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Angiotensin II receptor subtypes and cardiac function

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All the components of the renin-angiotensin system have been identified in the heart including the angiotensin II receptor subtypes AT_1 and AT_2.

In the normal human heart, there is a decreasing receptor density from the right atrium to the left ventricle. In right atrial membranes prepared from pathological hearts, the percentage of AT_1 receptor decreases with the severity of cardiac dysfunction whereas that of AT_2 receptor increases. Treatment of hypertrophic rats with AT_1 receptor antagonists inhibits cardiac hypertrophy and reverses the increase receptor density, indicating involvement of this Ang II receptor subtype. The role of the AT_2 receptor is still largely unknown but it may be involved in cell growth and proliferation. The cloning of both AT_1 and AT_2 receptors as well as the availability of potent and selective antagonists will help us to understand better the functional role of Angiotensin II in cardiovascular disorders.

Introduction

It was previously thought that the renin-angiotensin system (RAS) was a purely endocrine system acting entirely through the peptide hormone angiotensin II (Ang II) in the blood. During the past decade, the existence of a tissue RAS has become more firmly established[1]. Angiotensinogen renin, angiotensin converting enzyme (ACE) and Ang II have all been demonstrated in various tissues including the heart[2–4]. Use of molecular biology has proven beyond doubt the extra-renal expression of the RAS specific messenger RNAs (mRNA) and therefore the local synthesis of the members of the cascade, thereby excluding sequestration from the circulation[5,6]. Generation of the biologically active peptides can also be induced in isolated perfused hearts, indicating that it is independent of the plasma RAS[7]. Both cultured neonatal rat cardiomyocytes and ventricular fibroblasts have been shown to express angiotensinogen and renin mRNA as well as ACE and are able to synthesize Ang II[8,9].

The existence and functional significance of Ang II receptors in the heart have also been demonstrated[10–12]. When radioactive Ang II is injected into the left ventricle of adult rats, it localizes preferentially around the nucleus of cardiac muscle cells, suggesting the existence of Ang II nuclear binding sites[13]. More recently, Ang II binding sites have been observed in purified liver nuclei[14,15] and have been shown to modulate the transcription of mRNA for renin and angiotensinogen, suggesting a role of Ang II in intracellular regulation of the RAS[16].

These findings therefore clearly indicate that there is intra-cardiac synthesis of RAS components, and clinical evidence for an active RAS in the heart has been obtained from the effective use of ACE inhibitors in the treatment of congestive heart failure, heart hypertrophy, coronary atherosclerosis, myocardial infarction and post-infarction remodelling[3,6,17–19].

The purpose of this short review is to summarize the present knowledge on the Ang II receptor in the heart and to discuss the potential role of the Ang II receptor subtypes in the pathophysiology of heart diseases.

Location and characterization of Ang II receptors in the heart: animal studies

The first radioligand binding report using Ang II and 19-day old chick embryo cardiocytes was made by Moore in 1980[20]. Since then, Ang II binding sites have been identified in rabbit, guinea-pig, bovine, chicken, rat, pig, monkey and human heart[21]. In the early studies, one or two binding sites with high and low affinity were reported and guanine nucleotides modulated their affinity, suggesting a coupling with a G-protein[10]. It is only recently that two different receptor subtypes, called AT_1 and AT_2 have been described using selective ligands[22,23]. Losartan (DuP 753) binds specifically to the AT_1 receptor, whereas CGP 42112 and PD123177 have a high affinity for the AT_2 receptor. AT_1 and AT_2 are essentially distinguished by two other characteristics: firstly, sensitivity to reducing agents like dithiothreitol (DTT) which decrease binding of Ang II by the AT_1 receptor, whereas binding by the AT_2 receptor is increased[24]. Secondly, in contrast to the AT_1 subtype, GTPγS has been shown to have no effect on the AT_2 binding characteristics, thus indicating a lack of coupling of this receptor to a G protein[25].

Using the same criteria, two receptor subsets were identified in rabbit ventricular membrane[26]. The AT_1 subtype

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has a Kd of 31 nM for losartan, whereas the AT_2 subtype shows a Kd of 0·5 nM for CGP 42112. The two receptor populations have an almost identical affinity for Ang II (1·5 and 1·2 nM for the AT_1 and AT_2, respectively) and the ratio of AT_1/AT_2 is nearly equal (60:40). Chang and Lotti confirmed this ratio in the rabbit heart and also that of the monkey[27]. In an undefined strain of rat, however, these authors reported a high proportion of AT_1 receptor subtype (90%), suggesting an heterogeneity amongst species[28]. In contrast, equal proportions of AT_1 and AT_2 were seen in 'in situ' binding assay on tissue sections obtained from fetal, neonatal and adult Sprague Dawley rats[29]. Interestingly, the density of the receptor was at least twice as high in the neonatal as in the adult heart, indicating that the receptor expression is developmentally regulated. Myocytes, freshly isolated from neonatal rats through a Percoll column, express roughly 20% of the AT_2 receptor subtype. The AT_2 receptors, however, seem to disappear rapidly during primary culture (Rogg, Ramjoué and Whitebread, unpublished observation). This observation is important in the interpretation of results obtained from such culture preparations for detection and quantitation of Ang II receptors subtypes.

As in other tissues[24], the AT_1 and AT_2 receptor subtypes in the heart can be characterized by their different sensitivities to disulfide reducing agents, the binding of the AT_2 receptor being increased and that of AT_1 decreased after DTT treatment[26].

Cellular location in the heart

It is known that the heart is a heterogeneous tissue and cardiomyocytes occupy nearly 76% of the structural space, but represent only one third of all cells[30]. The remaining cells (2/3) are made up from fibroblasts, endothelial cells, vascular smooth muscle cells, macrophages, nerve cells, and specialized cells of the conduction system. Membrane binding assays cannot determine whether the AT_1 and AT_2 receptor are co-expressed in the same cells, or if the two receptor subtypes are expressed in different cells. Knowledge of where and via which receptors Ang II exerts its cardiac action could give important clues as to the function of the receptor subtypes. Early studies have shown low density binding in the myocardium, moderate to high density binding throughout the conduction system, and high density binding in the autonomic nervous system[31–33]. Iwami *et al.*[34] separated cardiomyocytes and non-myocytes from neonatal rat heart and evaluated the AT_1 mRNA levels in these fractions. Interestingly, the AT_1 receptor mRNA was primarily located in non-cardiomyocyte fractions in adult rat heart[34]. We have performed in vitro binding to localize Ang II receptors at the light microscopic level on sections of rabbit heart using film or emulsion autoradiography for detection. As illustrated in Fig. 1, a high density of Ang II receptors was detected in nerve cells next to intramural coronary arteries. Subtype-selective agents have been used in competition with radiola-

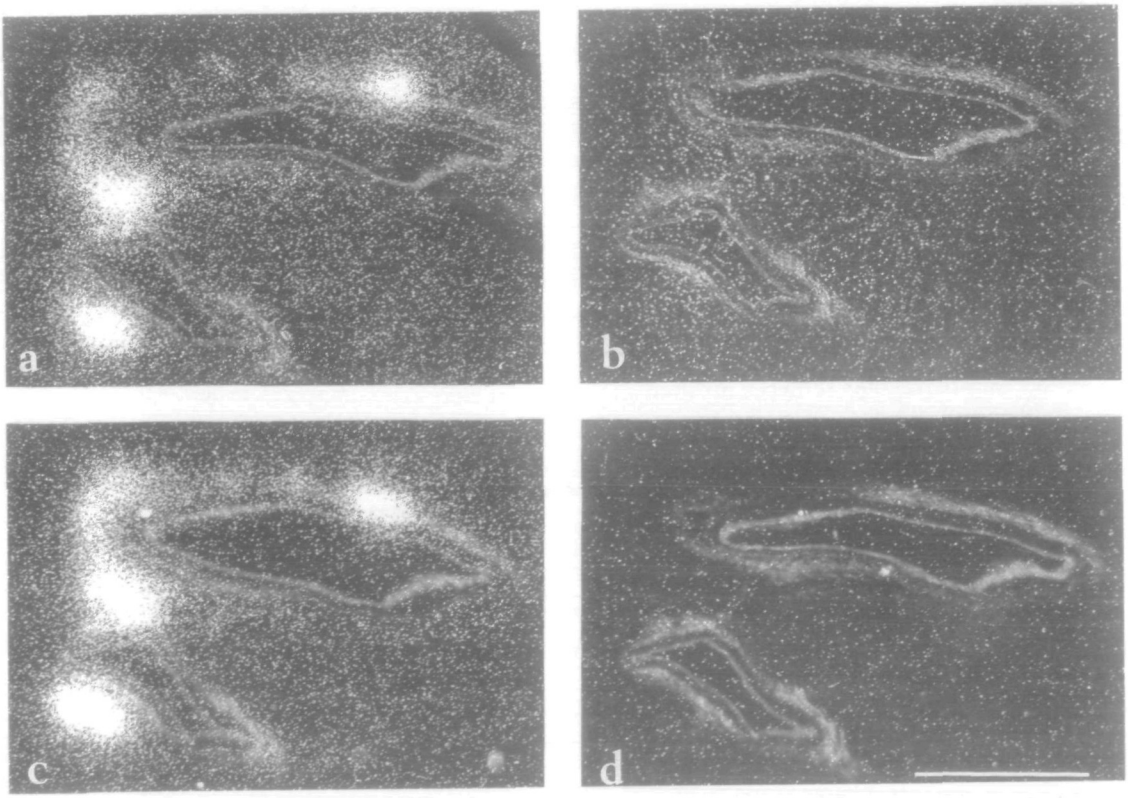

Figure 1 Dark-field micrographs showing the location of Ang II receptor subtypes in rabbit heart parasympathetic nerve cells situated next to intramural coronary arteries. Adjacent sections were incubated with 0·25 nM ^{125}I-[Sar^1, Ile^8] Ang II in the absence (a), or presence of 1 μM Ang II (b), 100 nM CGP42112 (c) or 1 μM losartan (d). Binding was visualized using the coverslip emulsion autoradiograhy method[65]. All Ang II receptors are of the AT_1 subtype. (bar = 100 μm).

belled [Sar¹,Ile⁸] Ang II to determine the receptor subtype expressed in the parasympathetic nerves. The binding was totally inhibited by the AT_1 antagonist losartan and not by the AT_2 competitor CGP42112, indicating that the nerve cells contain only the AT_1 receptor subtype. Subtyping has also been performed in the conduction system of the rat heart. Saavedra *et al.*[35] detected only the AT_1 receptor subtype in both sinoatrial (SA) and atrioventricular (AV) nodes, whereas Sechi *et al.* found the same ratio of AT_1 to AT_2 in cardiac tissue and in the myocardium of the rat with a greater density of the receptor in AV rather than in SA nodes[29].

Ang II receptors in the human heart

Binding studies have also been performed in human cardiac tissue and the presence of both the receptor subtypes observed in rat and rabbit heart has been confirmed[36].

Atria, right and left ventricles from five normal people not eligible as heart donors and right atrial tissues from 35 patients undergoing heart surgery, were investigated. In normal heart tissue, the density of the receptor (mean SEM) decreased from the right atrium (323 ± 35 fmol/mg protein; n = 5) to the left ventricle (118 ± 30; n ± 5). The amount of Ang II receptor measured in this study is significantly higher than that which was reported earlier for human heart by Urata *et al.*[32]. The difference may be due to the tissue collection procedure and the preparation of the membrane. The two different subtypes can be distinguished, the AT_1 receptor accounting for 60% in both chambers. In right atrial membranes from pathological hearts, Ang II receptors (mean ± SEM; n = 35) were present in high density (406 ± 60 fmol/mg protein) and affinity (1·4 ± 0·18 nM). The two different subtypes (Fig. 2) were also detected with a relative abundance of AT_1 receptor, of 33 ± 2% (mean ± SEM; n = 35). These results indicate a considerable change in the AT_1/AT_2 ratio between normal and diseased hearts[37].

The Ang II receptors and cardiac function

There is no doubt as to the implications of Ang II in post-infarction remodelling, in cardiac hypertrophy and in heart failure. However, the Ang II receptor subtypes involved remains to be established.

The potential relationship of the Ang II receptor subtypes to parameters of cardiac function was investigated in the right atria of 35 patients undergoing coronary bypass surgery and/or valve replacement. The left ventricular ejection fraction was measured before, and right and left atrial pressure were recorded during surgery. There was no correlation between any of the measured cardiac functions and total Ang II receptor density or affinity. However, the percentage of AT_1 receptors was significantly higher in the atria of patients with normal right atrial pressure ($r = 0·901$; $P < 0·001$) and left ventricular ejection fraction ($r = 0·74$; $P < 0·001$) while the percentage of the AT_2 receptor was positively correlated with the levels of left atrial pressure ($r = 0·853$; $P < 0·001$). Hence, the ratio of AT_1 and AT_2 receptors in atria shows a good correlation with right atrial and left ventricular function[38]. Also, with

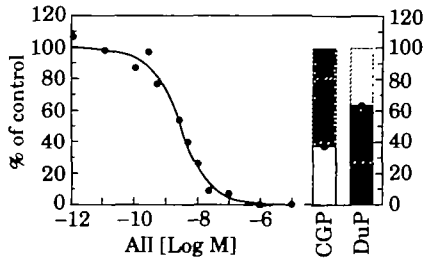

Figure 2 Inhibition of ^{125}I-Ang II binding to membranes of human atria and quantitation of the receptor subtypes. The radioligand and varying amounts of unlabelled Ang II or subtype selective ligands were incubated with the membrane fraction (5 μg of protein), and the receptor binding studies were performed and quantitated as described[26]. The solid circles represent the values obtained from the tissue of one patient determined in duplicate. The total receptor density was 405 ± 60 $fmol^{-1}mg$ protein (mean ± SEM; n = 35) and the Kd value for Ang II was 1·4 nM. The proportions of Ang II receptor subtypes were determined in competition binding experiments by their sensitivity to subtype selective concentrations of losartan (formerly named DuP 753; selective for the AT_1 subtype) and CGP 42112 (selective for AT_2). The CGP column represents the data obtained in the presence of CGP 42112 (0·3 μM). The white area, the resistant section, represents the AT_1 receptors and the hatched area the sensitive section represents the AT_2 receptors. DuP shows the inverse experiment using losartan (3 μm) where the resistant section represents the AT_2 receptors (hatched area) and the sensitive section the AT_1 receptors (white area). The mean of these two cross-experiments was used to calculate the AT_1/AT_2 ratio (33 ± 2/66 ± 2; mean SEM; n = 35). In the patient represented in this Fig. the ratio was 37/63.

respect to the absolute density of AT_1 receptors, a significant difference was found in atrial tissue from patients with normal vs pathological left ventricular function (Fig. 3). Similarly, the relative abundance (mean ± SEM) of AT_1 receptor was lower in left ventricles of heart from cardiac transplant recipients (40 ± 4; n = 9) compared with normal hearts which were not used for transplantation (60 ± 4; n = 5), suggesting a selective down-regulation of the AT_1 receptor in these patients. From these data the proposal

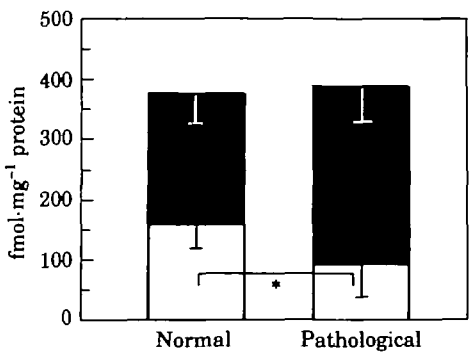

Figure 3 The proportion of AT_1(□) and AT_2(■) receptors in atrial membranes of patients with normal ejection fraction (EF > 60%; n = 8) and pathological left ventricular function (EF < 49%; n = 16). The asterix indicates a significant difference (P < 0·05) in the AT_1 receptor density ($fmol^{-1}$.mg protein).

can be put forward that the more severe the heart failure, the lower the AT_1 and the higher the AT_2 receptor proportion or density[38]. The same observation has been made in rats hearts after aortic banding in which cardiac AT_1 receptors are down-regulated in pressure-overloaded left ventricular hypertrophy[39].

Our working hypothesis described above could be challenged in the light of some studies reported in the literature. For example, in contrast to previous observations in human and rat heart, an up-regulation of both AT_1 and AT_2 receptor expression was reported in rat cardiac hypertrophy[40,41]. A threefold increase in ventricular AT_{1A} mRNA levels, an isoform of the AT_1 receptor expressed in vascular tissues[42], and 1·7-fold rise in Ang II receptor densities were observed in spontaneously (SHR) and renovascular hypertensive (WKY) rats[40,41]. In these studies, the proportion of AT_1 and AT_2 receptors measured with selective ligands was nearly equal and remained unchanged during the progression of the hypertrophy. Regression completely reversed the increased levels of mRNA and receptor density to the control levels. We cannot yet explain the discrepancy between human and these animal studies but it may be related to the experimental models.

An increased density of the Ang II receptors was also observed in isolated myocytes obtained from rat hearts with myocardial infarction after coronary artery ligation[43]. Only the AT_1 receptor subtype was detected, but as has been previously stated, the AT_2 subtype rapidly disappears during the primary culture (Rogg *et al.* unpublished data) and so the effect of the culture conditions must be taken into account.

The role of the Ang II receptor in cardiac pathophysiology

Many reports suggest that the AT_1 receptor subtype plays a major role in cardiac pathophysiology. The Ang II receptor antagonist L 158809 administered to rats in drinking water for 7 days after coronary ligation reduced left ventricular systolic and end diastolic pressure as well as the length and diameter of left ventricular myocytes[43]. In the same model, early treatment with subcutaneous losartan (15 $mg.kg^{-1}.day^{-1}$) only slightly reduced DNA synthesis but, in contrast to captopril, did not affect cardiac output[44]. This suggests that the effects of captopril may not be entirely dependent on AT_1 receptor-mediated mechanisms. Losartan administered to 3-day-old piglets decreased the left ventricle/body weight ratio and RNA/DNA ratio in the left ventricle, indicating the involvement of theAT_1 receptor in the growth of the heart after birth[45,46]. Losartan also inhibits the Ang II-induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts[47,48]. The implication of the AT_1 receptor in reactive growth processes after myocardial infarction has been documented[49]. For example, losartan blocks the induction by Ang II of immediate–early genes (*c-fos, c-myc, c-jun*), late fotal genes (α-actin, ANP receptor genes) and growth factor genes TGFβ)[50]. Finally, the positive inotropic response to Ang II in isolated papillary muscles from rabbit heart[51] or right and left atria from guinea-pigs[52] were competitively antagonized by losartan. There was no apparent functional consequence of Ang II interaction with the AT_2 receptor. Therefore, most, if not all, of the beneficial effects observed with ACE inhibitors on haemodynamic parameters and cardiac hypertrophy will probably be seen after treatment with AT_1 receptor antagonists.

The involvement of the AT_2 receptor in cardiac pathophysiology remains largely ill defined at the moment. However, Daemen *et al.* using the model of coronary ligation in the rat have recently described an effect of AT_2 receptor blockade[53]. After infusion of PD 123319, a specific AT_2 antagonist, for 2 weeks by subcutaneous implanted osmotic minipumps at a dose of $3mg.kg^{-1}.day^{-1}$, the cumulative BrdU labelled fraction in the non-infarcted septum was significantly decreased by 60%. This result suggests that the AT_2 receptor subtype is involved in the regulation of DNA synthesis in interstitial cells. The cell-type involved, e.g. fibroblast or endothelial cells, is currently under investigation.

Conclusion

The importance of the RAS in cardiovascular disease, both experimentally and clinically, has been well illustrated by the beneficial effect observed with ACE inhibitors on inhibiting the development of atherosclerosis, cardiac hypertrophy and remodelling. The Ang II AT_1 receptor antagonists discussed here represent a new class of drugs which appear to have the same indications as the ACE inhibitor but possibly a better tolerability profile[54]. Their efficacy in human subjects has been proven in hypertension but has still to be demonstrated for other indications.

The existence of two Ang II receptor subtypes in the heart has been demonstrated. As reported previously, blockade of the AT_1 receptor alone, in animal and in man, is accompanied by a compensatory increase of circulating Ang II[55–58]. This may result in Ang II exerting some hitherto unknown, possibly unwanted effects via the unblocked non-AT_1 receptors. The functional importance and clinical relevance of recently identified receptors other than AT_1 and AT_2, such as AT_3 and AT_4[59,60] is not yet known and therefore requires further investigation.

There are some conflicting data in the literature concerning the implication of the AT_2 receptor in cardiovascular disease. This leads one to question the validity of the various animal and in vitro models. It appears, however, that there is a 'balance' between the number and distribution of AT_1 and AT_2 receptors in the human heart suggesting a role for AT_2 in the pathophysiology of cardiac dysfunction[61].

Recent advances in the treatment of cardiovascular disorders have paralleled our increase in knowledge about the RAS. With the cloning of both AT_1 and AT_2 receptors[62–64] and the availability of potent and selective AT_1 and AT_2 blockers, tools are now available to understand better the biology of Ang II and most probably to open new therapeutic perspectives.

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