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The angiotensin AT_1 receptor antagonist irbesartan has near-peptide affinity and potently blocks receptor signaling

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Abstract

The angiotensin II type 1 (AT_1) receptor plays a pivotal role in the regulation of blood pressure and electrolyte balance, and is involved in the control of specific ingestive behaviours. Irbesartan (SR 47436/BMS 186295) is a recently developed angiotensin AT_1 receptor antagonist, chemically described as 2-butyl-3-([2′-{1*H*-tetrazol-5-yl}biphenyl-4-yl]methyl)-1,3-diazaspiro (4,4)non-1-en-4-one. Irbesartan displays higher affinity for its target receptor than other similar antagonists. In radioligand binding assays performed on membranes from WB-Fischer 344 (WB) rat liver epithelial cells, irbesartan was able to displace [^{125}I]angiotensin II with a K_i of 4.05 nM as compared to losartan (DuP 753) and tasosartan (WAY 126756), which had K_i values of 25.2 nM and 46.6 nM, respectively. Similarly, in functional assays, irbesartan exhibited the highest functional potency to block angiotensin II-induced inositol trisphosphate (IP_3) turnover. The improved affinity of irbesartan for the angiotensin AT_1 receptor does not coincide with a concomitant increase in affinity for the angiotensin AT_2 receptor, as irbesartan and losartan exhibited the same low potency to displace [^{125}I]angiotensin II in radioligand binding assays performed on membranes from PC-12w cells. In binding assays performed on peripheral tissues in rat, irbesartan bound to the angiotensin AT_1 receptor expressed in liver, adrenal, kidney and pituitary with an overall affinity closely approaching that of the high affinity peptidic antagonist [Sar^1, Ile^8]angiotensin II. Due to the higher affinity of irbesartan over other similar antagonists for the angiotensin AT_1 receptor in many tissues and its greater potency to block receptor activation, irbesartan may be quite useful in the study of the angiotensin AT_1 receptor and its role in controlling ingestive behaviours and, furthermore, shows great potential to improve the treatment of hypertension and other cardiovascular disease states.

Keywords: Angiotensin II; Angiotensin AT_1 receptor; Inositol trisphosphate; Irbesartan; Peripheral tissue

1. Introduction

The octapeptide hormone angiotensin II is a potent regulator of electrolyte balance and blood pressure, as well as one of the most potent dipsogens in mammals, birds and reptile species (Fitzsimons, 1998) Angiotensin II interacts directly with membrane-bound receptors expressed within the various target tissues throughout the body and central nervous system (Steckelings et al., 1992; Bernstein and Berk, 1993). By binding to and stimulating these receptors, angiotensin II produces its pressor effects through multiple physiological mechanisms — by causing constriction of vascular smooth muscle (Griendling et al., 1997), by its chronotropic (Nakashima et al., 1982) and inotropic (Kobayashi et al., 1978) actions on the heart, and by promoting aldosterone secretion from the adrenal cortex and fluid reabsorbtion from the kidneys (Vallotton, 1987).

Angiotensin II is the active component of the renin–angiotensin system, and is formed through a cascade of enzymatic reactions leading to the formation of angiotensin II. More specifically, circulating angiotensinogen is cleaved by the enzyme renin to produce the inactive precursor decapeptide angiotensin I, which is in turn cleaved by the angiotensin-converting enzyme to form the active octapeptide angiotensin II. The targeting of the angiotensin-converting enzyme as a point of therapeutic intervention led to the development of anti-hypertensive drugs which block the formation of angiotensin II by

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inhibiting angiotensin-converting enzyme activity (Lees et al., 1992), an approach that was more therapeutically specific as compared to the usage of β-adrenoceptor antagonists, diuretics and other earlier antihypertensives. Angiotensin-converting enzyme inhibitors, such as captopril and enalapril, have been shown to be effective in the lowering of blood pressure and thus have been useful in the treatment and management of congestive heart failure (Cody, 1986), hypertension (Williams, 1988) and diabetic neuropathy (Unger and Gohlke, 1994). However, angiotensin-converting enzyme lacks specificity for its substrate, angiotensin I, and also cleaves other endogenous peptides, among them substance P (Skidgel and Erdos, 1987) and bradykinin (Timmermans et al., 1995). The side effects which are often associated with angiotensin-converting enzyme inhibitors, e.g., dry cough, agusia, angioedema (Gavras and Gavras, 1988), might be the result of the actions of these drugs outside of the renin–angiotensin system. Thus, efforts in anti-hypertensive drug development have also focused on the angiotensin II receptors themselves as more specific therapeutic targets (Wong and Timmermans, 1996).

The first angiotensin II receptor antagonists, like [Sar^1, Ala^8]angiotensin II and [Sar^1, Ile^8]angiotensin II, were peptide analogs of angiotensin II. Owing to their similarities to angiotensin II, these ligands possessed partial agonist properties (Anderson et al., 1977) and, due to their peptidic nature, also exhibited relatively poor oral bioavailability and short plasma half-lives (Pals et al., 1971). The development of losartan (DuP 753), one of the first non-peptide angiotensin II receptor antagonists, made available a long-acting and orally active antagonist that was devoid of agonistic activity (Chiu et al., 1991). It also allowed for the first clear pharmacological distinction between angiotensin II receptor subtypes: the angiotensin AT_1 receptor (losartan-sensitive) and the angiotensin AT_2 receptor (losartan-insensitive) (Chiu et al., 1989). While subtype selective ligands for the angiotensin AT_2 subtype have also been developed (Blankley et al., 1991; Whitebread et al., 1991), the angiotensin AT_1 receptor recognized by losartan mediates practically all the known physiological responses to angiotensin II, including its pressor effect (Timmermans et al., 1993).

Irbesartan (SR 47436/BMS 186295) is a more recently developed antagonist, 2-butyl-3-([2′-{1*H*-tetrazol-5-yl}biphenyl-4-yl]methyl)-1,3-diazaspiro (4,4) non-1-en-4-one, that has high affinity for the angiotensin AT_1 receptor (Cazaubon et al., 1993). Previous studies on the effects of irbesartan have characterized its ability to block the angiotensin II-induced pressor response in a number of animal systems (Cazaubon et al., 1993; Roccon et al., 1994; Christophe et al., 1995).These studies have clearly demonstrated that irbesartan displays a higher potency towards blocking the actions of angiotensin II than does losartan. In the present study, we have evaluated the potency of irbesartan to block angiotensin II-induced inositol trisphosphate (IP_3) hydrolysis, a cellular event in the signal transduction pathway of the angiotensin AT_1 receptor (Peach and Dostal, 1990) which is more proximal to receptor activation, and therefore perhaps a more direct and simple measurement of potency than the pressor response measured in previous studies. Using this technique, in combination with radioligand binding assays, we have compared the potency of irbesartan to the structurally related, clinically tested antagonists losartan and tasosartan (WAY 126756), as well as to the non-specific yet highly potent antagonist [Sar^1, Ile^8]angiotensin II. In addition, we report on the affinity with which irbesartan binds to a variety of tissues through which angiotensin II acts to induce its physiological effect on blood pressure and body fluid osmolarity.

2. Materials and methods

2.1. Chemicals

Tissue culture medium and supplements were obtained from Life Technologies (Gaithersburgh, MD), except for Richter's Improved Modified Eagle's Medium (MEM), which was obtained from Irvine Scientific (Santa Ana, CA). Tissue culture flasks and instruments were purchased from Fisher Scientific (Pittsburgh, PA). Bicinchoninic acid (BCA) protein quantification kit was obtained from Pierce (Rockford, IL). [^{3}H]inositol was obtained from American Radiolabeled Chemicals (St. Louis, MO) and [^{125}I]angiotensin II was obtained from NEN/DuPont (Boston, MA). Irbesartan (SR 47436/BMS 186295) was the generous gift from Dr. Sal Lucania (Bristol Myers Squibb); losartan (DuP 753) was a gift from Dr. Ronald Smith (DuPont-Merck); and tasosartan (WAY 126756) was a gift from Dr. Dale Hartupee (Wyeth-Aeyrst). PD 123319 was a gift from Dr. David Dudley (Parke-Davis) and CGP 42112A was purchased from RBI (Natick, MA). Other peptide ligands were obtained from Peninsula Labs (Belmont, CA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted.

2.2. Cell culture

Cultured cells were grown in polystyrene tissue culture dishes in a humidified atmosphere of 5% CO_2 and 95% O_2 at 37°C. WB-Fischer 344 (WB) rat liver epithelial cells were grown in medium consisting of Richter's Improved MEM supplemented with 5% fetal calf serum, 20 mM HEPES, 25 mM sodium bicarbonate, 50 units/ml penicillin and 50 μg/ml streptomycin. PC-12w (rat pheochromocytoma) cells were grown in Dulbecco's MEM supplemented with 10% heat-inactivated horse serum, 5% fetal bovine serum, 50 units/ml penicillin and 50 μg/ml streptomycin.

2.3. Animals and housing

Adult, male Sprague–Dawley rats were purchased from Harlan Industries Labs (Indianapolis, IN). They were individually housed in stainless steel cages and in a temperature-controlled room (20°C–24°C). They were maintained on a 12/12, light/dark cycle. Animals received ad libitum access to tap water, 0.5 M NaCl solution and Purina Rodent Chow. Animals weighed 300–400 g at the time of sacrifice and tissue collection.

2.4. Inositol trisphosphate assay

Cultured WB cells were grown in 100 mm dishes and loaded with [^{3}H]inositol (6 μCi/ml D-MEM) for 18 h prior to assay. Cells were then stimulated with 20 nM angiotensin II for 30 s in the presence or absence of antagonist, rinsed once with ice cold phosphate-buffered saline and then rapidly lysed in 1 ml of 10% trichloroacetic acid. Insoluble materials were pelleted at 16,000 × *g*. The pellets were solubilized in 400 μl of 1% sodium dodecylsulfate in 0.1 M NaOH for protein quantification. The supernatant from each lysate was extracted five times with 2 volumes of water-saturated ether. Following the final extraction, the aqueous layers were neutralized by addition of sodium bicarbonate and EDTA to final concentrations of 6 mM and 15 mM, respectively. The aqueous supernatants were added to 1-ml AG 1-X8 anion exchange resin columns (Bio Rad Labs, Hercules, CA) and inositol phosphates were separated by stepwise elution with increasing concentrations (0 to 1 M) ammonium formate in 0.1 M formic acid (Berridge et al., 1982). The amount of IP_3 eluted from each column was quantitated by liquid scintillation counting in Tru-Count scintillation cocktail (IN/US Systems, Tampa, FL). Data were analyzed using GraphPad Prism software (GraphPad Software, San Diego, CA, USA) and statistical analysis was performed using SuperANOVA software (Abacus Concepts, Berkeley, CA, USA).

2.5. Radioligand binding assay

WB and PC-12w cells were harvested by scraping into phosphate-buffered saline and pelleting the cells by centrifugation at 23,000 × *g* for 10 min. The cells were then resuspended in assay buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$, 0.3 TIU/ml aprotinin and 100 μg/ml 1,10-phenanthroline) and lysed by polytron homogenization. Following a second centrifugation at 40,000 × *g* for 20 min to pellet the cell membranes, the final pellet was re-suspended in assay buffer and protein content was determined spectrophotometrically using the BCA protein assay. Peripheral tissues gathered from adult male rats were homogenized separately in assay buffer (above) supplemented with 0.32 M sucrose, 1 mM EDTA, 5 mM EGTA and 1 mM PMSF. Cell membranes were first isolated in the supernatant, away from unbroken cells, nuclei and mitochondria which were pelleted by centrifugation at 3000 × *g* for 10 min. After discarding the pellets, the cell membranes were then pelleted from the supernatant by centrifugation at 38,000 × *g* for 20 min. The final tissue membrane pellet was re-suspended in unsupplemented assay buffer and protein content was determined. The binding assays were initiated by addition of the desired amount of membrane protein (80–100 μg for WB membranes; 5–10 μg for PC-12w membranes; 50 μg for liver and pituitary; and 100 μg for kidney and adrenal) to assay mixture containing various concentrations of [^{125}I]angiotensin II and unlabelled competitors. Non-specific binding was defined as the amount of radioligand binding remaining in the presence of 1 μM [Sar^1, Ile^8]angiotensin II. All binding assays done on adrenal tissue were done in the presence of 1 μM PD 123319 (to occupy angiotensin AT_2 receptors coexpressed in adrenal

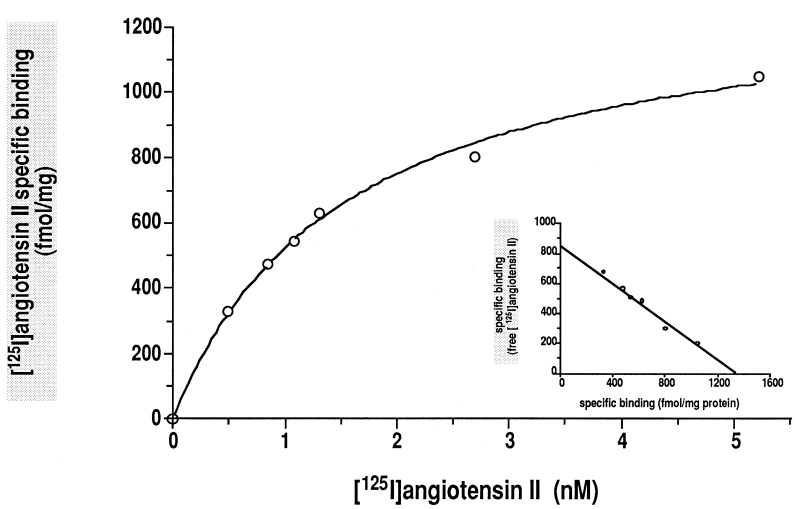

Fig. 1. Saturation binding of [^{125}I]angiotensin II to WB cell membranes. Radioligand binding was determined as described using [^{125}I]angiotensin II in concentrations ranging from 0.5 to 5 nM. Non-specific binding was defined in the presence of 1 μM [Sar^1, Ile^8]angiotensin II. Values reported represent the mean ± standard error of three independent determinations. A representative saturation isotherm and the corresponding Scatchard transformation (inset) are shown.

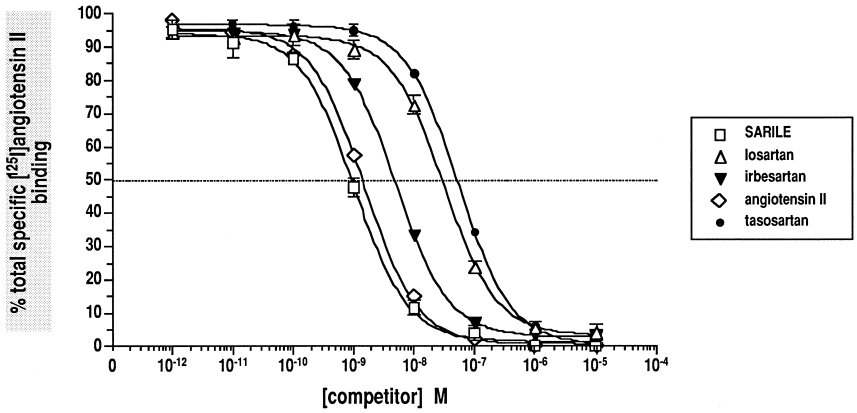

Fig. 2. Competition binding of irbesartan to WB cell membranes. Radioligand binding was performed as described using [^{125}I]angiotensin II at a concentration of approximately 0.6 nM and irbesartan or other receptor ligands at the concentrations depicted. Non-specific binding was defined in the presence of 1 μM [Sar^1, Ile^8]angiotensin II. Values reported represent the mean ± standard error of 5 to 7 independent experiments.

tissue). The binding assays proceeded for 60 min and were terminated by rapid dilution and filtration onto Whatman GF/B filters using a Brandell harvester. Tissue bound radioligand was quantitated by gamma counting of the filters. Ligand binding data were analyzed using GraphPad Prism software (GraphPad Software).

3. Results

3.1. Relative affinity of irbesartan for the angiotensin AT$_1$ receptor

The affinity of irbesartan for the angiotensin AT_1 receptor was tested and compared to that of a number of other angiotensin II receptor ligands. Radioligand binding assays were performed using [^{125}I]angiotensin II and crude membranes from WB cells, which endogenously express the angiotensin AT_1 receptor but are devoid of angiotensin AT_2 receptor binding. Saturation isotherms measuring the affinity of the angiotensin AT_1 receptor for [^{125}I]angiotensin II revealed a K_D of 1.50 ± 0.17 nM and a B_{max} of 1.10 ± 0.16 pmol [^{125}I]angiotensin II bound per mg total membrane protein (Fig. 1). Competition binding assays using irbesartan or a variety of other angiotensin AT_1 receptor ligands to displace [^{125}I]angiotensin II from the angiotensin AT_1 receptor revealed that irbesartan possessed the highest affinity for the angiotensin AT_1 receptor out of all the angiotensin AT_1-selective receptor ligands tested (Fig. 2). Irbesartan demonstrated significantly greater affinity ($K_i = 4.05 \pm 0.48$ nM) at the angiotensin AT_1 receptor than either of the angiotensin AT_1-selective antagonists losartan ($K_i = 25.2 \pm 4.6$ nM) or tasosartan ($K_i = 46.6 \pm 3.6$ nM). The only ligands with greater affinity

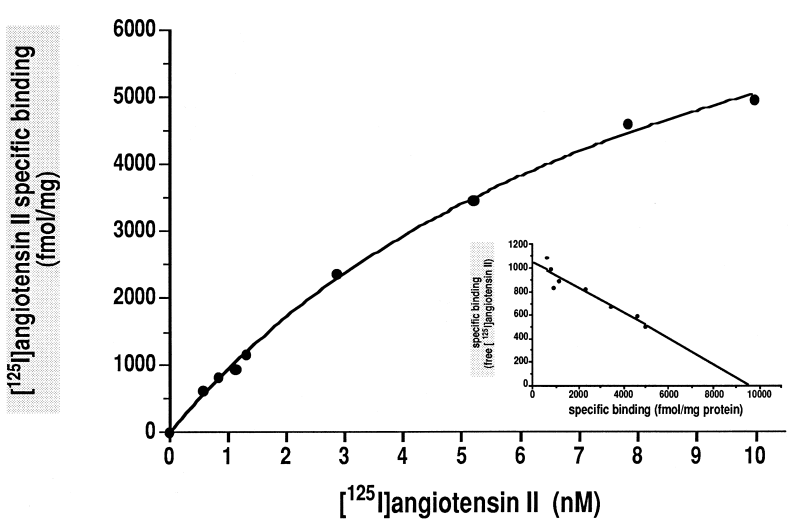

Fig. 3. Saturation binding of [^{125}I]angiotensin II to PC-12w cell membranes. Radioligand binding was determined as described using [^{125}I]angiotensin II in concentrations ranging from 0.5 to 10 nM. Non-specific binding was defined in the presence of 1 μM [Sar^1, Ile^8]angiotensin II. Values reported represent the mean ± standard error of three independent determinations. Shown are a representative saturation isotherm and the corresponding Scatchard transformation (inset).

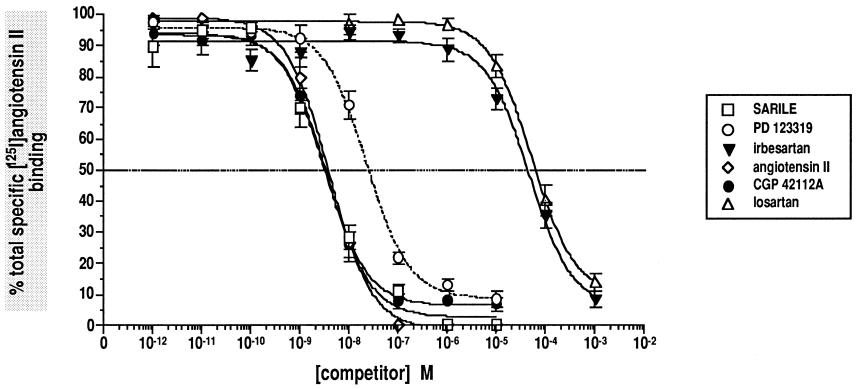

Fig. 4. Competition binding of irbesartan to PC-12w cell membranes. Radioligand binding was performed as described using [^{125}I]angiotensin II at a concentration of approximately 0.6 nM and irbesartan or other receptor ligands at the concentrations depicted. Non-specific binding was defined in the presence of 1 μM [Sar^1, Ile^8]angiotensin II. Values reported represent the mean ± standard error of 4 to 5 independent experiments.

than irbesartan for the angiotensin AT_1 receptor were the peptides [Sar^1, Ile^8]angiotensin II and angiotensin II itself, both of which are non-selective angiotensin II receptor ligands and therefore exhibit a high affinity for the angiotensin AT_2 receptor subtype as well.

3.2. Relative affinity of irbesartan for the angiotensin AT_2 receptor

The selectivity of irbesartan for the angiotensin AT_1 receptor subtype and its lack of high affinity for the angiotensin AT_2 receptor was demonstrated by radioligand binding analysis using crude membranes from PC-12w cells, which endogenously express only the angiotensin AT_2 receptor subtype. Saturation binding of [^{125}I]angiotensin II to the angiotensin AT_2 receptor in PC-12w cells resulted in a K_D of 5.81 ± 1.86 nM and a B_{max} of 10.1 ± 3.2 pmol [^{125}I]angiotensin II bound per mg total membrane protein (Fig. 3). In competition binding assays (Fig. 4), irbesartan failed to displace [^{125}I]angiotensin II at concentrations lower than 10 μM (K_i = 50.0 ± 17.5 μM), similar to results obtained with losartan (K_i = 46.7 ± 13.9 μM). The angiotensin AT_2-selective agonist CGP 42112A (K_i = 3.19 ± 1.88 nM) and the angiotensin AT_2-selective antagonist PD 123319 (K_i = 16.7 ± 3.2 nM) exhibited high affinity, as did the non-selective peptides angiotensin II and [Sar^1, Ile^8]angiotensin II (K_i = 3.70 ± 2.26 nM and 3.18 ± 1.79 nM, respectively).

3.3. Relative potencies of angiotensin AT_1 antagonists to block receptor activation

The results of radioligand binding revealed that irbesartan shows high affinity for the angiotensin AT_1 receptor at nanomolar concentrations while exhibiting no affinity for the angiotensin AT_2 receptor in the same concentration range. In order to determine the functional potency of irbesartan as an antagonist, the ability of irbesartan to block the stimulatory effects of angiotensin II at the angiotensin AT_1 receptor in WB cells was examined. Stimulation of the angiotensin AT_1 receptor in WB cells with angiotensin II leads to a rapid activation of phospholipase C, causing increases in the intracellular level of free IP_3. In the WB cells, angiotensin II stimulates IP_3 production with an EC_{50} of approximately 22 nM (data not shown). The potency of irbesartan to block this stimulation of IP_3 in response to angiotensin II was tested and compared to that of losartan and tasosartan in the same system (Fig. 5). Half-maximal inhibitory concentrations (IC_{50}) were determined from log-dose response curves by nonlinear regression analysis. Irbesartan possessed an IC_{50} of 62.2 ± 7.4 nM, whereas losartan had an IC_{50} of 103.1 ± 19.4 nM and tasosartan had an IC_{50} of 155.8 ± 10.0 nM. These IC_{50} values were significantly different ($F(2,6) = 37.38$; $P = 0.0004$): Student–Newman–Keuls post-hoc analysis showed that the IC_{50} for irbesartan was significantly different from those of losartan and tasosartan ($P < 0.01$); and that the IC_{50} for losartan was significantly different from that of tasosartan ($P < 0.01$). The results indicate that

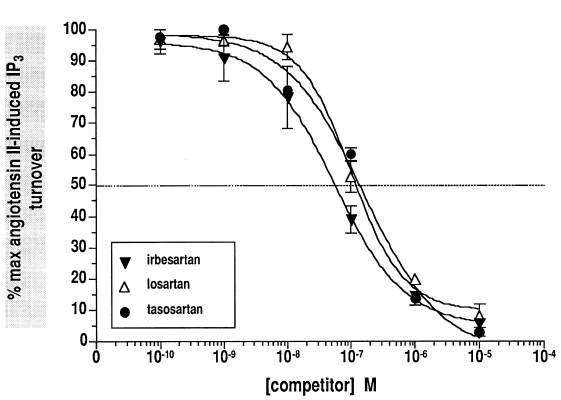

Fig. 5. Inhibition of angiotensin II-induced IP_3 turnover in WB cells by angiotensin AT_1 antagonists. The concentration of angiotensin II used was 50 nM. Values reported represent the mean ± standard error of three independent experiments.

Table 1
Affinities of angiotensin AT_1 receptor antagonists for peripheral tissues
The K_i values (nM) represent the mean ± standard error of three independent determinations. Radioligand binding was performed using approximately 0.6 nM [^{125}I]angiotensin II and irbesartan or other receptor antagonists at concentrations ranging from 0.1 nM to 1 μM. All binding done on rat adrenal was done in the presence of 1 μM PD 123319. Non-specific binding was defined in the presence of 1 μM [Sar^1, Ile^8]angiotensin II.

	Liver	Kidney	Pituitary	Adrenal
[Sar^1, Ile^8]-angiotensin II	1.76 ± 0.90	1.87 ± 1.10	0.46 ± 0.06	1.17 ± 0.40
irbesartan	6.90 ± 2.36	4.00 ± 1.23	1.83 ± 0.51	3.80 ± 1.83
losartan	20.9 ± 10.2	15.6 ± 4.28	9.43 ± 2.80	27.2 ± 10.8
tasosartan	41.2 ± 18.3	47.7 ± 5.55	22.6 ± 10.3	68.7 ± 29.0

irbesartan is indeed the most potent of the three antagonists with respect to blocking receptor activation of IP_3 production.

3.4. Binding of irbesartan in peripheral tissues

In the periphery, angiotensin II increases blood pressure by multiple physiological mechanisms through its interaction with a number of tissues (kidney, adrenals, vascular smooth muscle, etc.) which express the angiotensin AT_1 receptor subtype. Thus, potent angiotensin AT_1-selective antagonists show increasing promise as anti-hypertensive therapeutics. In all the peripheral tissues examined (Table 1), irbesartan possessed a significantly greater affinity for the angiotensin AT_1 receptor than either losartan or tasosartan. In the kidney, the affinity of irbesartan for the angiotensin AT_1 receptor ($K_i = 4.00 \pm 1.23$ nM) was nearly four-fold greater than that of losartan ($K_i = 15.6 \pm 4.28$ nM), and almost 12-fold greater than that of tasosartan ($K_i = 47.7 \pm 5.6$ nM). The increased affinity of irbesartan relative to the other antagonists was more dramatic in the adrenals, where irbesartan ($K_i = 3.80 \pm 1.83$ nM) displayed a seven-fold greater affinity than losartan ($K_i = 27.2 \pm 10.8$ nM) and an 18-fold greater affinity than tasosartan ($K_i = 68.7 \pm 29.0$ nM) for the angiotensin AT_1 receptor. In many of the tissues, most notably the adrenals and the kidney, the affinity of irbesartan for the angiotensin AT_1 receptor approached that of the highly potent, non-selective antagonist [Sar^1, Ile^8]angiotensin II.

4. Discussion

The octapeptide hormone angiotensin II is able to elicit a variety of different physiological reactions to regulate body fluid volume and blood pressure. It does so by interacting with receptors located on the surface of its intended target cells. However, despite the existence of at least two angiotensin II receptor subtypes (Chiu et al., 1989) and perhaps more (Kiron and Soffer, 1989; Tsutsumi and Saavedra, 1992; Chaki and Inagami, 1993; Yee et al., 1997), practically all of the physiological effects of angiotensin II are apparently mediated by the angiotensin AT_1 receptor subtype. Thus, the continued development of receptor ligands with a high affinity and selectivity for the angiotensin AT_1 subtype is of great benefit to both the basic scientist and the clinical investigator alike. The therapeutic potential of such compounds in the treatment of hypertension and congestive heart failure have made the use of angiotensin AT_1 receptor antagonists an area of great promise in the management of these conditions (Unger and Gohlke, 1994). Likewise, high affinity, subtype selective receptor ligands are perennially useful tools for research in the fields of pharmacology, physiology and protein chemistry.

Irbesartan is a recently developed angiotensin AT_1 receptor antagonist. Initial pharmacological studies done on rat liver and adrenal tissues (Cazaubon et al., 1993) showed that irbesartan possessed considerable affinity for rat angiotensin AT_1 receptors, and that the presence of a sulfhydryl-reducing agent like dithiothreitol could greatly reduce irbesartan binding in these tissues. In the present study, we examined the ability of irbesartan to bind to each of the two different angiotensin II receptor subtypes individually expressed in the clonal cell lines WB (which expresses the AT_1 subtype exclusively) and PC-12w (which expresses the angiotensin AT_2 subtype exclusively). The homogeneous nature of these cultured cells enabled us to study the affinity of irbesartan for each angiotensin II receptor subtype in complete isolation from the other. Furthermore, the robust angiotensin II receptor expression levels typical of these cells provided an enriched assay system with considerably lower non-specific binding, and therefore a much higher signal-to-noise ratio, relative to that obtainable in procured tissue sources.

Radioligand binding assays performed on crude WB cell membranes confirmed that irbesartan possessed a very high affinity for the angiotensin AT_1 receptor. Of all the non-peptidic antagonists tested, irbesartan had by far the highest affinity for the angiotensin AT_1 receptor: in competition radioligand binding assays, the affinity of irbesartan for the receptor was approximately six-fold greater than losartan, the prototypical angiotensin AT_1-selective antagonist, and approximately 10-fold greater than tasosartan, another clinically tested antagonist. Irbesartan was surpassed in affinity for the angiotensin AT_1 receptor only by the endogenous ligand, angiotensin II, and its analog [Sar^1, Ile^8]angiotensin II; neither of which are subtype selective and therefore possess affinities for the angiotensin AT_2 of a similar magnitude, as demonstrated in radioligand binding assays performed on crude PC-12w cell membranes. Likewise PD 123319 and CGP 42112A, two different ligands, showed high affinity for the angiotensin AT_2 receptor while irbesartan demonstrated virtually no affinity at concentrations up to 1 μM. Irbesartan managed to displace significant amounts of [^{125}I]angioten-

sin II from the angiotensin AT_2 receptor only at concentrations of 10 μM or greater.

Stimulation of the angiotensin AT_1 receptor by angiotensin II can lead to a number of changes at the cellular level, ranging from the rapid mobilization of intracellular Ca^{2+} stores (Peach and Dostal, 1990) to the activation of intracellular kinases (Molloy et al., 1993; Lu et al., 1996a) to the much slower induction of changes in the expression level of specific genes, such as tyrosine hydroxylase (Yu et al., 1996), the norepinephrine transporter (Lu et al., 1996b), c-*myc* and the A-chain for platelet-derived growth factor (Naftilan et al., 1989). One of the earliest biochemical steps in the signaling pathway of the angiotensin AT_1 receptor is the hydrolysis of phosphatidylinositol bisphosphate in the plasma membrane into IP_3 and diacylglycerol. A previous study (García-Sáinz et al., 1997) demonstrated that 1 μM irbesartan was as efficacious as an equally high concentration of losartan at blocking angiotensin II-induced IP_3 turnover in guinea pig hepatoctyes. In agreement with the results of our radioligand binding assays, irbesartan demonstrated the greatest potency to block the angiotensin II-stimulated increase in IP_3 turnover in WB cells when compared to the structurally related antagonists losartan and tasosartan. Curiously, while irbesartan displayed a six-fold greater potency over losartan and 10-fold greater potency over tasosartan in binding assays done on WB membranes, irbesartan's potency over the other antagonists in the functional assay was less dramatic. The reason behind these differences is unclear, but the results underscore the importance of performing both radioligand binding and functional assays in order to form a more comprehensive pharmacological determination of the effectiveness of an antagonist. Nevertheless, the rank order of potency observed in the binding assays is preserved in the functional assay, and this is especially encouraging when contemplating both the usefulness of irbesartan in the study of drinking and feeding behaviours and the therapeutic potential of irbesartan in the management of hypertension and other related disease states.

In order to induce the many physiological changes that often act in concert with each other to regulate blood pressure and body fluid volume, angiotensin II interacts with angiotensin AT_1 receptors located in various tissues throughout the body and in various areas of the brain. Peripheral tissues containing high levels of angiotensin AT_1 expression and thought to be involved in mediating the pressor effect of angiotensin II include the adrenals and the kidney (Vallotton, 1987), the heart (Kobayashi et al., 1978), pituitary (Aguilera et al., 1983), and vascular smooth muscle (Griendling et al., 1997). Competition radioligand binding assays on a number of these tissues showed that out of all the non-peptidic antagonists tested, irbesartan possessed the highest affinity for the angiotensin AT_1 binding sites in these tissues, and came very close to achieving the same high affinity as the peptide antagonist, [Sar^1, Ile^8]angiotensin II, which is among the most potent angiotensinergic ligands known. The differences in affinity between irbesartan and the other non-peptidic antagonists were evident in all tissues, notably the adrenals, where irbesartan displayed a seven-fold greater affinity for the angiotensin AT_1 compared to losartan, and an 18-fold greater affinity compared to tasosartan. Differences in affinity of such a magnitude in these tissues, taken together with it's significantly greater potency to block receptor activation, points towards irbesartan as a potentially superior anti-hypertensive agent.

Recently published studies have begun to evaluate the usefulness of irbesartan in the management of human cardiovascular disease states (Man in't Veld, 1997; Reeves et al., 1998). Studies have shown that irbesartan has a significantly greater oral bioavailability and extended half-life compared to other angiotensin AT_1 receptor antagonists (Brunner, 1997). In addition, irbesartan may have beneficial effect on cardiac function, aortic cholesterol content and renal injury (Powell et al., 1998).

Unlike losartan and tasosartan, irbesartan does not require metabolic activation in order for it to achieve its highest affinity state for the angiotensin AT_1 receptor, and hence its maximal therapeutic potential (Brunner, 1997). Whereas much of the angiotensin AT_1 inhibition by antagonists like losartan and tasosartan can be attributed to activated metabolites that are the result of oxidation by the liver, irbesartan already exists in its highest affinity state and is eliminated primarily via glucuronidation rather than oxidation (Perrier et al., 1994). This pharmacological aspect of irbesartan may prove especially advantageous in the treatment of patients with liver impairment (Marino et al., 1998). Similarly, irbesartan may find a growing usefulness in veterinary or lab animal medicine. It has been clearly documented that certain species, including canines and monkeys, are unable to convert losartan to its more activated form since they utilize different metabolic pathways for drug activation and elimination (Perrier et al., 1994). Moreover, the angiotensin AT_1 receptor found in some non-human species possesses greatly reduced affinity for losartan (Balla et al., 1991; Burns et al., 1994) when compared to the human angiotensin AT_1 receptor. Thus, a drug like irbesartan, which is more potent than losartan and other similar antagonists and which requires no metabolic activation may be a superior therapeutic in non-humans as well.

In summary, we have examined the selectivity and high affinity with which irbesartan binds to the angiotensin AT_1 receptor in clonal cell lines and in rat tissues, and its potency in blocking immediate downstream signaling events following angiotensin AT_1 receptor activation. Irbesartan possessed the highest affinity of all the nonpeptide angiotensin II-receptor antagonists tested, approaching the very high affinity previously exhibited only by the non-selective peptidic ligands. For these reasons, irbesartan should continue to be the focus of investigations aimed at improving the treatment of hypertension.

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