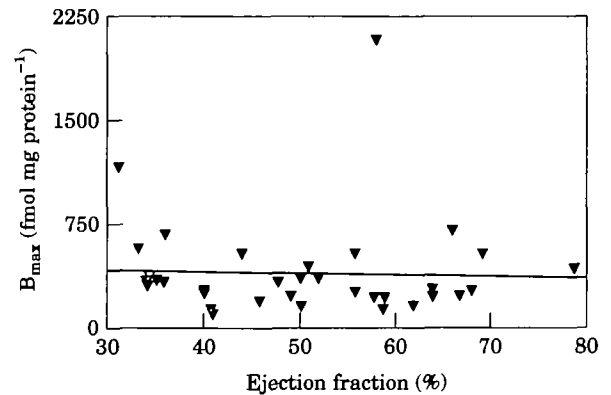
Figure 5 illustrates the lack of correlation between the density of total AII receptors and the ejection fraction.



**Figure 3** Inhibition of  $[^{125}\text{I}]\text{-AII}$  binding, by AII (■), CGP 42 112 A (●) and Losartan (△) to  $\text{AT}_1$  and  $\text{AT}_2$  receptors in human right-atrial membranes. (a) The  $\text{AT}_1$  receptor was unmasked by blocking  $\text{AT}_2$  receptors with CGP 42 112 A ( $0.3 \mu\text{M}$ ). (b) The  $\text{AT}_2$  receptor was unmasked by blockade of  $\text{AT}_1$  receptors using Losartan ( $3 \mu\text{M}$ ). The experiments were carried out in the presence of  $0.5 \text{ nM}$   $[^{125}\text{I}]\text{-AII}$ , and the data given are representative of two experiments each. The two sets of experiments show the pharmacological profile of the  $\text{AT}_1$  receptor (binding affinity order:  $\text{AII} \gg \text{Losartan} \gg \text{CGP 42 112 A}$ ) and the  $\text{AT}_2$  receptor ( $\text{CGP 42 112 A} \gg \text{AII} \gg \text{Losartan}$ ).



**Figure 4** Effect of various concentrations of dithiothreitol on the binding of  $0.5 \text{ nM}$   $[^{125}\text{I}]\text{-AII}$  to subtypes of AII receptors of human atrial tissue. The experiments were carried out in the presence of  $0.3 \mu\text{M}$  CGP 42 112 A (○) and  $3 \mu\text{M}$  Losartan (■), respectively, to investigate the effect of DTT on subtypes 1 and 2 separately (see Figs 2 and 3). The data given are representative of two experiments each.

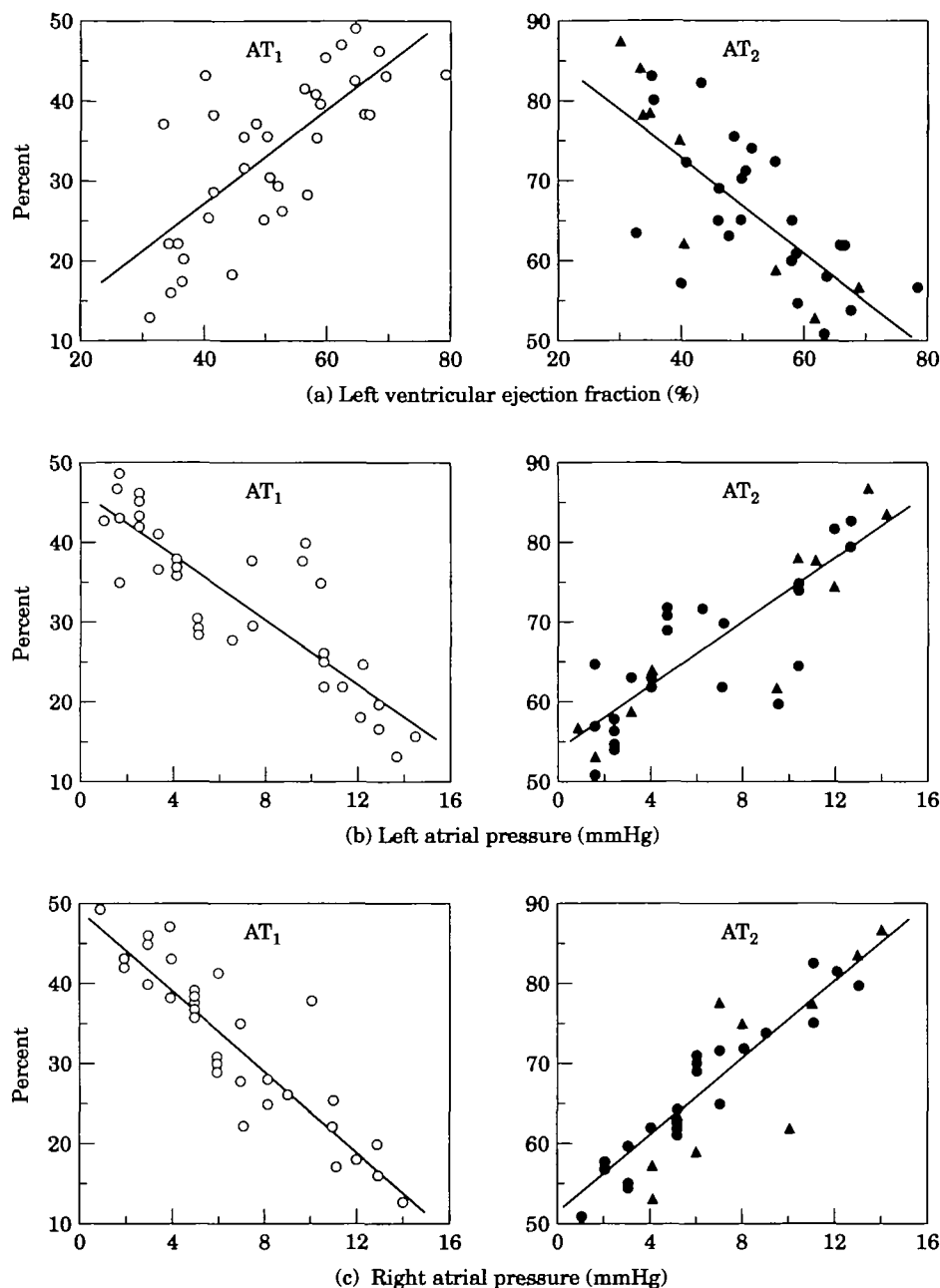


**Figure 5** Lack of relation of between density of total AII receptors and ejection fraction.

Moreover, there was no demonstrable association between alterations in left- or right-atrial pressure and changes in the density of total AII receptors, or their affinity for the hormone (data not shown).

Both AII-receptor subtypes were detected in the atria of all 35 patients. Although the mean distribution comprised  $33 \pm 10\%$   $\text{AT}_1$  receptors and  $67 \pm 10\%$  of the  $\text{AT}_2$  type, there was a fairly wide range of interindividual variation: some patients had almost identical proportions of the two subtypes, but in extreme cases the ratio of  $\text{AT}_2/\text{AT}_1$  was up to 7:1. The analysis of the data revealed a striking association between the proportions of the receptor subtypes and the various cardiac parameters measured (Fig. 6). It was found that the proportion of  $\text{AT}_1$  receptors increases significantly with the left-ventricular ejection fraction (Fig. 6(a)). There was a highly significant, direct correlation between the height of the left-atrial pressure and the proportion of  $\text{AT}_2$  receptors (Fig. 6(b)). Furthermore, the height of right-atrial pressure and the percentage of  $\text{AT}_2$  receptors were positively correlated (Fig. 6(c)). In summary, these findings indicate that the proportion of  $\text{AT}_1$  receptors in right-atrial tissue decreases, and concomitantly the proportion of  $\text{AT}_2$  receptors increases with the degree of impairment of the cardiac functions measured.

Analysis of relationships between the absolute density of the receptors was hampered by the variation in the total receptor number, mainly caused by two extreme values with densities exceeding  $1000 \text{ fmol} \cdot \text{mg}^{-1}$  of protein (see Fig. 5). Hence, with the exception of two individuals the patients had a receptor density with a variation expected in biological samples. We carefully investigated all characteristics including medication of two 'outliers' and found no explanation for this phenomenon. Re-analysis of the data after exclusion of these two values revealed significant correlations between the absolute density of  $\text{AT}_1$  receptors ( $\% \text{AT}_1 \times B_{\text{max}}$ ) and alterations in the various cardiac functions: the height of the ejection fraction correlates positively ( $r=0.44$ ,  $P<0.02$ ,  $n=32$ ) and the heights of both left- and right-atrial pressures showed an inverse correlation ( $r=-0.45$ ,  $P<0.02$ ,  $n=31$ ) and ( $r=-0.40$ ,



**Figure 6** Relationship between the proportion of receptor subtypes and cardiac parameters. The data show significant correlations between the proportions of AII receptor subtypes and ejection fraction (a), left-atrial pressure (b) as well as right-atrial pressure (c). In the panels depicting the fraction of AT<sub>2</sub> receptors, the circles indicate the values from patients who underwent coronary-bypass surgery and the triangles those from patients who underwent valvular surgery. The data indicate a decrease in the proportion of AT<sub>1</sub> receptors with increasing degree of dysfunction of all cardiac parameters measured (and a reciprocal situation for the AT<sub>2</sub> receptors) irrespective of the underlying disease state. For (a),  $r=0.740$  and  $P<0.001$ ; (b),  $r= -0.853$  and  $P<0.001$ ; (c)  $r= -0.901$  and  $P<0.001$ .

$P<0.03$ ,  $n=33$ ). This indicates that a decrease in AT<sub>1</sub> receptor numbers in the right atrium is associated both with increased right-atrial pressure and with impaired left systolic and diastolic function. The AT<sub>2</sub> receptor density showed an opposite tendency [ejection fraction:

$r= -0.20$  (ns); left-atrial pressure:  $r=0.34$ ,  $P=0.06$  (ns), right-atrial pressure:  $r=0.40$ ,  $P<0.03$ ]. This was expected, since the data on total receptor density and the parameters of cardiac function showed no correlation. However, no significant correlation between any

receptor parameters and the patients' ages or body weights was demonstrable (data not shown).

In conclusion, these data indicate that the relative proportions of AT<sub>1</sub> and AT<sub>2</sub> receptors in human atrial tissue show a good correlation with right-atrial pressure and left-ventricular functions in these patients.

## Discussion

In right-atrial tissues from 35 patients undergoing cardiac surgery, we have demonstrated the presence of a dense population of high-affinity AII receptors, comprising both AT<sub>1</sub> and AT<sub>2</sub>-receptor subtypes, and found the proportions as well as the absolute density of these two subtypes to be related to the height of right- and left-atrial pressures and the left-ventricular ejection fractions. Our findings contrast with the results of previous studies in human cardiac tissue<sup>[10,42]</sup>, in which very low densities of AII receptors ( $B_{max} < 7 \text{ fmol} \cdot \text{mg}^{-1} \text{ protein}$ ) were detected in normal subjects and patients with idiopathic or dilated cardiomyopathy: the values quoted for right-atrial tissue (the highest density recorded) were more than 40 times less than those determined in our patients. This discrepancy may have been due to methodological differences, such as dissimilarities in the purity of the membranes, or possibly to denaturation of the receptors, since the cardiac tissue had been kept for several hours in ice-cold cardioplegic solution before being frozen<sup>[10]</sup> whereas in the present study fresh, snap-frozen tissue was used. In addition, we investigated the receptors in atrial tissue, which in rabbit hearts has been shown to possess an AII receptor density about 10 times higher than that of ventricular tissue<sup>[5]</sup>. In very preliminary experiments this difference has also been found in human hearts in our laboratory (H. Rogg, unpublished observations). However, the reported absence of any clear-cut difference in the density of total AII receptors in normal and failing hearts agrees with our findings showing a lack of correlation between this parameter and the various cardiac functions.

This first study of human cardiac AII receptors was carried out prior to the initial report of the existence of AII-receptor subtypes<sup>[31]</sup> and the discovery of their coexistence in rabbit ventricular tissue<sup>[36]</sup>, and the tissues investigated were cardiac explants. In our study, we examined specimens excised during heart surgery. The possibility that the anaesthetics or the cardiac drugs administered prior to surgery may have influenced the results cannot be excluded. Although the tissue originated from patients on multiple drugs, specimens exposed to single drugs were too few in number to be analysed separately. At least no obvious effect of angiotensin-converting enzyme inhibitors (n=5 of 35 patients) on the proportions of AII receptor subtypes was detectable (data not shown).

The analysis of the 35 specimens revealed a direct relation between the proportion of AII receptor subtypes and right- and left-atrial pressures, as well as left-ventricular ejection fraction. Since these three

parameters were interrelated in the patients studied, the influence of a single abnormal parameter cannot be determined separately.

The main outcome of our study is the demonstration of the occurrence of the two AII-receptor subtypes in human atrial tissue and their close relations to various cardiac functions. The properties of the two atrial subtypes are indistinguishable from those in other tissues such as uterus, adrenal glands and vascular smooth-muscle cells<sup>[31]</sup>, as well as in rabbit ventricles<sup>[36]</sup>. The order of affinity of the AT<sub>1</sub> subtype (AII > Losartan > CGP 42 112) clearly differentiates it from the AT<sub>2</sub> subtype (CGP 42 112 A > AII > Losartan). Furthermore, the two subtypes have different susceptibilities to the disulphide-reducing reagent dithiothreitol in human atria and other tissues<sup>[31,34,36]</sup>: the AT<sub>1</sub> receptors is inhibited, whereas the binding of the hormone to the AT<sub>2</sub> receptor is stimulated by DTT. This furnishes additional evidence for the distinct nature of the two subtypes of AII receptors in human atrial tissue.

Atrial tissue was chosen for these investigations of human cardiac AII-receptor subtypes because it can be obtained fresh and because earlier reports indicated that atria may be the cardiac tissue with the highest receptor density, both in animals and in man<sup>[5,10]</sup>. The demonstrable correlations between different proportions of the two AII-receptor subtypes and right-atrial pressure, and especially left-ventricular systolic and diastolic function, afford the first indication of the possible involvement of these entities in a pathophysiological process. The individuals investigated have the characteristics of a large proportion of cardiac patients suitable for heart surgery. The receptor profile of patients with either valvular disease or coronary artery disease did not deviate from the correlations (Fig. 6). However, none of them had other heart disease such as dilated cardiomyopathy. Accordingly, our finding cannot be extrapolated to such conditions. Although at this stage it is obviously impossible to tell whether these changes are a cause or a consequence of cardiac dysfunction, it is tempting to speculate that the cardiac AT<sub>1</sub> receptor, which is known to be coupled to a G protein<sup>[43]</sup>, may be down-regulated with the degree of cardiac dysfunction, in a similar fashion to the alterations in beta-adrenergic receptors in these conditions<sup>[44]</sup>. Thus, the effects of AT<sub>1</sub> receptor, known to be mediated via phosphoinositol breakdown<sup>[34]</sup> leading to an elevation of intracellular free calcium<sup>[35]</sup>, might conceivably result in sluggish calcium transients and hence reduced contractile force in patients with altered left-ventricular systolic function as a consequence of receptor down-regulation.

An increase in angiotensin-converting enzyme activity and mRNA expression has been reported in rats with pressure-overload left-ventricular hypertrophy<sup>[45]</sup>. This indicates that in some forms of cardiac dysfunction the local renin-angiotensin system may be activated and linked to cardiac hypertrophy. Moreover, AII is thought to play a permissive or regulatory role in modulating cardiac growth and development<sup>[14-18]</sup>. The reported alteration in the proportion of the AII-receptor subtypes

associated with cardiac dysfunction in this study may have both theoretical and practical implications. The possibility cannot be excluded that these alterations may play an important role in the progression of structural changes in the myocardium, either linked to ischaemic heart disease or to chronic pressure overload. Unfortunately, the effector pathway(s) of the AT<sub>2</sub> receptors, and their function is still controversial. Therefore, it would be premature to speculate on a permissive or restrictive involvement of AT<sub>2</sub> receptors in the regulation of growth. However, the high density of this subtype found in the fetus may play a functional role in development<sup>[46,47]</sup>.

The association between both the proportion and the density of the AT<sub>1</sub> receptor subtype and cardiac dysfunction reported in this study may be of direct therapeutic relevance. The non-peptidic AT<sub>1</sub>-specific receptor antagonist Losartan and other compounds of the same class are in preclinical or clinical development. Recent initial reports of the effects of Losartan in man describe a marked elevation of circulating AII<sup>[48]</sup>. In a recent study comparing the effects of Losartan and an angiotensin-converting enzyme inhibitor in rats, it was demonstrated that the latter compound causes no appreciable change in circulating AII<sup>[49]</sup>. It is consequently not admissible to draw inferences regarding the effects of AT<sub>1</sub>-selective inhibition from the cardiac effects of angiotensin-converting enzyme inhibition. Given the presence of both the AT<sub>1</sub> and AT<sub>2</sub> receptor subtypes on human cardiac tissue, blockade of the AT<sub>1</sub> subtype by treatment with such drugs exposes the AT<sub>2</sub> subtype to elevated AII levels, with unpredictable, either desirable or undesirable, long-term cardiac effects.

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