Annu. Rev. Pharmacol. Toxicol. 1992. 32:135–65

PHARMACOLOGY OF NONPEPTIDE ANGIOTENSIN II RECEPTOR ANTAGONISTS

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KEY WORDS: angiotensin II, AII receptor antagonists, losartan, renin, angiotensin system

INTRODUCTION

Nonpeptide angiotensin II (AII) receptor antagonists are a new class of drugs whose actions as experimental tools and potential therapeutic agents result from a specific blockade of the actions of AII (1–3). AII is the primary humoral mediator of the renin angiotensin system (RAS). In this well-characterized system, angiotensinogen is produced by the liver (4) and converted to the decapeptide angiotensin I by the action of renal renin at both renal and extrarenal sites (5, 6). Angiotensin I is essentially inactive itself but is converted by the action of converting enzyme in the kidneys, lungs, and other sites to the active effector, AII. Angiotensin III and angiotensin 1–7 also have biological activity, but the principal effector of the RAS is AII (7). With the new nonpeptide antagonists represented by Losartan (Losartan potassium; DuP#753; MK-954; 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole, potassium salt) it is possible for the first time to block the RAS at the angiotensin receptor without the confounding partial agonist effect of peptide AII receptor antagonists such as saralasin or the nonspecific angiotensin-converting enzyme (ACE) inhibitors such as captopril or enalapril. ACE inhibitors, for example, block the RAS

0362-1642/92/0415-0135$02.00

but also inhibit kininase II and thereby potentiate the vasodilator action of bradykinin. Losartan, by contrast, blocks only the action of AII at its receptor (1–3).

The nonpeptide nature of the new AII receptor antagonists also facilitates the long-term oral treatment of animals, which will probably lead to clarification of the physiologic and pathophysiologic role of AII. The pharmacology of this new class of agents will be explored primarily on the basis of the experience with Losartan. Since this compound became available to researchers outside Du Pont at the end of 1989, over 200 presentations and manuscripts have been published. It is hoped that this chapter will capture the essence of the rapidly unfolding story of how a pharmacologic discovery can lead to greater physiological understanding and ultimately to new therapy.

DISCOVERY

Losartan represents the culmination of a long-term cooperative effort between medicinal chemists and biologists at E. I. du Pont de Nemours & Co. (now The Du Pont Merck Pharmaceutical Company) to identify a nonpeptide AII receptor antagonist (3, 8–10). The ACE inhibitors had confirmed the importance of the RAS in hypertension (11, 12), but early attempts, including those at Du Pont, to discover a receptor antagonist which was nonpeptide, or which lacked intrinsic (AII-like) activity, were unsuccessful. The breakthrough came when Furukawa et al (13, 14) disclosed a series of patents of antihypertensives that antagonized AII. These compounds were shown to lack sufficient binding affinity for the AII receptor but, importantly, to have specificity for AII (15, 16). New compounds based on this chemical lead were tested for AII receptor affinity in rat adrenal microsomes, functional AII antagonism in isolated rabbit aorta, and intravenous (i.v.) and oral (p.o.) efficacy in renal hypertensive rats (3, 8–10). There were several milestones in the progress from the Takeda leads (S-8307 and S-8708) to Losartan. The first milestone was EXP6155, which demonstrated an approximately 10-fold increase in binding affinity (50% inhibitory concentration [IC_{50}] = 1.6×10^{-6} M) and AII antagonism (pA_2 = 6.54); this was followed by EXP6803, a compound with an additional 10-fold increase in affinity (IC_{50} = 1.4×10^{-7} M; pA_2 = 7.2) (2). Then came EXP7711 (17), the first potent nonpeptide angiotensin receptor antagonist to display significant oral activity, producing a dose-related decrease in blood pressure in conscious renal artery–ligated rats and high-renin (furosemide-treated) dogs (17). The final step leading to Losartan was the synthetic work to replace the aromatic carboxylic acid function of EXP7711 with more lipophilic isosteres such as a tetrazole (8–10). It is the biphenyl tetrazole moiety that is common to the newly disclosed AII receptor antagonists (18, 19).

Concurrent with the discovery of Losartan, another series of compounds (such as PD123177) was synthesized independently by Warner Lambert (20, 21; Figure 1). Although patented as antihypertensive AII receptor blockers, these compounds did not lower blood pressure in renal hypertensive rats (22). PD123177 (also designated EXP655 and XD329) did, however, interfere with AII binding in various tissues, and its affinity for these AII-binding sites was found to be specific for tissue, species, and age of animal (see Defining AII Receptor Heterogeneity, below). The biology of two new receptor antagonists, DuP 532 (18) and L-158,809 (19), has been reported. Both of these compounds have greater in vitro affinity and in vivo potency than Losartan. DuP 532 differs from Losartan in being a noncompetitive (pseudoirreversible) antagonist of AII in isolated tissue, decreasing the maximum response to AII in a dose-related way (18, 23). These three compounds have a common biphenyltetrazole moiety. A new series of nonbiphenyltetrazoles represented by SKF108566 has recently been described (24)

PHARMACOLOGY OF SPECIFIC AII RECEPTOR BLOCKADE

The in vitro and in vivo effects of Losartan are primarily a result of blockade of AII at its receptor as evidenced by competition/antagonism studies with exogenously added AII and with endogenously formed AII.

In vitro competition assays with [^{3}H]AII, [^{125}I]AII, or [^{125}I]Sar^1-Ile^8-AII clearly show that Losartan has affinity for the AII receptor in microsomal or membrane preparations from a diverse number of AII target tissues with IC_{50}s or K_is of 2×10^{-8} to 2×10^{-9} M (in preparations with IC_{50}s or K_is for unlabeled AII of 1×10^{-9} to 2×10^{-10} M). Importantly, concentrations of Losartan of $\geq 10^{-5}$ M do not interfere with the α-1 receptor ([^{3}H] prazosin) or Ca^{2+} channel (^{3}[H]nitrendipine) binding (25) and do not show affinity for a panel of receptors including opioid (μ, γ, κ) dopamine-2, serotonin-2,

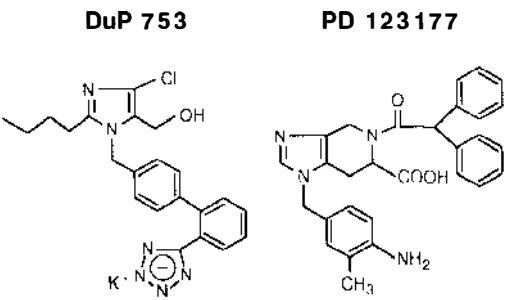

Figure 1 Chemical structures of Losartan and PD123177.

phencyclidine, neurotensin, cholinergic (muscurinic and nicotinic), glycine (strychnine-insensitive), and basic FGF growth factor (26). Characteristically, however, Losartan and DuP 532 do not completely inhibit AII binding in rat adrenal cortex, leaving 20–30% resistant sites (18, 27). These Losartan-resistant sites are the basis of current efforts to explore AII receptor heterogeneity (see Defining AII Receptor Heterogeneity, below). The Losartan-sensitive sites are designated AT_1 receptors (28).

The Losartan-sensitive (AT_1) binding sites are functional receptors for AII as evidenced by the results of second-messenger and isolated-tissue studies (Table 1). AII stimulates phospholipase C (through a G protein), the production of inositol 1,4,5-triphosphate (IP_3) and diacylglycerol, and the mobilization of intracellular calcium (29). In rat aortic smooth muscle cells in culture (30), bovine adrenal cells (31), and rat liver cells (32) the AII-induced calcium fluxes are blocked by Losartan (2×10^{-8} to 1×10^{-4} M). In the bovine adrenal cells (31), rat mesangial cells (33), clone 9 rat liver–derived cells (34), rat liver cells (32), and 7315c cells derived from murine pituitary tumor (35), Losartan (4×10^{-9} to 1×10^{-4} M) blocked the AII-induced increases in IP_3 formation. In the 7315c cells and the rat hepatocytes, AII both stimulated IP_3 formation and inhibited adenylate cyclase activity (32, 35). Both second-messenger responses to AII were blocked by Losartan.

In isolated tissue, the nonpeptide AII receptor antagonist Losartan selectively attenuates contractile responses of isolated vascular and nonvascular smooth muscle and cardiac muscle to AII (Table 1). The responses of rabbit aortic strips or rings to AII were competitively antagonized by Losartan, with pA_2 ranging from 7.98 to 8.48 (30, 36). Likewise, the contractions of turtle aorta induced by AII, but not norepinephrine, were blocked by Losartan (37). Losartan blocked the actions of AII on rat portal vein, stomach, and urinary bladder; rabbit jugular vein and aorta; and human colon, intestine, and urinary bladder (38). The pA_2s for Losartan were quite similar, ranging from 8.19 to 8.66, whereas the responses of these tissues to other agonists (e.g. norepinephrine, acetylcholine, bradykinin, and bombesin) were unaffected (38). Collectively, these results suggest that Losartan and presumably other similar nonpeptide antagonists, are competitive and selective AII receptor antagonists which lack agonist activity characteristic of the peptide antagonists.

In the pithed rat, AII (0.03–10 $\mu g\ kg^{-1}$) produced a dose-related increase in diastolic blood pressure. Losartan at 3 and 10 mg kg^{-1} i.v. "competitively" antagonized AII by right-shifting the dose–response curve while not decreasing the maximum response (39). The peptide antagonist saralasin (4 and 12 $\mu g\ kg^{-1}\ min^{-1}$ i.v.) right-shifted the AII dose–response curve but decreased the maximum pressor response. The noncompetitive antagonism of saralasin

Table 1 In vitro responses to AII (AT_1) blocked by Losartan

Response[a]	Species	Organ/Tissue	DuP 753	Reference
(+) Contraction	Alligator	Aorta	10^{-6} M	193
	Turtle	Aorta	10^{-6} M	37
	Rat	Aorta	85 nM (IC_{50})	34
	Rabbit	Aorta	8.45 (pA_2)	25
	Rabbit	Aorta	8.27 (pA_2)	38
	Rat	Pulmonary artery	8.4 (pA_2)	194
	Rabbit	Pulmonary artery	8.27 (pA_2)	38
	Rat	Portal vein	8.66 (pA_2)	38
	Rat	Uterus	8.75 (pK_B)	83
	Rabbit	Uterus	$\sim 10^{-9}$ M	34
	Rabbit	Jugular vein	8.27 (pA_2)	38
	Rat	Stomach	8.66 (pA_2)	38
	Rat	Urinary bladder	8.66 (pA_2)	38
	Guinea pig	Ileum	8.59 (pA_2)	25
	Guinea pig	Urinary bladder	10^{-7} M	138
	Guinea pig	Gastrointestinal tract	10^{-6} M	195
	Alligator	Isolated perfused heart	10^{-6} M	193
(+) Aldosterone release	Rat	Adrenal capsular cells	1.3×10^{-8} M (K_B)	177
(+) Catecholamine release	Rat	Perfused adrenal	10^{-6} M	22
	Rat	Perfused kidney	10^{-6} M	90
(−) Renin release	Rat	Kidney slice	5×10^{-5} M	72
(+) Ca^{2+} mobilization	Rat	Aortic smooth muscle cells	2×10^{-8} M (IC_{50})	30
	Rat	Hepatocytes	10^{-5} M	32
	Cow	Adrenal medullary cells	10^{-4} M	31
	Mouse	Neuroblastoma	[redacted]	152
	Human	Astrocytes	1.9×10^{-8} M	228
(+) Inositol metabolism	Rat	Mesangial cells	3.8×10^{-9} M	33
	Rat	Hepatocytes	10^{-5} M	32
	Rat	Hepatocytes	10^{-6} M	197
	Rat	Liver clone 9 cells	1.9×10^{-8} M	34
	Cow	Adrenal medulla cells	10^{-4} M	31
	Mouse	7315c cells (pituitary tumor)	1.75×10^{-7} M (K_i)	35
	Rat	Neonatal astrocytic glia	10^{-6} M	198
	Rat	Adult astrocytic glia	10^{-6} M	199
(±) cAMP	Rat	(−)Liver membranes	10^{-5} M	32
	Mouse	(−)7315c cells (pituitary tumor)	2×10^{-8} M (K_i)	35
	Rat	(+)Rat fetal fibroblasts	[redacted]	183
(+) Thymidine incorporation	Rat	Aortic smooth muscle cells	10^{-5} M	105
	Human	Mesangial cells	10^{-8} M	117

[a](+) Stimulation, (−) inhibition.

was reversed by Losartan (36), as was the noncompetitive antagonism by EXP3892 (40), presumably owing to conformational changes at the receptor (40). Losartan was specific for AII as it did not alter the pressor responses to norepinephrine or vasopressin or the depressor response to isoproterenol (39). Similar findings were made with the Losartan metabolite EXP3174 (41) and the new nonpeptide AII receptor antagonist DuP 532 (23).

In conscious rats and dogs, Losartan produced a dose-related blockade of the blood pressure response to AII. In normotensive rats, Losartan at 1 or 3 mg kg^{-1} i.v. blocked the pressor response to AII but had no effect on the basal blood pressure (39). This lack of intrinsic agonist (AII-like) activity is in sharp contrast to the dose-related increase in basal blood pressure following saralasin infusion (39). Similar findings have been made in normotensive rats with EXP3174 (41), DuP 532 (23), and the novel structure L-158,809 (19). In spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) cats, Losartan, but not PD123177, blocked the pressor responses to AII (42). In conscious dogs with normal renin levels, Losartan at 1–10 mg kg^{-1} i.v. produced a dose-related decrease in the pressor response to AII but not to phenylephrine or vasopressin (43). In sodium-depleted dogs (high renin), Losartan infused at 30–1000 μg kg^{-1} min^{-1} for 180 min, lowered blood pressure, antagonized AII, and increased the response to norepinephrine (44).

The pharmacokinetics of Losartan are species specific. In rats and humans, Losartan is active itself, but is also converted to an active metabolite, EXP3174, which contributes to its overall effect (45). By contrast, Losartan is not converted to EXP3174 to any significant extent in dogs (46). Furthermore, the elimination half-life of Losartan (3 mg kg^{-1} i.v.) is shorter in dogs (41 ± 10 min) (46) than in rats (5.7 ± 2.0 h) (45). In rats, Losartan and EXP3174 have similar $t_{1/2}$ values following p.o. administration (45). DuP 532 and L-158,809 do not have significant active metabolites.

BLOOD PRESSURE–LOWERING EFFECTS

The extent to which nonpeptide AII receptor antagonists such as Losartan lower arterial blood pressure is dependent upon the status of activation of the RAS in the particular animal. This important principle is based on the specificity of the AII antagonistic effects and the lack of agonist effects of these compounds. In conscious normotensive rats (Sprague-Dawley and WKY rats), Losartan, EXP3174, DuP 532, and L-158,809 (19, 23, 39, 41) do not alter basal blood pressure, neither lowering pressure (indicative of AII receptor antagonism) nor raising it (indicative of AII receptor agonism). In long-term experiments, Losartan produces a modest lowering blood of pressure in normal rats (47).

In sodium-depleted rats, with high renin levels, Losartan at 10 mg kg^{-1} i.v.

lowers blood pressure significantly (48). The maximum hypotensive response was similar for a peptide AII antagonist, renin inhibitor, and ACE inhibitor (48). Likewise, Losartan acutely lowered blood pressure in sodium-depleted conscious (44) or anesthetized (49) dogs.

In renal hypertensive rats, Losartan significantly lowers arterial pressure [total ligation of one artery (50); 2K1C (51–53) or 1K1C (47)]. Blood pressure was lowered to the maximum extent in the short term by 3 mg kg^{-1} i.v. or 10 mg kg^{-1} p.o. (50). That these doses maximally lowered blood pressure was evidenced by the lack of an additional effect of captopril. In renal hypertensive cynomologus monkeys, Losartan at 1,3, or 10 mg kg^{-1} reduced blood pressure in a dose-related manner. As in renal hypertensive rats, no tachycardia was noted in these animals, but the duration of action was less than 6 h in the monkeys compared with more than 24 h in the rats (54).

SHR have low plasma renin activity (55), but their renal RAS activity contributes to maintenance of arterial pressure. This has been shown by both short- and long-term experiments with RAS inhibitors (48, 50, 56), antibodies to AII (57), and nephrectomy (56, 58). Losartan i.v. (3–30 mg kg^{-1} or p.o. (3 or 10 mg/kg^{-1}) produced a dose-related reduction in mean arterial pressure in SHR, and captopril (10 mg/kg^{1} i.v.) gave no additional effect (56). The maximum hypotensive effect (ca. 50 mmHg) was somewhat greater than that observed in a similar preparation in which the maximum response to Losartan at 10 mg kg^{-1} i.v., a renin inhibitor and ACE inhibitor, was ca. 15 mmHg (48). The effects of L-158,809 in SHR appeared to be enhanced by pretreatment with hydrochlorothiazide (59). In chronic studies in 3-week-old SHR dosed for 4 weeks, Losartan at 15 mg kg^{-1} day^{-1} or captopril at 100 mg kg^{-1} day^{-1} via the drinking water lowered blood pressure (Losartan group, 104 ± 10 mmHg; captopril group 93 ± 7 mmHg; control group, 130 ± 15 mmHg) and reduced the cardiac hypertrophy (60). Unlike captopril, however, the 4-week Losartan treatment did not reduce the vascular hypertrophy. Subsequently, these investigators have dosed the SHR for 10 weeks and shown that the vascular hypertrophy was reversed (J. J. Morton, personal communication), suggesting that 15 mg kg^{-1} day^{-1} may be on the low end of the dose–response curve.

In deoxycorticosterone salt hypertensive rats, a low-renin model, Losartan at 10 mg kg^{-1} i.v. had no effect on blood pressure (50). By contrast, Losartan, like ACE inhibition, lowered blood pressure in the REN2 transgenic rat (61). In Dahl S rats fed a high-salt diet, Losartan at 30 mg kg^{-1} day^{-1} did not alter the development of pressure but decreased mortality (62). In stroke-prone SHR (SPSHR) given saline to drink, a severe hypertension developed which was not blocked by Losartan (63). In SPSHR fed a 4% NaCl diet, where hypertension was less severe, at 30 mg kg^{-1} significantly blunted the rise in blood pressure (64). EXP7711, a Losartan predecessor, has also

been shown to reverse the hypertension caused by long-term, low-dose AII infusion in rats (65).

BLOCKADE OF ALDOSTERONE RELEASE AND DRINKING BEHAVIOR

AII has a powerful stimulant effect on aldosterone release (66), and this can be blocked by peptide AII receptor antagonists (67). Nonpeptide AII receptor antagonists also block this response, as Losartan at 10 mg kg^{-1} i.v. blocked the AII-induced rise in plasma aldosterone levels in normotensive rats (68). Siegl et al (19) reported similar findings with L-158,809. In SHR and WKY rats, AII induces aldosterone release, which is blocked by Losartan (69). Losartan was three times more potent in blocking the aldosterone response in SHR than in WKY rats and was three times more potent in blocking the AII-induced aldosterone response than in blocking the AII pressor response (69).

The physiologic role of AII in drinking behavior has been extensively reviewed (70, 71). Administration of AII subcutaneously or directly into the brain (i.c.v.) elicits a marked drinking response in rats (68, 72). Losartan at 3 and 10 mg kg^{-1} subcutaneously (s.c.) produced a dose-related blockade of the drinking response to AII (200 μg kg^{-1} s.c.). Likewise, EXP7711, a Losartan predecessor, at 25 or 50 nmol i.c.v., blocked the drinking response to AII at 100 pmol i.c.v. (72). Losartan i.c.v. also blocks the drinking response to i.c.v. AII without affecting the drinking response to carbechol (73). Acclimation to cold induces a drinking response in rats that is thought to involve AII mechanism. This response was blocked by Losartan (M. J. Fregly, personal communication).

RENAL FUNCTION

The effects of AII receptor antagonists on renal function are necessarily as complex as the role of AII itself in maintaining arterial blood pressure and fluid and electrolyte balance (74–76). The extrarenal actions of AII on systemic vascular resistance and aldosterone secretion and the antagonism by Losartan were discussed above. There are also multiple possible intrarenal effects of AII and of receptor antagonists. AII modulates intrarenal blood flow through actions on efferent and afferent arterioles and mesangial cells (74) acting in concert with other vasoactive peptides (75), by increasing the reabsorption of sodium and water in the proximal convoluted tubule (77), by enhancing renal sympathetic nerve transmission (77, 78), and by suppressing renin release (79). Nonpeptide receptor antagonists may act to modulate the actions of AII at each of these sites.

In isolated perfused rat kidneys, Losartan had no effect of its own on renal vascular resistance (RVR) but completely reversed the vasoconstriction induced by AII (80). In isolated hydronephrotic kidneys, Losartan (10^{-7}–10^{-5}M) completely blocked both the renal efferent and afferent arteriolar effects of AII (81). In Münich Wistar rats, with the use of microperfusion, free-flow micropuncture, and clearance techniques, Losartan, like captopril, was shown to markedly reduce proximal-tubule (S1 segment) fluid and electrolyte transport (82). In vivo, in volume-expanded Wistar Kyoto rats (WKY) and SHR and in euvolemic Münich Wistar rats, Losartan (2 or 10 mg kg^{-1} i.v.) had limited effects on renal function (84). At the highest dose in the SHR, Losartan, like captopril, lowered blood pressure and renal vascular resistance but produced little effect on sodium and water excretion (84). The renal response to Losartan appears to be partially systemic pressure independent. In one-kidney, one-clip hypertensive rats, Losartan lowered the pressure but decreased the glomerular filtration rate (GFR) in the clipped kidney and increased the GFR in the normal kidney (53).

In conscious SHR, L-158,809 (1.0 mg kg^{-1} i.v.) increased the urinary volume and electrolyte output throughout a 6 h period (59). In anesthetized rabbits, Losartan (2 mg kg^{-1} bolus plus 1 mg kg^{-1}/h^{-1} i.v. for 20 min) decreased arterial pressure and increased renal blood flow (RBF) (85, 86). Captopril further increased RBF, suggesting that ACE inhibitors have additional effects on the kidney, presumably by potentiating bradykinin (87). However, in normotensive guinea pigs, comparable blood pressure–lowering doses of Losartan and the ACE inhibitor MK-521 produced similar changes in GFR, RBF and urinary output (88). In that study the renin inhibitor RO42582 surprisingly produced significantly greater increases in RBF (88). In conscious genetic hypertensive dogs, Losartan (1–30 mg kg^{-1} p.o.) blocked the pressor responses to AII and produced a dose-dependent increase in GFR and RBF (89).

AII potentiated the response to renal sympathetic nerve stimulation, and this response was antagonized by Losartan in the isolated perfused rat kidney (90). Likewise, Losartan increased efferent renal sympathetic nerve activity by 20–30% in anesthetized rabbits (91) and dogs (92). This effect of AII may be expressed both pre- and postsynaptically (90). In the rat, Losartan blocked the potentiated response to exogenous norepinephrine, suggesting a postsynaptic effect (90). Losartan given to furosemide-treated rabbits increased renal nerve activity and reduced baroreceptor sensitivity (93).

Renin release is modulated by sympathetic nerves, sodium levels in plasma, and AII action via a negative-feedback mechanism (79, 94). Interfering with the synthesis of AII (ACE inhibitors) or blocking the action of AII at its receptor results in a compensatory rise in plasma renin activity (PRA) and AI levels for ACE inhibitors and PRA, AI, and AII for nonpeptide AII receptor

antagonists (95). This effect of Losartan has not been tested directly, but it is clear that it acutely raises PRA like ACE inhibitors in rats (96), monkeys (96), guinea pigs (88), and humans (97). On chronic dosing in stroke-prone SHR, the initial rise in PRA returned toward baseline values by 12 weeks (98). This fall in PRA may reflect increased salt intake and/or blunted sympathetic activity (99). EXP7711 has been shown to reverse the inhibitory effect of AII on renin release in kidney slices (72).

EFFECTS ON CELL GROWTH

The role of AII as a mitogen that stimulates the growth of smooth muscle (100), cardiac (101), and other (102) cells in culture has been demonstrated by the use of inhibitors of the RAS (both peptide AII receptor antagonists and ACE inhibitors) (102–104). Although less well studied, the experience to date with Losartan appears to confirm the complex role of AII in modulating growth in concert with other growth promoters.

In cultured rat aortic smooth muscle cells, Losartan (10^{-5} M) blocked the AII-induced intracellular calcium mobilization, increased protein synthesis ([^{3}H]leucine incorporation) and DNA synthesis ([^{3}H]thymidine incorporation), and increased the hypertrophic response (105). In a similar rat aortic smooth muscle cell preparation, Losartan (IC_{50} = 5.8 nM), Sar^{1}Ile^8-AII (IC_{50} = 1 nM), and dithiothreitol (DTT) (IC_{50} = 0.2 mM) blocked the 5.5-fold AII-induced increase in [^{3}H]thymidine incorporation (106). The myoproliferative response to balloon injury was blocked by Losartan (10 mg kg^{-1} day^{-1}) given at the time of injury (107). This is significant because ACE inhibitors must be given prior to the injury (103). Vascular hypertrophy seen in SHR was blocked by Losartan (15 mg kg^{-1} day^{-1} in the drinking water) after 10 weeks (J. J. Morton, personal communication) but not after 4 weeks (60). Rarefaction, or the decrease in the number of arterioles, in renal hypertensive rats (1K1C) has been shown to be blocked by captopril (104). Losartan, 10 mg kg^{-1} day^{-1} for 4 weeks, caused little lowering of blood pressure and did not reverse the hypertrophy of the aortic wall. However, this dose of Losartan did decrease the rarefaction of small arterioles, indicating inhibition of growth (47). A still lower dose of Losartan (4.6 mg kg^{-1} day^{-1}) administered to rats on a low-sodium diet had no effect on blood pressure or microvessel density, while blocking the pressor response to AII (108). These data suggest that the dose–response relationships for lowering of blood pressure, antagonism of AII pressor effects, and inhibition of vascular growth may be different.

AII can act (directly or indirectly) in concert with other mitogens to promote hypertrophy of cardiac tissue (101, 102, 109). The observations that lowering pressure (mechanical stress) did not necessarily reduce hypertrophy

(110, 111) and that an ACE inhibitor (presumably by lowering AII levels) could reduce hypertrophy at a dose that did not affect blood pressure (112) have strongly implicated AII. In adult rats given 7- or 14-day AII infusions, Losartan blocked the AII-induced increases in left ventricular mass whereas enalapril and hydralazine were ineffective, even though the pressures were normalized by hydralazine (113). A low dose of Losartan (3 mg kg^{-1} day^{-1} for 6 weeks) was, however, less effective than ramipril in preventing or reversing the cardiac hypertrophy following aortic banding in rats (114). In the SHR, cardiac hypertrophy was blocked after 4 weeks of treatment with Losartan at 15 mg kg^{-1} day^{-1} or captopril at 100 mg kg^{-1} day^{-1} (60). In myocardially infarcted rats, Losartan, like captopril, tended to reduce the hypertrophic response (115, 116). These data suggest that the hypertrophy is AII receptor mediated and that ACE inhibitors are acting to decrease AII formation.

AII has been shown to stimulate the growth of various cell lines in vitro, and this growth is blocked by RAS inhibitors (102). Losartan (10^{-8} M), like ACE inhibitors, blocks the AII-induced (insulin-dependent) increase in [^{3}H]thymidine incorporation in human mesangial cells (117). In cultured human SHSY54 neuroblastoma cells, Losartan and PD123177 (see Defining AII Receptor Heterogeneity, below) reduced the [^{3}H]thymidine incorporation to a similar extent (118). As with the mesangial cells, the AII response in neuroblastoma cells was insulin dependent. In cultured murine proximal tubule cells, high-glucose media increased [^{3}H]Leucine incorporation, which indicates an increased protein synthesis. AII further increases the protein synthesis and the associated increase in cell size, and these effects are blocked by Losartan (10^{-6} M) (119). It can be concluded that AII antagonists will inhibit AII-induced cell growth proportionally to the magnitude of the AII response itself.

Recently, the *mas* oncogene and its product have been patented as targets for peptide antagonists for use in cancer therapy (120). Likewise, "pharmaceutical antagonists" of the RAS such as enalapril were shown to markedly increase survival in rats implanted with a Walker 256 carcinosarcoma and were patented for inhibiting tumor growth (121).

OTHER EFFECTS OF AII

Losartan has been shown to act on nerve (central and peripheral) transmission, on animal "behavior," on nonvascular smooth muscle, and on intraocular pressure. Each action is presumed to be the result of specific AII receptor blockade.

Losartan has no direct electrophysiologic effects on the specialized conducting tissue of the heart (122). Losartan does, however, antagonize the

AII-enhanced response to nerve stimulation in renal (90–92) or mesenteric (123, 124) artery preparations. By contrast, in rabbit vas deferens AII enhanced the "adrenergic" neurotransmission and reduced the "nonadrenergic" neurotransmission. Losartan blocked only the nonadrenergic component (125). The concurrent increases in phosphatidylinositol and phosphatidylcholine lipase activities elicited by AII in the vas deferens were both blocked by saralasin, but only the phosphatidylinositol lipase activity was blocked by Losartan (126). In specific areas of the brain (e.g. the para ventricular nucleus of the hypothalamus), AII raised levels of norepinephrine without affecting dopamine (DA), 3,4-dihydroxyphenylethylene glycol (DOPEG), or 3,4-dihydroxyphenylacetic acid (DOPAC), and this effect was blocked by Losartan (127). In vitro field stimulation of rat brain striatal slices induces DA release, as evidenced by increased DOPAC levels. Losartan (10^{-5}–10^{-6} M) reduced this response (128). By contrast, long-term treatment of normal rats with Losartan (10 mg kg^{-1} day^{-1} s.c. for 21 days) produced a 1.64-fold increase in DOPAC levels (128).

The behavioral effects of AII have been defined largely by the responses to ACE inhibitors. ACE inhibition has been shown to reverse atropine-induced hypermotility (nootropic effect) (129), to antagonize scopalamine-induced impairment in light/dark discrimination (cognition effect) (130), and to reverse the escape deficits following a foot shock (learned-helplessness) procedure (131). The "behavioral" effects of Losartan have not been extensively characterized, but it appears to have effects similar to those reported for ACE inhibitors. Losartan in extremely low doses (0.1–100 μg kg^{-1}, p.o.) inhibited the suppressed behavior of mice in a light/dark aversion test (anxiolyticlike action) (132). In a learned-helplessness paradigm in rats, Losartan (0.5–2 mg kg^{-1}) reversed the behavioral deficit and significantly potentiated the effects of imipramine (P. Martin, personal communication). Losartan at 100 mg kg^{-1} s.c., like ACE inhibitors, reversed apomorphine-induced stereotyped behavior (increased licking, gnawing, and motor activity) in rats (133). However, the AT_2-selective agent PD123177 (see Defining AII Receptor Heterogeneity, below), at 30 mg kg^{-1} s.c., had a similar effect in antagonizing apomorphine (133). These preliminary findings encourage further study of the central role of AII and of Losartan in behavior. The central bioavailability of Losartan has not been established, although it has recently been reported that Losartan given i.v. to rats displaces AII binding in brain areas outside the blood–brain barrier (F.A.O. Mendelsohn, personal communication). This is unexpected because of the high water solubility of Losartan and previous results showing that Losartan at 10 mg kg^{-1}, p.o. did not block the pressor effect to i.c.v. AII (39).

AII can have direct and indirect effects (contraction, secretion, and transport) on nonvascular smooth muscle tissues (134, 135). Inhibitors of the RAS

are potent antagonists of these effects in vitro, but the in vivo effects (e.g. the gastrointestinal side effects of the ACE inhibitors) have been limited (136). Losartan very selectively antagonized the AII contractile responses of rat stomach and urinary bladder and human colon, intestine, and urinary bladder (137). Likewise, Losartan (0.1 μM) completely blocked the ATT-induced contraction of circular rings from guinea pig bladders (138).

Other reported effects of Losartan include intraocular pressure-(IOP) lowering effects in rabbits (139), calcium ion channel effects in isolated canine Purkinje fibers (140), antiarrhythmia effects in isolated perfused rat hearts (141), and prostaglandin release (142). A lack of effect of Losartan was noted in monocrotoline-induced pulmonary hypertension (143).

Inhibition of the intraocular RAS by ACE inhibitors or renin inhibitors has been shown to lower IOP in rabbits and/or monkeys (133, 144). Similarly, a 1% Losartan solution administered topically to the eyes of conscious rabbits produced a small but significant and long-lasting reduction in IOP (139), whereas the AT_2-selective agent XD329 (PD123177) was inactive. The exact nature of the IOP-lowering effects of these agents is unknown, but the eye has a RAS system. ACE activity has been found in ocular fluids of many species, including humans (145). Furthermore, GTP-regulated AII receptors have also been identified in the aqueous humor–generating tissue (the ciliary process and the iris and ciliary body) of the rabbit (146).

Losartan (1 μM) had no effect on L-type calcium current in canine ventricular myocytes but blocked the increase in the calcium current induced by AII (140). In isolated canine Purkinje fibers, Losartan (10 μM) had no effect on transmembrane action potentials (122). In isolated perfused rat heart, Losartan (2 μM) given before ischemia reduced the median duration of ventricular fibrillation (147) (see the discussion below).

The contribution of arachidonic acid products to the actions of AII is not fully understood. It has been shown that AII releases prostacyclin (6-keto-$PGF_{1\alpha}$) in cultured rat mesenteric and aortic smooth muscle cells (148). Inhibiting prostaglandin synthesis in an in situ rat cremaster muscle preparation blocked the dilator but not the constrictor response to AII (149). By contrast, inhibiting lipoxygenase in rats reduced both the in vitro and in vivo vascular effects of AII (150). However, in cultured human vascular endothelial cells (umbilical cord vein), AII did not increase prostaglandin I_2 (PGI_2) synthesis even though captopril had a concentration-related inhibitory effect (10^{-9}–10^{-3} M) (151). Recently, it has been reported that AII releases PGE_2 and PGI_2 from cultured human astrocytes, rat C6 glioma cells, and porcine smooth muscle cells (142). Both responses were blocked by Losartan (10^{-7} M) in C6 glioma. Losartan, however, was reported to produce a concentration-related (10^{-7}–10^{-5} M) increase in PGI_2 release in the human astrocytes, C6 glioma cells, and porcine smooth muscle cells (152). In

subsequent studies in our laboratories, using cultured porcine smooth muscle cells, AII binding sites and AII-induced PGI_2 release were demonstrated. In these cells, however, Losartan had no PGI-releasing effect but completely antagonized the AII response (153). Furthermore, in rat C6 glioma cells and porcine and bovine endothelial cells, no response to AII or Losartan was observed (153). A lack of effect of Losartan on prostaglandin release was also noted in the isolated vas deferens of the rabbit (125).

The involvement of AII in the etiology of pulmonary hypertension has been supported by the observations that the ACE inhibitors cilazapril (154) and captopril (155) partially prevent or ameliorate monocrotaline-induced cardiopulmonary injury in rats. In a rat model of chronic hypoxia known to be associated with pulmonary hypertension, inhibiting the RAS with cilazapril reversed the medial thickening of the pulmonary arteries but did not decrease pulmonary artery pressure or right ventricular weight (156). In an initial report, Losartan at 10 mg kg^{-1} s.c. had no effect on pulmonary vascular remodeling or pulmonary hypertension when administered daily for 21 days beginning 24 h after monocrotaline treatment (143). It should be noted that in the previous studies, the ACE inhibitors were given at the same time as the monocrotaline (154, 155). Additional studies are needed to clarify the role of AII in the etiology of pulmonary hypertension and of the potential usefulness of inhibitors of the RAS.

PHARMACOLOGY IN MODELS OF HUMAN DISEASE

The primary therapeutic indications for Losartan and other nonpeptide AII receptor antagonists have been defined by ACE inhibitors, namely hypertension and heart failure (11, 157). Other uses of inhibitors of the RAS system, including proteinuria, scleroderma renal crisis, idiopathic edema, Raynaud's syndrome, and hypertensive emergencies, are being explored with ACE inhibitors (11, 157). Experimentally, the beneficial effects of these drugs are being evaluated in animal models of stroke, diabetes, and myocardial ischemia.

The antihypertensive effects of Losartan in "renin-dependent" models of hypertension or in sodium-depleted normal animals have been described above (see Blood Pressure–Lowering Effects). The potential key to the importance of RAS inhibitors, including Losartan, in the long-term treatment of hypertension is their ability to block vascular hypertrophy that may be independent of arterial pressure per se (112). In addition, early treatment with these agents may significantly delay the onset of hypertension. Dosing of SHR in utero with RAS inhibitors, for example, produces long-lasting normalization of blood pressure (158), which suggests that future treatment of the young may permanently alter the course of the disease. This prospect must

be weighed against the risk of altering the critical role of AII in maintaining blood pressure and renal function in the fetus (159).

The anti–cardiac failure effects of Losartan have been assessed by two separate laboratories, using rats with coronary artery ligation–induced myocardial infarctions (115, 116). In the first, Losartan at 40 mg kg^{-1} day^{-1} by gavage for 14 days, like captopril, reduced left ventricular end-diastolic pressure and volume index while increasing venous compliance (115). These rats had electrocardiographic evidence of large myocardial infarction (>40%) of the left ventricle 3 weeks after the coronary ligation and were anesthetized at the time of hemodynamic evaluation. In the second series of experiments, Losartan was administered at 15 mg kg^{-1} day^{-1} by osmotic pumps for either days 1–21 or days 21–35 postinfarction (115, 160). It was found that Losartan decreased cardiac hypertrophy but did not improve performance in conscious rats as determined by monitoring the response to an infusion of Ringer's solution (115). DNA synthesis was also not blocked in their experiments (160). These data suggest that the dose was too low, since in SHR, Losartan at 15 mg kg^{-1} day^{-1} took 10 weeks to block both the vascular and cardiac hypertrophy (60). In addition, interpretation is made difficult by the observation that captopril is the only ACE inhibitor that increased performance (161). In SHR (60), aortic coarcted rats (114), and newborn pigs (162), Losartan acted like ACE inhibitors in blocking cardiac growth or hypertrophy, suggesting that the action of both classes of agents is to antagonize the actions of AII.

In stroke-prone SHR fed a high-sodium diet or given saline to drink, Losartan dramatically reduces morbidity and mortality (64, 163). In these animals the RAS is activated with increased mortality associated with renal and cerebrovascular lesions (164). In the diet-fed group, Losartan at 30 mg kg^{-1} day^{-1} via the drinking water increased survival at 12 weeks (84% in the Losartan group versus 26% in the untreated group). The Losartan treatment delayed the onset of hypertension but did not completely block the rise in pressure (64). In a separate study, Losartan at 15 mg kg^{-1} twice daily by gavage prevented the stroke and proteinuria associated with providing SPSHR with saline to drink (163). None of the vehicle-treated SPSHR survived the 18-day experiment, whereas all of the Losartan-treated animals survived (163). These results are similar to those observed with high-dose enalapril in the same model (165), which suggests that AII is the mediator of the stroke. Losartan and ACE inhibitors increased survival without preventing the development of hypertension. Likewise, in salt-loaded Dahl S rats, Losartan at 30 mg kg^{-1} day^{-1} in the drinking water greatly enhanced survival after 10 weeks (63% of the Losartan and 31% of the untreated controls), even though the blood pressures were not different in the two groups (62). The nature of this protective effect is as yet unexplained.

In a model of renal disease, Losartan appears to have an important pro-

tective effect (166). In rats with 85% renal ablation (remnant kidney model), Losartan at 8–16 mg kg^{-1} day^{-1} p.o. for 7 days significantly lowered systolic blood pressure and urinary protein excretion (74 ± 41 mg day^{-1} for Losartan versus 186 ± 70 mg^{-1} day^{-1} for the untreated controls) (166). In this setting, the protective effect of Losartan may relate to the reduction in pressure, in contrast to that observed in the SPSHR, in which pressure was not reduced. In rats with passive Heymann nephritis, Losartan at 6 mg kg^{-1} day^{-1} had little effect on blood pressure but, like enalapril, significantly decreased the albuminuria (167). In diabetic rats, Losartan lowered blood pressure and normalized glomerular pressure (168).

The use of RAS inhibitors as myocardial antiischemia agents has been suggested by a number of studies that show that ACE inhibitors reduce ventricular fibrillation in rats, pigs, and, importantly, humans (20). It has been suggested that in rats, part of this effect of ACE inhibitors is related to their ability to potentiate bradykinin (169). Losartan (2 μM), which blocks only the action of AII at its receptor, has been shown to reduce the median duration of ventricular fibrillation produced in isolated rat hearts by reperfusing after 40 min of no flow (147). Similar results were shown with the ACE inhibitor enalaprilat (20 nM) and the rat renin inhibitor CGP44099A (20 nM). The creatine phosphokinase release and recovery of left ventricular developed pressure was, however, not affected by any of the treatments (147).

The possible direct effects of AII on cardiac tissue have been suggested by the observation that there is a moderate to high density of AII-binding sites throughout the conducting system of the heart including the sinus node, atrioventricular node, and atrioventricular bundle (170, 171). Inhibitors of AII may exert part of their effect on the heart via an action on these conducting tissues. No direct effect of AII or of Losartan was noted, however, in isolated canine Purkinje fibers (122). Direct inotropic and chronotropic effects of AII have been demonstrated (172), and it has been recently suggested that in patients undergoing long-term treatment with ACE inhibitors, AII may play an important role (173). According to this hypothesis, the high circulating AI levels seen in these patients are converted to AII by an alternative (non-ACE) pathway, as evidenced by the ability of captopril to inhibit only 11% of the AII "forming activity" in tissue from normal and pathologic human hearts (173). The importance of this action of AII should be answered by the clinical trials of the nonpeptide AII receptor antagonists, which will presumably antagonize the effects of AII regardless of the source.

DEFINING AII RECEPTOR HETEROGENEITY

Losartan and PD123177 are the nonpeptide AII receptor antagonists which have become the prototypical AT_1 and AT_2 selective agents (3, 28, 174).

These two antagonists have been instrumental, along with the new peptide AT_2-selective peptide antagonist CGP42112A (175), in elucidating the AII "receptor" subtypes. The discovery of these selective antagonists has initiated a wealth of research directed toward identifying the occurrence and function of each subtype (see Table 3). As discussed above, Losartan blocks virtually all of the effects of AII but it does not completely inhibit AII binding (Tables 1 and 2). Although this research is in its early stage, several observations concerning the occurrence of these Losartan-sensitive (designated AT_1) and Losartan-resistant (designated AT_2) sites can be made: (*a*) the relative amount of AT_2 sites varies with both the species and the specific tissue being studied, (*b*) the AT_1 sites are localized in very discrete parts of the brain, and (*c*) the fetus contains high levels of AT_2-binding sites in tissues that do not express these sites in the adult animal.

The use of the new antagonists to define AII receptor heterogeneity and receptor subtypes (Table 3) stemmed from the observation that Losartan did not completely inhibit [^{3}H]AII or [^{125}I]AII binding to isolated adrenal cortical membranes not containing DTT (174–176). Losartan was shown to inhibit AII binding virtually 100% in some tissues (e.g. the rat aorta), virtually 0% in other tissues (e.g. the rat adrenal medulla) and 59–60% in yet other tissues (e.g. the heart) (27, 174, 175). Several different designations have been used for these AII receptor subtypes, including type A and B (175), type 1 and 2 (177), AII-1 and AII-2 (174), and AII_α and AII_β (178). Recently, an ad hoc committee of the High Blood Pressure Countil–American Heart Association proposed a new nomenclature in which the abbreviation for the angiotensin receptor is AT with the subtypes being designated by numbered subscripts,

Table 2 In vivo responses to AII (AT_1) blocked by Losartan

Response[a]	Species	Dose (mg kg^{-1})	Reference
Pressor response	Rats, pithed	10 i.v.	22
	Rats, conscious	1, 3 i.v.; 1, 3, 10 p.o.	39
	Rats, conscious SHR & WKY	?	69
	Rats, myocardial infarcted	40 mg kg^{-1} day^{-1}, p.o.	115
	Dogs, conscious (salt depleted)	30, 100, 300, 1000 μg kg^{-1} min^{-1} i.v.	44
	Dogs, conscious hypertensive	3, 10, 30 p.o.	89
(+) Aldosterone release	Rats, conscious	10 i.v.	68
	Rats (SHR and WKY rats)	?	69
(−) Renin release	Rats, monkeys	5, 10 i.v.	96
(+) Vasopressin release	Rat, conscious	0.7 μg[b], i.v.t.	200
(+) Drinking	Rats, conscious	3, 10 s.c.	68
Sympathetic nerve functions	Dogs, renal artery	10, 30, 100 μg kg^{-1}, i.a.[b]	92

[a] (+) Stimulation, (−) inhibition.
[b] Total dose: i.v.t. = intraventricular; i.a. = intraarterial

Table 3 Occurrence of receptor subtypes defined by inhibition of binding studies with nonpeptide AII receptor antagonists

Tissue	Predominant AII receptor subtype		
	AT_1	AT_2	Both AT_1 and AT_2
Kidney			
Cortex	Rat (96), rabbit[a]		Monkey (96)
Glomeruli	Rat (201), rabbit (207)		
	Human (202)		
JG apparatus			Monkey (96)
Mesangial cells	Rat (203, 204) human[b]		
Vascular			
Aorta	Rat (205), rabbit (206)[a]		Rat[a], monkey[a]
	Pig (153)		
Pulmonary artery	Rat (194)		
Adrenal (whole)	Rabbit (34)		Rat (177)
Cortex	Rabbit, monkey[a]		Rat (175, 177)[a]
	Rat, dog (208)		Human (175)
Medulla	Cow (31, 208)	Rat (174, 209)	Rat (177)
Heart	Rat[c], monkey[a]		Rat (182), rabbit (210)[a]
Brain (whole)	Rabbit, monkey[a]	Rat (194)	Rat[a]
Circumventricular organs (SFO)	Rat (179)		
Nucleus solitary tract	Rat, human (201)		
Pituitary	Rat (211)		
Ventrolateral medulla	Human (201)		
Hypothalamus	Rat (166)		
Superior colliculus		Rat (158, 201)	

Thalamus		Rat (211)	
Subthalamic nucleus	Human (201)	Rat (158, 201)	
Locus ceruleus	Human (201)	Rat (158, 201)	
Inferior olive		Rat (158, 211)	
		Human (201)	
Cerebellum		Rat (179), cow (180), human (201)	
Reproductive organs			
Ovarian granulosa cell		Rat (212)	
Uterus	Rat (209)	Rabbit (34), human (175, 206)	Rat (175)
Liver	Rat (34, 178, 209)		
Miscellaneous			
Fetus		Rat (213)	
Fibroblasts (fetal skin)			(183)
7315C cells	(35)		
Swiss 3T3 cells		(214)	
PC12 cells		(178)	
NG108-15 undifferentiated	(152)		
NG108-15 differentiated		(152)	
Neuronal cells (1 day)		Rat (198)	
Astrocytic gial cells	Rat (198, 199)		

[a]R.S.L. Chang, personal communication.
[b]R. Ardaillou, personal communication.

e.g. AT_1 or AT_2 (28). Losartan and DTT bind preferentially to AT_1 receptor subtype, whereas PD123177, CGP42112A, and pNH_2-Phe^6-AII preferentially bind to AT_2 receptor subtype.

With these selective antagonists as tools, two results have emerged. First, the relative proportion of AT_1 and AT_2 subtypes varies greatly across species and across tissues within species (Table 3). Second, most all of the known effects of AII are functionally coupled to the AT_1 receptor subtype (3, 25, 27).

AT_1 receptor subtypes predominate in virtually all vascular tissue, in the adrenal cortex of most species, and certain areas of the brain such as the circumventricular organs, e.g. the subfornical organ (27, 174, 175, 177). AT_2 receptor subtypes predominate in the adrenal medulla of the rat and specific parts of the brain such as the locus ceruleus, thalamus, and cerebellum (179, 180). It should be noted that the distribution of AII receptor subtypes differs significantly between rats and humans, e.g. the subthalamic nucleus and locus ceruleus contains AT_1 sites in humans and AT_2 sites in rats (Table 3). In most other tissues, such as adrenal cortex and kidney, there is a mixture of the two receptor subtypes. Importantly, the occurrence and distribution of AT_1 and AT_2 receptor subtypes change markedly with maturation (181, 182). Some tissues abundantly express AT_2 sites in the fetus but do not express these sites soon after birth (181). The ratio of AT_1 to AT_2 may also change with the "passage" of cultured cells, as has been shown with fetal fibroblasts (183).

Functionally, the well-known actions of AII are coupled to the AT_1-binding site (Tables 1 and 2). Furthermore, as discussed above, the actions of AII on the second-messenger cyclic AMP (cAMP), phosphoinositol, and Ca^{2+} systems are virtually all antagonized by Losartan (30, 32, 33, 35). The potential importance of an alternative functional site, however, remains high, and efforts continue to explore further receptor subtypes that are Losartan or PD123177 insensitive, such as the *mas* oncogene (184) and the amphibian AII receptors expressed in *Xenopus* oocytes (185).

Possible intracellular actions of AII have been proposed (186). The potent binding affinity of Losartan and subsequent nonpeptide antagonists for AII receptors may allow the monitoring of AII receptor through its synthesis, membrane insertion, and recycling. A cytosolic AII (actually Sar^{1}Ile^8-AII)-binding site from rat liver homogenates has been identified, but this site is not bound by either Losartan or PD123177 (187). Losartan-sensitive sites in the nuclear fraction of rat liver cells have also been identified (188). The functional importance of these sites is not known, but it suggests that still further research may be warranted.

Losartan and PD123177 also play a role in characterizing the products of angiotensin receptor cloning. The angiotensin receptors from rat vascular smooth muscle cells (189), bovine adrenal cortical cells (190), and kidney

cells from SHR (191) have been sequenced. The binding of AII and the AII-induced CA^{2+} transients of these "receptors" expressed in COS7 cells are antagonized by Losartan but not by PD12377.

HUMAN PHARMACOLOGY

Losartan is currently undergoing clinical trials and some early results have been reported (97, 192). In this initial trial in healthy male volunteers, Losartan at 40 mg day^{-1} p.o. produced similar reductions of the AII pressor response on days 1, 4, and 8 (192). There was a dose-related increase in plasma renin activity, as seen with ACE inhibitors (95). Losartan was well tolerated, and an evaluation in hypertensive patients is under way. On the basis of the animal data and the early clinical results, it seems highly probably that Losartan will lower blood pressure in renin-dependent (ACE inhibitor–sensitive) hypertension. It will be some time before sufficiently large comparative studies with ACE inhibitors to establish any advantage of efficacy or safety are completed.

FUTURE PERSPECTIVES

Losartan is the first nonpeptide AII receptor antagonist that has reached clinical trial, and it is much too early to know whether it will become an important therapeutic agent. It is clear, however, that new compounds will follow, such as the biphenyl tetrazoles DuP 532 and L-158,809 and the nonbiphenyl tetrazole SKF 108566 (24). What will the newer compounds add? "Improved" pharmacokinetics and/or tissue selectivity will be issues with newer angiotensin receptor antagonists, as they are with the current rush of ACE inhibitors (11). Although Losartan is clearly AT_1 selective and there is no clear function for the AT_2, efforts to identify AT_2-specific antagonists will continue. Would an inhibitor that blocked both receptor subtypes be preferable? Are there AT_1 receptor subtypes that could be selectively inhibited? Are there as yet unknown angiotensin receptor subtypes? Answering these questions will continue to fuel AII receptor antagonist discovery programs for the foreseeable future. Each new receptor antagonist may then be used to further clarify the actions of AII and the therapeutic consequence of blocking these actions.

ACKNOWLEDGMENTS

We acknowledge the assistance of Jacque Connor in acquiring the information and in preparing the manuscript.

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