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Angiotensin II Receptors and Angiotensin II Receptor Antagonists

PIETER B. M. W. M. TIMMERMANS, PANCRAS C. WONG, ANDREW T. CHIU, WILLIAM F. HERBLIN, PAMELA BENFIELD, DAVID J. CARINI, ROBERT J. LEE, RUTH R. WEXLER, JO ANNE M. SAYE, AND RONALD D. SMITH*

The Du Pont Merck Pharmaceutical Company, Wilmington, Delaware 19880–0400

* Address for correspondence: Discovery Research, Experimental Station, P.O. Box 80400, Wilmington, DE 19880–0400.

I. Introduction

The cloning and sequencing of Ang II† receptors (Murphy et al., 1991; Sasaki et al., 1991) and the discovery of selective Ang II receptor antagonists (Duncia et al., 1992; Blankley et al., 1991) has brought a new level of understanding to the biology of Ang II. It is now possible to specifically characterize the multiple actions of Ang II at its specific receptor sites. Four epochs in the investigation of the role of the renin system in cardiovascular disease have been described with the fourth being the development of specific renin system probes (Cody, 1992). The multiplicity of endocrine, paracrine, autocrine, and possible intracrine effects of Ang II acting on virtually every cellular/tissue/organ system have now been defined (Rhee and Lee, 1991; Paul et al., 1992). Many of the technical difficulties that have limited the measurement of Ang II levels in plasma have been resolved (Nussberger et al., 1988; Chauveau et al., 1992). These new techniques have allowed the quantification of the basal and drug-induced changes in the angiotensin II system (both at the circulatory and tissue level) (fig. 1) (Schalekamp, 1991; Gohlke et al., 1989).

Our current concept of Ang II receptors has evolved through a great number of experimental observations with Ang II inhibitors (for reviews, see Peach, 1972; Phillips, 1987; Hall, 1991; Ferrario, 1990; and table 1 for highlights). The Ang II "inhibitors" include (*a*) inhibitors of Ang synthesis, e.g., renin inhibitors (Thaisrivongs, 1992; Greenlee and Siegl, 1991), ACE inhibitors (Raia et al., 1990; Salvetti, 1990); (*b*) inhibitors of Ang II itself, e.g., antibodies (Wong et al., 1991e; Dagenais and Escher, 1992); (*c*) modulators of receptor affinity state, e.g., DTT, guanosine 5′-O-(5-thiophosphate) (Chiu et al., 1989b; McQueen and Semple, 1991); (*d*) antibodies that compete for Ang II receptor-binding sites (Reilly et al., 1988); and (*e*) receptor-mimicking peptide (Budisavljevic et al., 1992). The nonpeptide Ang II receptor antagonists have proven especially useful tools with which to explore the actions of Ang II and to define Ang II receptors.

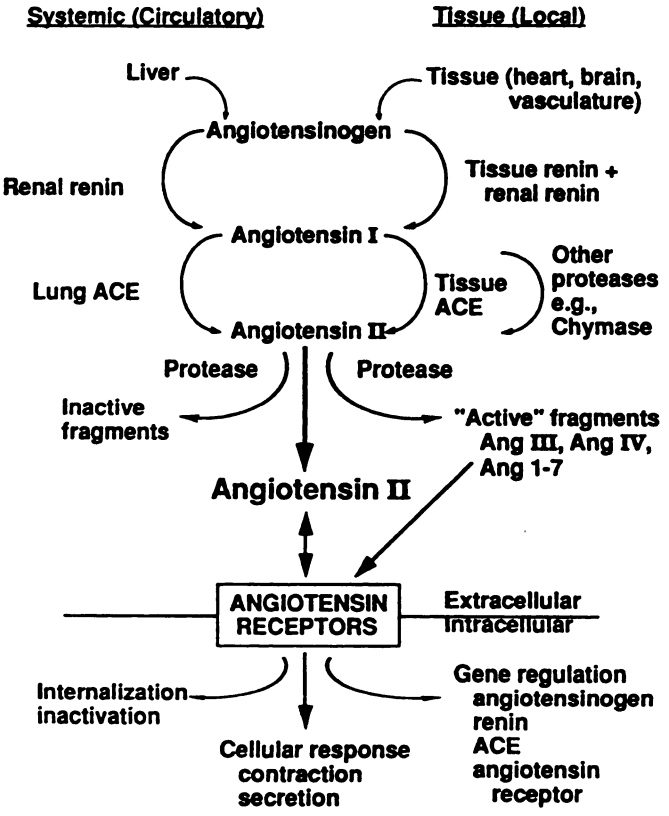


FIG. 1. The angiotensin system.

Although peptide analogs of Ang II were shown to be potent and specific antagonists of Ang II at its receptor (Peach and Chiu, 1974), their short action and partial agonist properties limited their application as research tools and therapeutic agents. The breakthrough came with the independent discovery of two unique series of nonpeptide compounds that interfere with Ang II at its binding site. The prototypes of these two series of compounds are losartan (DuP 753, MK-954) (Duncia et al., 1992) and PD123177 (and the related PD123319) (Blankley et al., 1991). Use of these compounds as research

† Abbreviations: Ang II, angiotensin II; ACE, angiotensin-converting enzyme; DTT, dithiothreitol; AT_2, AT_3. . .AT_n.? angiotensin II receptor subtypes; AT_1, angiotensin II receptor subtype sensitive to losartan; pA_2, the log of the molar concentration of antagonist that reduces the effect of a 2-fold higher concentration of agonist (Ang II) to that of a single concentration (Rhaleb et al., 1991), DOPAC, 3,4-dihydroxyphenylacetic acid; PRA, plasma renin activity; NO, nitric oxide; ANF, atrial natriuretic factor; GFR, glomerular filtration rate; i.c.v., intracerebroventricular; PVN, paraventricular nucleus; EDRF, endothelium-derived relaxing factor; NAME, L-*n*-monomethylarginine (N^G-monomethyl L-arginine); RVR, renal vascular resistance; RBF, renal blood flow; ANF, atrial natriuretic factor; $t_{1/2}$, half-life.

TABLE 1
Highlights of the evolution of current knowledge of Ang II and its receptor

Year	Highlight	Reference
1836	Postulated association between renal disease and hypertension	Bright, 1836
1898	Demonstrated pressor substance in renal extracts	Tigerstedt and Bergman, 1898
1934	Renal artery constriction produced hypertension in dogs	Goldblatt et al., 1934
1940	Independently showed that renin acted on plasma substrate to produce pressor hormone	Page and Helmer, 1940 "angiotensin" Braun-Mendez et al., 1940 "hypertension"
1956	"Hypertensin" peptide isolated	Peart, 1956
1956	Hypertensin II formed by action of converting enzyme	Skeggs et al., 1956
1957	Independently synthesized the octapeptide "Ang II"	Bumpus et al., 1957 Schwyzer et al., 1957
1968	Ang II shown to regulate aldosterone release	Gross, 1968
1971	Saralasin introduced as the first peptide Ang II receptor antagonist	Pals et al., 1971
1972	Pepstatin shown to inhibit the renin-Ang reaction	Gross et al., 1972
1972	Propranolol inhibition of renin release	Buhler et al., 1972
1972	Teprotide, the first peptide ACE inhibitor, described	Engel et al., 1972
1975	Ang II shown to stimulate cell growth in 3T3 mouse cells	Ganten et al., 1975
1977	Captopril, the first nonpeptide ACE inhibitor described	Cushman et al., 1977
1988	Losartan, the first orally active nonpeptide Ang receptor antagonist is described	Carini and Duncia, 1988
1989	Independently confirmed Ang receptor heterogeneity with nonpeptide antagonists	Chiu et al., 1989a Whitebread et al., 1989
1991	Ang II AT_1 receptor cloned	
	Rat vascular	Murphy et al., 1991
	Rat kidney	Iwai et al., 1991
	Bovine adrenal	Sasaki et al., 1991
1992	Ang II AT_1 receptor subtypes cloned	Kakar et al., 1992a Iwai and Inagami, 1992 Sandberg et al., 1992

tools has greatly expanded the concept of Ang II receptor heterogeneity (see section III).

The clinical efficacy of inhibiting Ang II synthesis with ACE inhibitors stimulated international interest in Ang II research, but the effects of these agents to potentiate bradykinin could not be ruled out (Zusman, 1987; Ujhelyi et al., 1989). The current data reviewed here suggest that the majority of the actions of ACE inhibitors are due to blocking Ang II synthesis (Smith et al., 1992a).

Molecular biological and biotechnological methods have been applied to confirm the presence of the components of the synthetic pathway of Ang II in specific tissues, to characterize the tissue (or local) Ang II system (Paul et al., 1992; Samani and Swales, 1991), and to clone and sequence the Ang II receptors from different species (Murphy et al., 1991; Sasaki et al., 1991). The gene for the human Ang II receptor has been cloned (Furuta et al., 1992). Most of what we know about Ang II is associated with AT_1 receptor subtypes (Smith et al., 1992a; Timmermans et al., 1992b). Although the AT_2 receptor has not been cloned and much of its physiological function remains obscure, a number of observations suggest possible functions (see section III.C).

Losartan is the first of a new class of pharmacological and therapeutic agents, and as such has been widely studied (Duncia et al., 1992). Many new nonpeptide AT_1-selective Ang II-selective antagonists have been synthesized (see section II.B; figs. 5 and 6), but the biological data are limited. The present discussion will focus primarily on the findings with losartan (DuP 753/MK-954) and with PD123177 (or PD123319), because they have been used to define Ang II heterogeneity and to study the physiological and pathophysiological roles of Ang II.

II. Nonpeptide Angiotensin II Receptor Antagonists

A. Discovery of Nonpeptide Receptor Antagonists

The Ang II receptor was the first target of efforts to inhibit the Ang II system, and many peptide analogs of Ang II itself were synthesized and shown to have varying degrees of agonist and antagonist properties. Saralasin (Sar^1Ile^8-Ang II) is a prototype of this type of receptor antagonist. It was extensively studied preclinically (Castellion and Fulton, 1979) and was shown to lower blood pressure in humans (Case et al., 1979). However, the need for parenteral administration and the presence of agonist effects in 23% of the patients (Anderson et al., 1977) limited the therapeutic utility of this drug. Early attempts to identify nonpeptide antagonists failed to yield acceptable compounds.

The breakthrough to the current series of Ang II receptor subtype-selective agents came with the publication of patents describing the hypotensive imidazole-5-acetic acid derivatives that antagonized Ang II vasoconstriction (Furakawa et al., 1982). These patent publications led two independent research groups to design novel series of molecules, which led to the discovery of losartan and PD123177.

The discovery of losartan at the Du Pont Merck Pharmaceutical Company was previously described in detail (Duncia et al., 1992; Timmermans et al., 1991). The highlights of the progress that was made in chemically modifying the lead compound are shown in figure 2.

The lead compounds, S-8307 and S-8308, that appeared in patents issued to Takeda Ltd. (Furakawa et al., 1982) had extremely weak antihypertensive effects but were specific for the Ang II receptor (Wong et al., 1988; Chiu et al., 1988). Analysis of these molecules led to the postulate that (*a*) both Ang II and the lead compounds bound at the same receptor site (fig. 3), (*b*) the core structure of the leads must be enlarged to better mimic Ang II and its points of attachment to the recep-

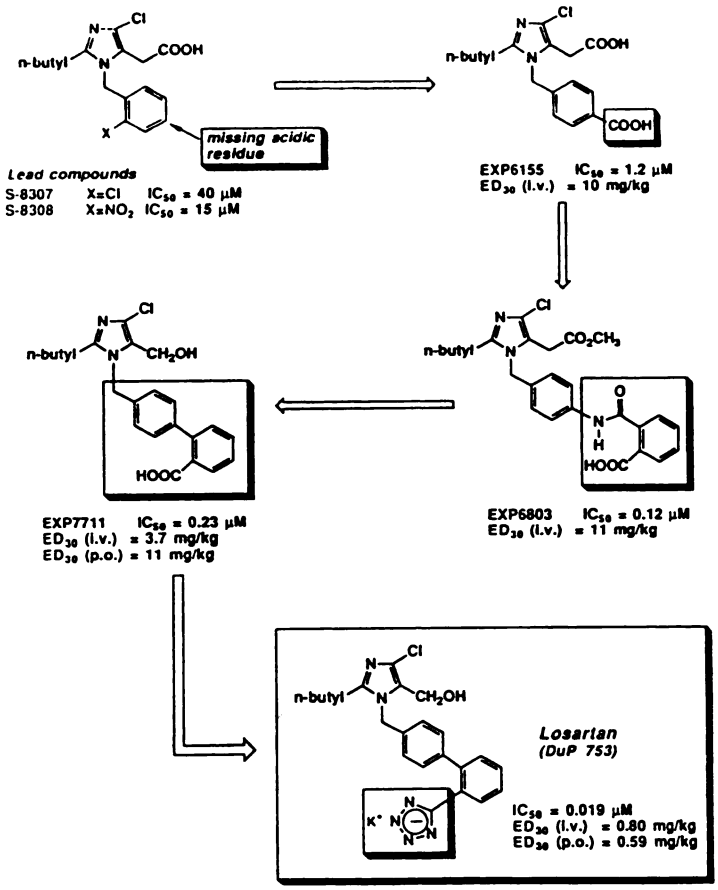


FIG. 2. The discovery of losartan.

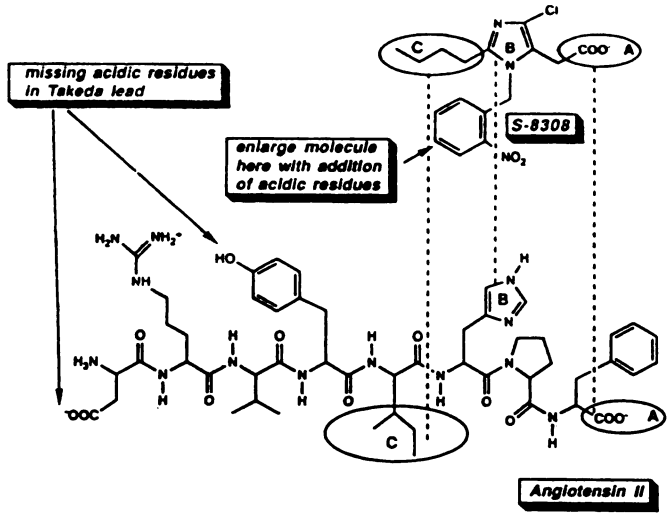


FIG. 3. Designing novel Ang II receptor antagonists based on the Takada lead and the structure of Ang II. Overlap of the lead compound S-8308 onto the structure of Ang II. A, B, and C denote areas of overlap.

tor, and (*c*) both the COOH group of the leads and the COOH-terminal group of Ang II exist as anions at physiological pH and most likely bind to a positive charge in the receptor (Duncia et al., 1990).

Both Ang II and the core structure of S-8307 and S-8308 were overlapped as closely as possible using computer modeling: (*a*) the carboxylate anions, both pointing to the positive charge in the receptor; (*b*) the imidazole rings of histidine and of the lead; and (*c*) the isoleucine side chain with the *n*-butyl side chain of the lead (Duncia et al., 1990). The benzyl group of the lead compound was pointed toward the NH_2 terminus of Ang II. It was believed that the receptor-binding information missing from the leads resided in the NH_2-terminal portion of Ang II. Because Ang II contains two acidic residues near the NH_2 terminus that are not mimicked by the lead compounds, namely, aspartic acid's β-COOH group and tyrosine's hydroxyl group, it was hypothesized that what was missing in the lead compounds was an additional acidic functionality (Duncia et al., 1990). Thus, substitution of an additional COOH group at the *para* position resulted in EXP6155, which had a binding affinity 10-fold greater than that of S-8308 (Duncia et al., 1990).

The addition of a phenyl ring, as occurs in EXP6803, increased the binding affinity by yet another order of magnitude (Duncia et al., 1990). Replacement of the amide bond with a carbon-carbon single bond yielded the more lipophilic biphenyl-containing EXP7711, which for the first time exhibited good p.o. activity (Carini et al., 1991). Finally, while searching to replace the polar COOH group with a more lipophilic or more metabolically stable isostere, Carini et al. (1991) discovered that the tetrazole group increases the intrinsic activity by an

order of magnitude while maintaining good p.o. bioavailability. This compound was designated DuP 753.

The second series of nonpeptide Ang II receptor antagonists was discovered at Warner Lambert-Parke Davis Pharmaceutical Company. They had previously found that 1-substituted-5-acyl derivatives of the natural product spinacine displayed Ang II receptor-binding activity (Blankley et al., 1991) (fig. 4). Following publication of the Takeda patent (Furakawa et al., 1982), they synthesized a series of 1-benzyl spinacine analogs. This series of tetrahydro-1H-imidazol[4,5-c]pyridine-6-carboxylic acids, exemplified by PD123177, was described as having antihypertensive activity via blockade of Ang receptors (Blankley et al., 1991). It is now known that these compounds selectively bind to AT_2-subtype receptors (Chiu et al., 1989a; Whitebread et al., 1989) and have no significant effect on blood pressure (Wong et al., 1990a). This series of AT_2-selective antagonists was chosen because the binding assay contained 5 mM DTT which interferes with AT_1 binding (Chiu et al., 1989b; Gehlert et al., 1991a). Although the therapeutic utility of these compounds is unknown, they have proved to be valuable tools in characterizing Ang II heterogeneity.

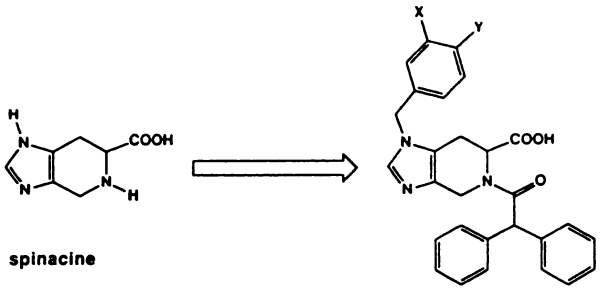
B. Evolving Class of Nonpeptide Angiotensin II Receptor Antagonists

The disclosure of the patent describing losartan (Carini and Duncia, 1992) resulted in an international effort to discover novel, nonpeptide antagonists. The methods utilized for the synthesis and preclinical and clinical testing for losartan have guided the development of these compounds (Smith et al., 1992b). As with the ACE inhibitors, these compounds are being selected on the basis of greater potency, greater bioavailability, and difference in tissue distribution. In the case of ACE inhibitors, these "differences" have not proven therapeutically important. It is too early to know whether the same will apply to nonpeptide receptor antagonists.

Losartan has a biphenyl tetrazole moiety that is critical for its Ang II-binding properties. Most of the early worldwide synthetic effort has been directed at molecules that contain this moiety (fig. 5). The imidazole portion of the losartan molecule has been substituted with a variety of heterocycles, and good Ang II binding is retained (see review of recent patents by Bovy and Olins, 1992).

Losartan has affinity for specific Ang II receptors, competitively inhibits the contractile response of vascular smooth muscle in vitro, competitively inhibits the pressor responses to exogenously administered Ang II in vivo, and lowers blood pressure in Ang II-dependent hypertension by blocking endogenously released Ang II at its receptor (Chiu et al., 1991b; Wong et al., 1991d). In addition, losartan is orally bioavailable and has no demonstrated agonist activity like that observed with the peptide antagonist (see section VII). The basic properties of losartan have provided the bench mark against which new compounds are compared (Table 2).

The imidazole carboxylic acid, DuP 532 (fig. 5) is a potent antagonist of Ang AT_1 receptors and possesses good antihypertensive activity with a long duration of action. DuP 532 potently inhibits the specific binding of ^{125}I-radiolabeled Ang II to rat isolated adrenal cortical microsomes with an IC_{50} of 3.1 nM (Chiu et al., 1991a). The contractile response of the isolated rabbit aorta to Ang II was selectively and noncompetitively antagonized by DuP 532 (Wong et al., 1991a). DuP 532 (0.03 to 1 mg/kg, i.v., or 0.3 to 10 mg/kg, p.o.) abolished the Ang II-induced pressor effect, aldosterone secretion, and drinking response in conscious normotensive rats but did not affect basal blood pressure or heart rate in doses up to 100 mg/kg. In conscious renal hypertensive rats, DuP 532 decreased mean arterial pressure when given i.v. and p.o. with ED_{30} values of 0.02 and 0.21 mg/kg, respectively (Wong et al., 1991a). The antihypertensive effect of 0.3, 1, and 3 mg/kg given p.o. lasted for at least 24 h. In conscious SHR and in conscious furosemide-treated dogs, DuP 532 dosed i.v. or p.o. (0.3 to 3 mg/kg) reduced



PD121981 (WL-19): X=CH_3; Y=OCH_3
PD123177 (WL-13): X=CH_3; Y=NH_2
PD123319 (WL-15): X=CH_3; Y=$N(CH_3)_2$
PD124125 (WL-75): X=CH_3; Y=H

FIG. 4. The development of the PD123177 series of nonpeptide AT_2-selective Ang II receptor antagonists from the structure of spinacine.

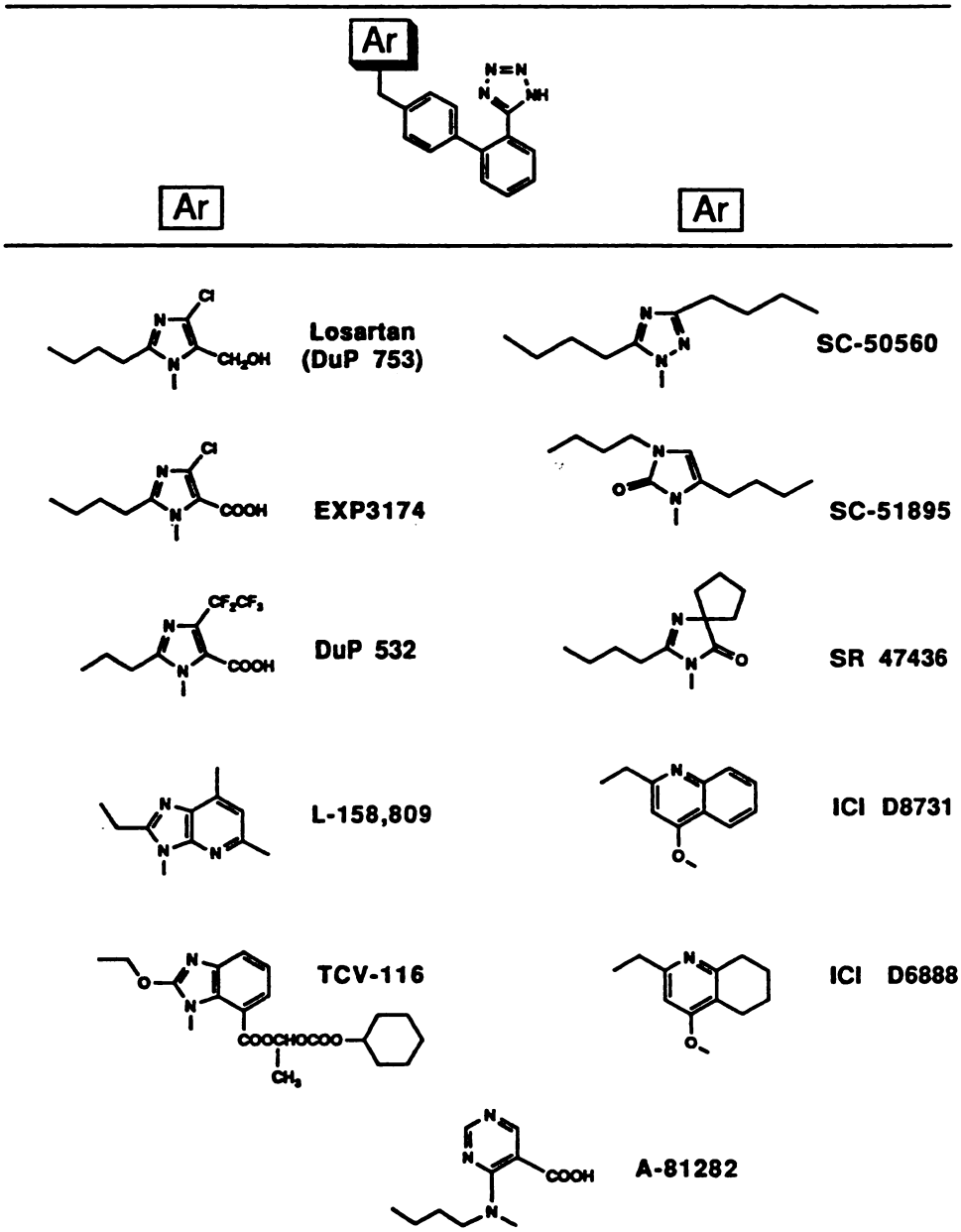


FIG. 5. Structures of losartan and other new biphenyl tetrazole nonpeptide Ang II receptor antagonists.

blood pressure dose dependently. These studies indicate that DuP 532 is a potent, orally active, and selective Ang II receptor antagonist. Its pharmacological properties are very similar to those of EXP3174, except that DuP 532 is more potent orally (table 2).

L-158,809 is a biphenyl tetrazole in which the imidazole ring is fused to another heterocyclic ring (fig. 5) (Mantlo et al., 1991) exhibiting a high potency for AT_1 receptors (displaced ^{125}I-Sar^{1}Ile^8-Ang II) in the vasculature, adrenal, heart, kidney, liver, and brain of several animal species including rat, rabbit, monkey, and human tissues (IC_{50} = 0.2 to 0.8 nM) (Chang et al., 1992). L-158,809 demonstrated a very high selectivity for AT_1 relative to AT_2 receptors (IC_{50} >10 μM in rat brain). L-158,809 lacked agonist activity and acted as a specific antagonist of Ang II-induced contractile responses in isolated rabbit aorta and rat pulmonary arteries as well as Ang II-mediated aldosterone release in rat adrenal cortical cells (pA_2 = 10.5) (Chang et al., 1992). In conscious normotensive rats, L-158,809 exhibited a dose-related inhibition of Ang II-induced pressor responses with a duration of action greater than 24 h after a single dose (0.1 mg/kg, i.v., or 0.3 mg/kg, p.o.). In rats with high renin hypertension (aortic coarctation), at single doses of 0.1 and 0.3 mg/kg, p.o., L-158,809 reduced the mean arterial blood pressure to normotensive levels (−60 and −80 mm Hg, respectively) with a duration of action exceeding 24 h. In the coarctated rat model and in volume-depleted rhesus monkeys, L-158,809 elicits hypotensive responses and efficacies similar to those of ACE inhibitors. L-158,809 did not elicit a hypotensive response in animals with low renin hypertension

TABLE 2

Pharmacological characterization of AT_1-selective nonpeptide Ang II receptor antagonists

Compound	Receptor binding IC_{50} [nM (tissue)]	Ang II antagonism rabbit aorta (nM)	Ang II antagonism in vivo rat "effective" dose		Blood pressure lowering renal hypertensive rat effective dose		Reference
			i.v.	p.o.	i.v.	p.o.	
Losartan	19 (rat adrenal cortex)	$pA_2 = 10$			0.78	0.59	Chiu et al., 1990c; Wong et al., 1991d
EXP3174	37 (rat adrenal cortex)	$K_B = 0.1$			0.04	0.66	Wong et al., 1990c
DuP 532	3.1 (rat adrenal cortex)	$K_B = 0.11$	0.03–1.0	0.03–10	0.02	0.21	Wong et al., 1991a; Chiu et al., 1991a
L-158,809	0.49 (rat whole adrenal)	$IC_{50} = 0.3$	0.029	0.023		0.1	Chang et al., 1992; Siegl et al., 1992
SC51895	12 (rat uterus)	$pA_2 = 8.6$		2.7 (intragastric)			Olins et al., 1992a
SC51316	5.1 (rat uterus)	$pA_2 = 8.62$					Olins et al., 1992b
SR47436	1.3 (rat adrenal)	$IC_{50} = 4$	0.1–3	0.3–30			Nisato et al., 1992
ICI D8731	30.7 (guinea pig adrenal)	$pA_2 = 8.3$	1.0	5.0			Oldham et al., 1992
GR117289	pKi = 7.5 (rat liver)	$pK_B = 9.8$			0.3–3 (intraarterial)	0.3–10	Marshall et al., 1991; Drew et al., 1992
SK&F 108566	1.5 (rat mesenteric artery)	$K_B = 0.26$	0.08	5.5 (intradermal)			Weinstock et al., 1991; Edwards et al., 1992b
TCV-116 (CV-11974)		$pA_2 = 9.97$	0.01	0.03		0.03	Shibouta et al., 1992; Wada et al., 1992
A-81282	$K_i = 2.98$ (rat liver)	$pA_2 = 9.64$		10	0.08	2.2	Lee et al., 1992; Buckner et al., 1992
CGP48933	8.9 (rat aorta)	$IC_{50} = 1.4$		3–10		3–10	Criscione et al., 1992

(deoxycorticosterone acetate-salt rats) (Siegl et al., 1992; Steckelings et al., 1992b). In conscious SHR, L-158,809 is a potent antihypertensive agent with long duration of action (exceeding 24 h), and it also possesses diuretic and natriuretic properties (Kivlighn et al., 1992).

SC-50560 is an example from a series of compounds in which the imidazole is replaced with a 1,2,4-triazole (Reitz et al., 1992). SC-50560 (fig. 5) is a potent Ang II antagonist both in vitro and in vivo (table 2). SC-51895 (fig. 5), an imidazolinone, is an example from several series of compounds in which the imidazole is replaced by an oxoheterocycle (Olins et al., 1992a). In both rat adrenal cortical and uterine membrane preparations, SC-51895 bound to the AT_1 receptor with an IC_{50} of 12 nM. In vitro, SC-51895 (0.1 mM) did not inhibit PRA or ACE. In anesthetized, ganglion-blocked rats, the pressor response to an infusion of Ang II was blocked by i.v. administration of the compound with an ED_{50} of 0.07 mg/kg. In SHR (30 mg/kg/day, intragastrically, for 4 days), SC-51895 lowered mean arterial pressure as effectively as enalapril (10 mg/kg/day, intragastrically, for 4 days) (Olins et al., 1992a).

SR47436, a 4-spirocyclopentane-imidazolin-5-one, is a potent and selective AT_1 receptor antagonist that inhibits the binding of ^{125}I-Ang II in rat liver membranes with an IC_{50} of 1.3 nM and does not inhibit the AT_2 receptors at concentrations up to 10 μM (rat adrenal cortical membranes). In the same preparation, the affinity of SR47436 (IC_{50} = 1.3 nM) exceeded that of losartan (IC_{50} = 14 nM) and was approximately equivalent to saralasin (IC_{50} = 2.4 nM). In conscious cynomolgus monkeys, SR47436 dosed at 1 mg/kg is at least 10-fold more potent than losartan. SR47436 antagonized the Ang II-induced hypertension by 89% and 66% after i.v. and p.o. administration, respectively, compared to losartan which, under similar conditions, at 10 mg/kg inhibited this hypertension by 83% and 20%, respectively (Nisato et al., 1992).

ICI D8731 is one of a series of quinolines that (fig. 5) is in phase II clinical trials as a selective AT_1 receptor antagonist (table 2). When dosed p.o. at 5 mg/kg, ICI D8731 inhibited the Ang II pressor response by 70.0 ± 5.4%, and 51.0 ± 5.5% at 1 and 5 h after dosing, respectively. In this model, the effectiveness of the compound was maintained when given once daily at 5 mg/kg/day for 10 days (Oldham et al., 1992).

ICI D6888 (fig. 5) represents an extension of ICI D8731 and also is in phase I clinical trials. The compound appears to have a greater binding affinity and potency in vitro (table 2). The p.o. efficacy of the two compounds in rats, however, is similar (Edwards et al., 1992a).

TCV-116 is an inactive ester prodrug that is rapidly converted in vivo to CV-11974. TCV-116 (CV-11974) is highly selective for Ang II receptors in vitro and produces long-acting Ang II antagonism (Shibouta et al., 1992) and antihypertensive effects in renal and genetically hypertensive rats (Wada et al., 1992). TCV-116 had no effect on blood pressure in deoxycorticosterone acetate-salt hypertensive or normotensive rats (Wada et al., 1992).

GR117289 (fig. 6) is a biphenyl replacement in which

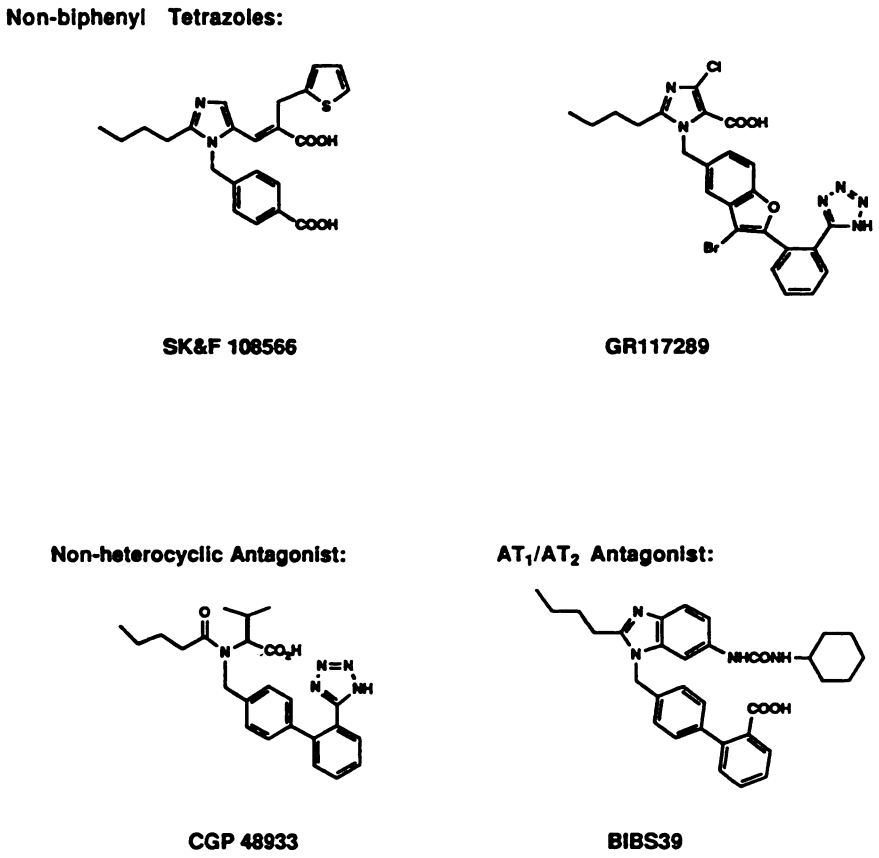


FIG. 6. Structures of novel non-biphenyl tetrazole nonpeptide Ang II receptor antagonists.

the inner phenyl ring is replaced with a bromobenzofuran. GR117289 is a potent and slowly reversible competitor for the AT_1-binding sites in the rat liver in competition-binding studies using ^{125}I-Sar^{1}Ile^8-Ang II (pK_i = 9.0) (Marshall et al., 1991). In rabbit aorta, GR117289 is a potent antagonist of the vasoconstrictor effects of Ang II but, unlike losartan, causes insurmountable suppression of the Ang II-induced contraction (Hilditch et al., 1991). In renal artery-ligated hypertensive rats, GR117289 (0.3 to 3 mg/kg, intraarterially, or 0.3 to 10 mg/kg, p.o.) caused dose-dependent, long-lasting decreases in diastolic blood pressure with minimal (<10%) effects on heart rate (Hilditch et al., 1991).

A-81282 (fig. 5) is one of a series of compounds in which the imidazole is replaced with a 4-aminopyrimidine. A-81282 has been identified as a potent and selective (Buckner et al., 1992) competitive AT_1 receptor antagonist (table 2). In renal artery-ligated hypertensive rats, A-81282 caused a dose-dependent decrease in mean arterial pressure. At 10 mg/kg, p.o., A-81282 normalized blood pressure in the renal artery-ligated rat for greater than 24 h with no effect on heart rate. At this dose, A-81282 had no effect on blood pressure in the conscious normotensive rat (Lee et al., 1992).

SK&F 108566 (fig. 6), an imidazole-5-acrylic acid derivative, is representative of one of the only series to date that was designed independently from the benzylimidazole reported by the Takeda group (Furakawa et al., 1982). SK&F 108566 deviated from the Fermandjian model for the bioactive conformation of Ang, and it was hypothesized that the N-benzyl and carboxyl group of the Takeda compounds corresponded to the Tyr^4 aromatic side chain and the Phe^8 carboxyl group of Ang II (Weinstock et al., 1991). By retaining the simple N-benzyl functionality and substituting the acetic acid side chain in position 5 of the imidazole with an α-thienylacrylic acid moiety, or, in other words, by more closely mimicking the COOH-terminal region of Ang II, they were able to enhance binding affinity substantially over that obtained with the Takeda compound and were also able to demonstrate p.o. antihypertensive activity. SK&F 108566 is a potent, AT_1-selective antagonist that exhibited competitive inhibition of ^{125}I-Ang II (0.08 nM) binding to rat mesenteric artery and adrenal cortical membranes (IC_{50} = 1.5 and 9.2 nM, respectively). In conscious normotensive rats, SK&F 108566 administered via bolus i.v. injections produced a dose-dependent inhibition of pressor responses to Ang II (250 ng/kg, i.v.) with an ID_{50} of 0.08 mg/kg. When administered intradermally, a dose-dependent inhibition of the pressor response to Ang II was observed with an ID_{50} of 5.5 mg/kg, and with the highest dose, 10 mg/kg, significant inhibition was still observed 3 h after dosing (Edwards et al., 1992b). In conscious Ang I-infused normotensive dogs (100 ng/kg/

min, i.v.), SK&F 108566 (3 mg/kg, i.v., or 10 mg/kg, p.o.) lowered mean arterial pressure from 160 to approximately 100 mm Hg, and at the high dose the reduction lasted at least 6 h. Agonist activity was not observed for SK&F 108566 (Weinstock et al., 1991).

CGP48933 (fig. 6) is one of the first nonheterocyclic Ang II receptor antagonists to be described. CGP48933 is a potent long-acting AT_1-selective Ang II receptor antagonist with a rapid onset of action (Criscione et al., 1992). In renal hypertensive rats, after single p.o. dosing (3 and 10 mg/kg) of CGP48933, systolic blood pressure decreased dose dependently. The maximum effect was achieved 2 to 4 h after dosing, and the antihypertensive effect lasted for 24 h. In sodium-depleted marmosets, CGP48933 was also effective at decreasing systolic blood pressure (at doses of 0.3, 1, and 3 mg/kg) for 6 h. In this model (10 mg/kg/day), CGP48933 lowered blood pressure by 25 mm Hg, and blood pressure remained at this level for the 8 days of treatment.

BIBS39 represents a series of cyclohexylaminocarbonyl aminobenzimidazoles that have affinity for both AT_1- and AT_2-subtype receptors (Zhang and VanZwieten, 1992; Wienen et al., 1992). This compound has high affinity for the AT_1 site (K_i = 29 nM) but less for the AT_2 site (K_i = 480 nM). In isolated rabbit aortic rings, BIBS39 is a competitive antagonist (shifted Ang II concentration-contractile response curves to the right in a parallel manner without a significant depression by the maximal response) with a pA_2 value of 8.2 (Zhang and VanZwieten, 1992; Wienen et al., 1992). In conscious renal hypertensive rats, BIBS39, when administered i.v., produced a significant antihypertensive effect (ED_{30} value of approximately 2 mg/kg) and a duration of antihypertensive action of only 25 min (Zhang and VanZwieten, 1992).

Currently, there is a search for nonselective or balanced (blocking both receptor subtypes) antagonists that can completely block the effects of Ang II. Circulating levels of Ang II are elevated in the presence of AT_1 receptor blockade, and virtually all of the known effects of Ang II are blocked by losartan (Smith et al., 1992a). It is possible, however, that AT_1-selective antagonists may show unwanted side effects due to exposure of the AT_2 sites to elevated Ang II levels. BIBS39, which has affinity for both sites, does not appear potent enough to test this concept. Because AT_2 function is not known, doses would be selected on the basis of the AT_1 effective dose, and, at that dose, the AT_2 receptors may not be blocked. Presumably, newer compounds will have nanomolar affinity for both AT_1 and AT_2 sites.

III. Defining Angiotensin II Receptor Heterogeneity

A. Nomenclature

The initial demonstrations of heterogeneity in the Ang II receptor system used a wide variety of designations for the observed "types." The functionally active receptor that was sensitive to inhibition by losartan was referred to as type B (Whitebread et al., 1989), Ang II-1 (Chiu et al., 1989a), site 1 (Chang and Lotti, 1990; Chang et al., 1990), and Ang II_a (Rowe et al., 1990a; Speth and Kim, 1990). The nomenclature was clarified in 1990 by a nomenclature committee established by the American Heart Association Council for High Blood Pressure Research (Bumpus et al., 1991). This committee proposed the designation AT_1 for the type inhibited by losartan and DTT that mediates the pressor effect of Ang II. The other type, which is inhibited by the nonpeptide PD123177 and its structural analogs, would be designated AT_2. The AT_2 type is also inhibited by the peptidic analog CGP42112A and potentiated by DTT. Its physiological role is yet to be defined. Additional receptor types would be designated AT_3, AT_4. . .AT_n. The committee also made allowance for the subdivision of the recognized types into subtypes AT_{1A}-AT_{1B} and so forth. Although other nomenclature is occasionally seen, almost all investigators have accepted these proposals.

B. Tissue Distribution of Receptor Subtypes

The differential distribution of receptor subtypes has been studied with radioligand membrane binding and with autoradiography. Membrane-binding measures the total sites per unit weight of tissue and can easily detect sites that are evenly, but sparsely, spread throughout the tissue. Autoradiography preserves the anatomical and morphological organization of the tissue and is much more sensitive to local concentrations of sites on particular cells or organelles. The two techniques are, therefore, complementary rather than equivalent and can sometimes give conflicting results.

The adrenal cortex was the standard source for membranes for Ang II binding and was the first tissue recognized as containing a mixture of the receptor types (Chiu et al., 1989a; Whitebread et al., 1989). Autoradiography of the adrenal confirmed the mixture in the cortex and demonstrated a relatively pure population of AT_2 sites in the rat adrenal medulla (Chiu et al., 1989a), showing that the distribution of the receptor types could vary greatly, even within a single organ. Because the medulla and cortex can be physically separated, ligand binding and autoradiography results have been virtually identical for these tissues (Herblin et al., 1991a).

One of the most studied organs is the rat brain, in which radioligand binding has demonstrated the existence of both AT_1 and AT_2 types and a heterogeneous distribution across brain areas (Leung et al., 1991b), with AT_1 sites predominating in the pituitary, AT_2 sites predominating in the thalamus-septum and the midbrain, and a mixture of the two subtypes in the hypothalamus. Autoradiographic techniques have demonstrated the localization of the types to very discrete nuclei (Wamsley et al., 1990). AT_1 sites are concentrated in the subfornical

organ, the circumventricular nuclei, and other brain regions associated with the central effects of Ang II (Song et al., 1992). Other regions, such as the colliculus, locus ceruleus, and inferior olive, contain predominantly the AT_2 type. These AT_2-rich regions tend to be associated with sensory information processing, but no direct involvement of the AT_2 sites has been demonstrated.

AT_2 sites have also been found to predominate in the rat fetus (Grady et al., 1991; Viswanathan et al., 1991; Tsutsumi et al., 1991a) or very young rats (Tsutsumi and Saavedra, 1991c). The solitary nucleus of the adult rat brain contains mainly AT_1 sites, but in fetal tissue, the level of AT_2 sites was at least 3-fold higher (Cook et al., 1991). There is a large transient expression of AT_2 sites in monkey and human fetus. These sites are classified as AT_2 based on the lack of effect of guanyl nucleotides (Zemel et al., 1990). This appearance of AT_2 sites in early stages of development has prompted the suggestion that AT_2 sites play a role in development (Zemel et al., 1990) or that the AT_2 site is actually a precursor of the AT_1 (Cook et al., 1991).

Given the involvement of the renin-Ang system in the regulation of blood pressure, the distribution of types in the kidney is of great interest. In the rat, the only subtype reported is AT_1 (Edwards et al., 1992c; Chang and Lotti, 1991; Song et al., 1991). AT_2 sites were seen in the rabbit but constituted a very minor proportion that was localized in the outer capsule (Herblin et al., 1991b; Chang and Lotti, 1991), whereas the monkey kidney exhibits a significant population of AT_2 sites that surround the glomeruli (Gibson et al., 1991). The human kidney appears to have a unique distribution in which AT_2 sites are present on the large preglomerular vessels of the renal cortex (Grone et al., 1992).

Numerous other organs of various species have now been examined for the differential distribution of AT_1 and AT_2 sites and a partial list of the findings is given in table 3. It is clear that the precise proportion of sites and even the predominant type can vary as a function of the tissue, the species, and the stage of development. The physiological significance of the receptor heterogeneity types has not been determined, as will be discussed, but the classifying and subclassifying of sites continues. Subtypes have been reported for both the AT_1 (Damon et al., 1992; Khosla, 1985) and the AT_2 subtypes (Tsutsumi and Saavedra, 1992).

C. Angiotensin II Receptor Subtype 2

Although there have been reports of functional activity mediated through AT_2 sites (table 4), there has been no definitive demonstration of a physiological role for these sites. It will be necessary to show that an effect specifically induced by Ang II is blocked by an AT_2-specific ligand, such as PD123177, and is not blocked by losartan. Cerebral arteries were reported to have only AT_2 sites, but no functional studies were done (Tsutsumi and Saavedra, 1991b). Similarly, the rabbit uterus (Whitebread et al., 1989) and ovarian granulosa cells (Pucell et al., 1991) appeared to contain only the AT_2 sites, but all effects induced by Ang II were blocked by losartan, indicating that functional activity was mediated through AT_1 receptors. PC12W cells appear to express a homogenous population of AT_2-binding sites, but the application of all Ang II produced no measurable effects (Webb et al., 1992), Using membranes from PC12W cells, another group (Bottari et al., 1992) demonstrated that Ang II induced a rapid dephosphorylation. This effect could be blocked by orthovanadate and not okadaic acid, suggesting the involvement of a protein tyrosine phosphatase. The effect was insensitive to losartan, eliminating the involvement of AT_1 receptors, but no data were reported for an AT_2-specific ligand.

PD123177 was shown to inhibit solute and water absorption in the proximal renal tubule (Cogan et al., 1991), but the effect was equal in magnitude to that of losartan and was not additive. In the vas deferens, losartan did not significantly alter the potentiation of adrenergic neurotransmission caused by Ang II, and this was taken as evidence that the effects of Ang II in this tissue were mediated by at least two types of Ang II receptors (Trachte et al., 1990), but there was no specific demonstration that AT_2 sites were involved.

A few effects of Ang II have been reported to be sensitive to specific inhibition by an AT_2 ligand and are, therefore, candidates for the AT_2 functional correlate. In neurons cultured from neonatal rat brain, the Ang II-induced decrease in basal cyclic guanosine monophosphate was significantly inhibited by 10 nM PD123177 but not by 10 μM losartan (Sumners et al., 1991). Neurons cocultured from the hypothalamus and brainstem exhibited a heterogeneous response to Ang II, with a minor population showing a decrease in K^+ currents and a larger population responding with an increase. The increase in K^+ currents was blocked by PD123177, but not losartan, and the decrease showed the opposite specificity (Kang et al., 1992). Both appeared to be dependent on intracellular calcium ions.

Several reports have appeared that describe effects of Ang II that are inhibited by both AT_2-specific ligands and losartan. It is unclear whether these effects are mediated by AT_2 receptors or atypical, nonselective sites. PD123177 was shown to delay and attenuate the Ca^{2+} spike induced by Ang II in cultured bovine adrenal medullary cells (Rasmussen-Ortega and Printz, 1991), which was also inhibited by losartan. The increase in [^{3}H] thymidine incorporation induced by Ang II in SHSY5Y human neuroblastoma cells was significantly reduced by PD123177, losartan, and an ACE inhibitor (Chen and Re, 1991). Human astrocytes respond to Ang II with an increased release of prostaglandins E_2 and I_2. The prostaglandin I_2 release is only blocked by CGP42112A,

TABLE 3
Localization of Ang II receptor subtypes

Species/tissue	>90% AT_1	Mixed	>90% AT_2
Rat			
Adrenal cortex		Chiu et al., 1989a; Whitebread et al., 1989; Wiest et al., 1991; Chang and Lotti, 1991; Song et al., 1991; DeGasparo et al., 1990	
Adrenal medulla		DeGasparo et al., 1990	Chiu et al., 1989a; Wiest et al., 1991; Song et al., 1991
Uterus		Whitebread et al., 1989; DeGasparo et al., 1990	
Brain		Wamsley et al., 1990; Rowe et al., 1991; Tsutsumi and Saavedra, 1991a; Leung et al., 1991b; Gehiert et al., 1990; Tsutsumi and Saavedra, 1990; Chang and Lotti, 1991; Gehlert et al., 1991a; Song et al., 1992; Cook et al., 1991; Millan et al., 1991; Obermuller et al., 1991; Rowe et al., 1990b; Tsutsumi et al., 1991b; Gehlert et al., 1991b; Tsutsumi and Saavedra, 1991a; Steckelings et al., 1992a; Rowe et al., 1992	
Liver	Dudley et al., 1990; DeGasparo et al., 1990		
Kidney	Edwards et al., 1992c; Song et al., 1991; Chang and Lotti, 1991; DeGasparo et al., 1990		
Aorta	Viswanathan et al., 1991		
Fetus		Tsutsumi et al., 1991a; Viswanathan et al., 1991	
Bovine			
Adrenal cortex	Balla et al., 1991		
Adrenal medulla			
Rabbit			
Adrenal cortex	Herblin et al., 1991b; Dudley et al., 1990; Chang and Lotti, 1991		
Uterus			Dudley et al., 1990
Brain	Chang and Lotti, 1991		
Kidney	Herblin et al., 1991b Chang and Lotti, 1991		
Heart		Rogg et al., 1990; Scott et al., 1992	
Aorta	Chang and Lotti, 1991		
Monkey			
Adrenal cortex	Chang and Lotti, 1991		
Brain		Chang and Lotti, 1991	
Kidney		Chang and Lotti, 1991	
Aorta		Chang and Lotti, 1991	
Human			
Adrenal cortex		Whitebread et al., 1989	
Brain		Barnes et al., 1991e	
Kidney glomeruli	Sechi et al., 1992 Chansel et al., 1992b; Grone et al., 1992	Grone et al., 1992	
Uterus			Whitebread et al., 1989
Placenta	Kalenga et al., 1991		

TABLE 4
*Reported functions of Ang II mediated by AT_2 receptors in vitro**

Selectively inhibited by PD123177/PD123319 (AT_2 only)
Increase in K^+ current in cultured rat brain neurons (Kang et al., 1992)
Decrease in cGMP in cultured neonatal rat brain (Sumners et al., 1991)
Inhibition of collagenase in cultured cardiac fibroblasts (Matsubara et al., 1992)
Inhibited by both losartan and PD123177 or CGP42112A (AT_1 and AT_2)
Solute and water absorption in rat proximal renal tubule (Cogan et al., 1991)
Scopolamine-induced amnesia in mice (Barnes et al., 1991b)
Modulation of Ca^{2+} transient in bovine medullary cells (Rasmussen-Ortega and Printz, 1991)
Increased thymidine incorporation in SHSY5Y neuroblastoma cells (Chen and Re, 1991)
Prostaglandin release from cultured cells (Jaiswal et al., 1991b)
Dilation of rat brain arterioles (Brix and Haberl, 1992)
Development of microvasculature in chick chorioallantoic membrane (LeNoble et al., 1992)
Secretion of luteinizing hormone and prolactin in rats (Stephenson and Steele, 1992)
Drinking response in rats (Hogarty and Phillips, 1991)

* From Timmermans et al., 1992b.

whereas the release of prostaglandin E_2 is blocked by both CGP42112A and losartan (Jaiswal et al., 1991b).

In mice, very low doses of both losartan and PD123177 enhanced performance in a habituation test and prevented the cognitive impairment induced by scopolamine (Barnes et al., 1990a, 1991c). Only losartan, however, was found to release the suppressed behavior of mice in a light/dark aversion paradigm (Barnes et al., 1990b). The endothelium-dependent dilation of rat pial arterioles induced by Ang II was attenuated by losartan and blocked by PD123319, both at 1 μM (Brix and Haberl, 1992). The drinking response induced in rats by the intraventricular administration of Ang II was blocked by the coadministration of either losartan or PD123319, but only losartan inhibited the increases in blood pressure and vasopressin release (Hogarty and Phillips, 1991). Similarly, both compounds inhibited the effects of Ang II on prolactin and luteinizing hormone in estrogen- and progesterone-treated ovarectomized rats (Stephenson and Steele, 1992).

There are several factors that hamper the characterization of the function of the AT_2 sites: high doses/concentrations of PD123177 or PD123319 inhibit AT_1 sites, and there are no bioavailability data available; CGP42112A is a modified peptide and can show partial agonist effects; and all of these AT_2 ligands could displace losartan from protein-binding sites, giving rise to higher free levels of losartan as has been shown in the renal hypertensive rat (Wong et al., 1992b). The function of the AT_2 receptor remains of interest because of its specific differential localization and enhanced expression in wound healing (Kimura et al., 1992; Viswanathan and Saavedra, 1992). Losartan and the other AT_1 antagonists under development significantly elevate circulating levels of Ang II which could act on unblocked AT_2 sites to produce unexpected effects. To date, no such effects have been observed in animals or humans.

D. Atypical Angiotensin II Receptors and Binding Sites

In addition to the two recognized types of the Ang II receptor, reports have appeared describing receptors or binding sites that do not fit the standard definitions (table 5). In the previous section, we described some sites that fail to show selectivity for AT_1- and AT_2-specific ligands. Binding sites for Ang II have been reported in the aorta of the domestic fowl that show unusually low sensitivity to [Sar^1,Ile^8]Ang II, divalent cations, and guanine nucleotides (Stallone et al., 1989), clearly distinguishing them from mammalian Ang II receptors. Neither the binding of Ang II to amphibian cardiac membranes (Sandberg et al., 1991) nor the Ang II-induced increase in cytoplasmic Ca^{2+} in amphibian follicular cells (Hong et al., 1990) could be inhibited by losartan or PD123177. Ang II-binding sites were also observed on differentiated mouse neuroblastoma cells (Neuro-2A) that were not inhibited by losartan, PD123177, or the guanosine triphosphate analogs, guanosine triphosphate-γ synthetase and Gpp(NH)p (Chaki and Inagami, 1992a). Residual binding of Ang II in tissues after combined treatment with AT_1 and AT_2 ligands has been reported in the rat fetus (Grady et al., 1991). Certain strains of *Mycoplasma* exhibited high-affinity Ang II binding that was sensitive to DTT but was resistant to saralasin, losartan, and CGP42112A (Bergwitz et al., 1991).

The MAS oncogene, when transiently expressed in *Xenopus* oocytes, was reported to encode an Ang II receptor that was not blocked by losartan or PD123177 (Jackson et al., 1988) but was inhibited by a tachykinin antagonist (Hanley, 1991). This receptor has affinity for [^{3}H]Ang II or Ang III but not ^{125}I-Ang II (Papdimitriou and Worcel, 1974). A MAS-related gene (*mrg*), cloned from a human genomic library, was injected into *Xenopus* oocytes and shown to increase the electrophysiological response to Ang II (Monnot et al., 1991). This interaction with an Ang II response was not observed in Chinese hamster ovary cells or COS cells, and it was concluded that the seven-transmembrane proteins encoded by the MAS gene family subserve a modulatory role in the activity of Ang II (Monnot et al., 1991).

The current view of the Ang II family of receptors and receptor subtypes (fig. 7) includes a growing number of members. As described before, these receptors can differ in structure, binding, and functional characteristics.

IV. Receptor Cloning and New Angiotensin II Receptor Subtype 1

A major breakthrough in Ang II receptor biology occurred with the isolation of cDNA clones that encode the rat vascular type 1 receptor (Murphy et al., 1991) and

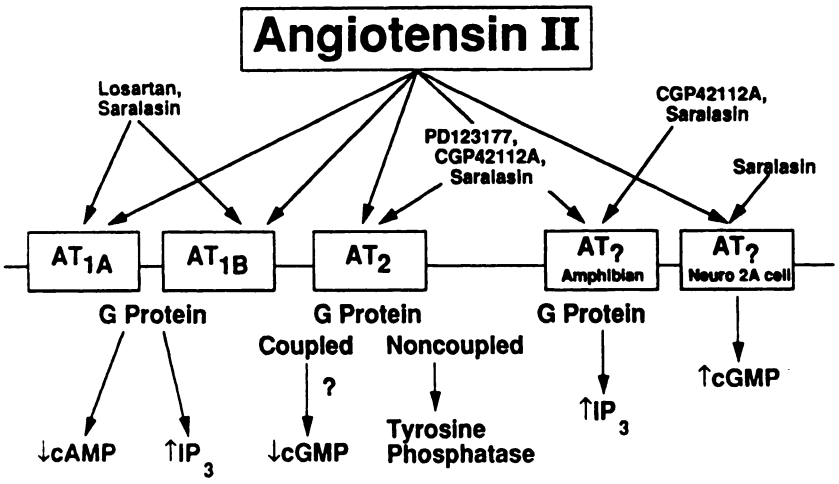


FIG. 7. The receptor-cellular response coupling of the current "family" of Ang II receptors (from Timmermans et al., 1992a). IP_3, inositol 1,4,5-trisphosphate; cGMP, cyclic guanosine monophosphate.

the type 1 receptor from bovine adrenal glomerulosa (Sasaki et al., 1991). Both receptor clones were isolated from cDNA libraries generated in the expression vector pCDM8 and screened by introduction into the receptor-negative COS-7 cell line. These data provide, for the first time, primary structural information about this receptor and materials to examine receptor subtype heterogeneity, expression patterns, and structure-function relationships. These initial reports have been followed-up with cDNA isolation of the rat kidney receptor (Iwai et al., 1991) and the receptor from human liver (Bergsma et al., 1992; Takayanagi et al., 1992).

Together, these reports have confirmed the prediction that the type 1 receptor belongs to the class of G-protein-coupled seven-transmembrane receptors. The receptor protein is 359 amino acids long, accounting for an unmodified molecular weight of approximately 41,000. The native receptor is probably subject to posttranslational modification, and multiple potential N-glycosylation sites are to be found in the extracellular domains (Murphy et al., 1991; Sasaki et al., 1991). Several structural motifs characteristic of this class of receptors are conserved, e.g., the aspartic-arginine-tyrosine sequence at the end of putative transmembrane domain 3. The extracellular domains contain cysteine residues that represent potential sites of disulfide bridge formation and probably account for the susceptibility of the native receptor to DTT. Consistent with this, these cysteine residues are essential for receptor function. Like many receptors of this class, the AT_1 receptor is sensitive to desensitization, and multiple serine and threonine residues in the intracellular domains present potential targets for desensitization by phosphorylation.

The availability of these cDNA probes has provided

TABLE 5
*Ang II receptor subtypes**

Subtype	Occurrence	Functional response	Reference
AT_1	Throughout body in all species	All principal responses to Ang II	See text
	Subtypes AT_{1A} and AT_{1B} in rats		Damon et al., 1992; Madhun et al., 1992
AT_2	Discretely localized in brain and adrenal, species variable	Possible role in neuronal ion channel modulation; angiogenesis, brain arteriolar dilation	See text
$AT_{n'}$	Neuro-2A cells	Mediates increase in cGMP	Chaki and Inagami, 1992a; Chaki and Inagami, 1992b
$AT_{n'}$	Avian (turkey, chicken)	Aldosterone, catecholamine release	Gottliebson et al., 1992
$AT_{n'}$	Amphibian	Unknown	Hong et al., 1990; Sandberg et al., 1991
$AT_{n'}$	*Mycoplasma* hyorhinis	Unknown	Bergwitz et al., 1991
$AT_?$	Mas gene family (mas, mrg)	Modulates intracellular Ang II actions	Jackson et al., 1988; Hanley, 1991; Monnot et al., 1991
$AT_?$	Cytoplasmic soluble binding protein	Unknown	Sugiura et al., 1992

* From Timmermans et al., 1992a.

reagents to probe receptor heterogeneity, and two different receptor subtypes, type 1A and type 1B, have been identified in rat (Iwai and Inagami, 1992; Kakar et al., 1992a,b; Sandberg et al., 1992). Comparison of the sequences for these two subtypes indicates a high degree of sequence conservation representing 94% sequence conservation at the protein level. Minor differences exist in the amino acid sequences between the published sequence for the rat AT_{1B} receptor, e.g., a total of 17 differences (Iwai and Inagami, 1992; Sandberg et al., 1992) and 18 differences (Kakar et al., 1992a). Most sequence changes are found in the COOH-terminal portion of the molecule and are concentrated in the extracellular and intracellular domains. The putative transmembrane domains are much less susceptible to variation. Most changes are highly conservative, particularly in the transmembrane regions. Exceptions include an alanine to isoleucine conversion in the first extracellular domain and a cysteine to phenylalanine change in the final extracellular domain. Cysteines in this domain have been shown to be palmitoylation sites in related receptors.

Only a single receptor type has been reported in the human, and Southern blot analysis suggests that the human contains only a single receptor gene that is highly related to the two rat receptor genes (Bergsma et al., 1992). The human receptor also shows sequence changes compared with the rat type 1A receptor (fig. 8). These changes are again concentrated in the COOH-terminal portion of the molecule and in the extracellular and intracellular domains. The pattern of sequence variation is nonrandom. The human and rat type 1B receptors share common sequence differences from the rat type 1A receptor, which are concentrated in the extracellular domains. In the transmembrane domains, and in the intracellular regions, the two receptors show independent sequence variation from the rat 1A receptor. Therefore, it is difficult to classify the human receptor as of either the 1A or 1B subtype based on this criterion.

All three receptor types have been introduced via expression vector constructs into receptor-negative cell lines, and this has allowed examination of their pharmacological properties. All three receptors support Ang II binding in the nanomolar range that can be blocked by type 1 receptor-specific blocking agents (losartan) but not by type 2 receptor-blocking agents (PD123177). This confirms that these clones encode a receptor of the type 1 class (AT_1).

For seven-transmembrane receptors the ligand-binding property of the receptors is contributed by the transmembrane regions with a variable contribution from the extracellular domains. This raises the possibility that receptor subtypes may exhibit subtle differences in ligand- and drug-binding properties. A 10-fold difference in the abilities of the rat AT_{1A} and AT_{1B} receptors to bind Ang I has been reported (Kakar et al., 1992a). Indeed, the observation that human and rat type 1B receptors have evolved common sequence differences from the rat type 1A receptor argues for a functional significance to this variation. This remains to be examined in detail, but the availability of cloned sequences provides the tools for such an investigation. Sequences on the intracellular segment of the molecule are probably involved in coupling to G-protein-mediated signaling pathways. Changes in the sequences in these domains may indicate different signaling mechanisms for the receptor subtypes that may even vary in different cell types and in response to different growth conditions. Mutations of the rat kidney AT_1 receptor, for example, have been recently shown to abolish both the guanosine triphosphate-induced shift to the low-affinity form and the Ang II-induced stimulated inositol 1,4,5-trisphosphate production (Ohyama et al., 1992).

The two sequences published for the rat type 1A receptor are identical in the protein-coding segments but differ in the 5′- and 3′-untranslated regions (Murphy et al., 1991; Sasaki et al., 1991). In the 5′-untranslated region, a variable insertion of 88 base pairs is found in the rat type 1A receptor (Murphy et al., 1991) and of 22 base pairs in the human receptor (Bergsma et al., 1992). Also, two different 3′-untranslated regions, one longer than the other, have been reported for the rat 1A receptor. Furthermore, Northern blot analysis of rat receptor RNA reveals species of 2.3 and 3.5 kb with the shorter species being most abundant (Murphy et al., 1991; Sasaki et al., 1991). Taken together, these data suggest that multiple messages are probably generated for the type 1A receptor. The coding segment for this receptor is uninterrupted by introns in the genomic DNA (Langford et al., 1992), suggesting that no additional receptor heterogeneity can be generated by alternate splicing. However, introns are found in the 5′- and 3′-untranslated regions in locations that suggest that diversity in these segments may be generated by alternate splicing. It seems likely that this alternate splicing may be related to the control of receptor expression either by control of receptor message stability or by translation efficiency. For example, the 3′-untranslated region of the receptor contains many ATTTA sequences. This sequence has been shown to affect message stability in other messages.

The complete genomic organization of the receptor has yet to be reported. However, promoter sequence has been presented for the rat type 1A receptor (Langford et al., 1992). Preliminary analysis and Southern blot data with 5′-untranslated region-specific probes suggest that the promoter may well be distanced by several kilobases from the coding portion of the gene by the presence of long introns in the 5′-untranslated region. Examination of the reported promoter sequence reveals typical promoter organization with an upstream consensus TATA box.

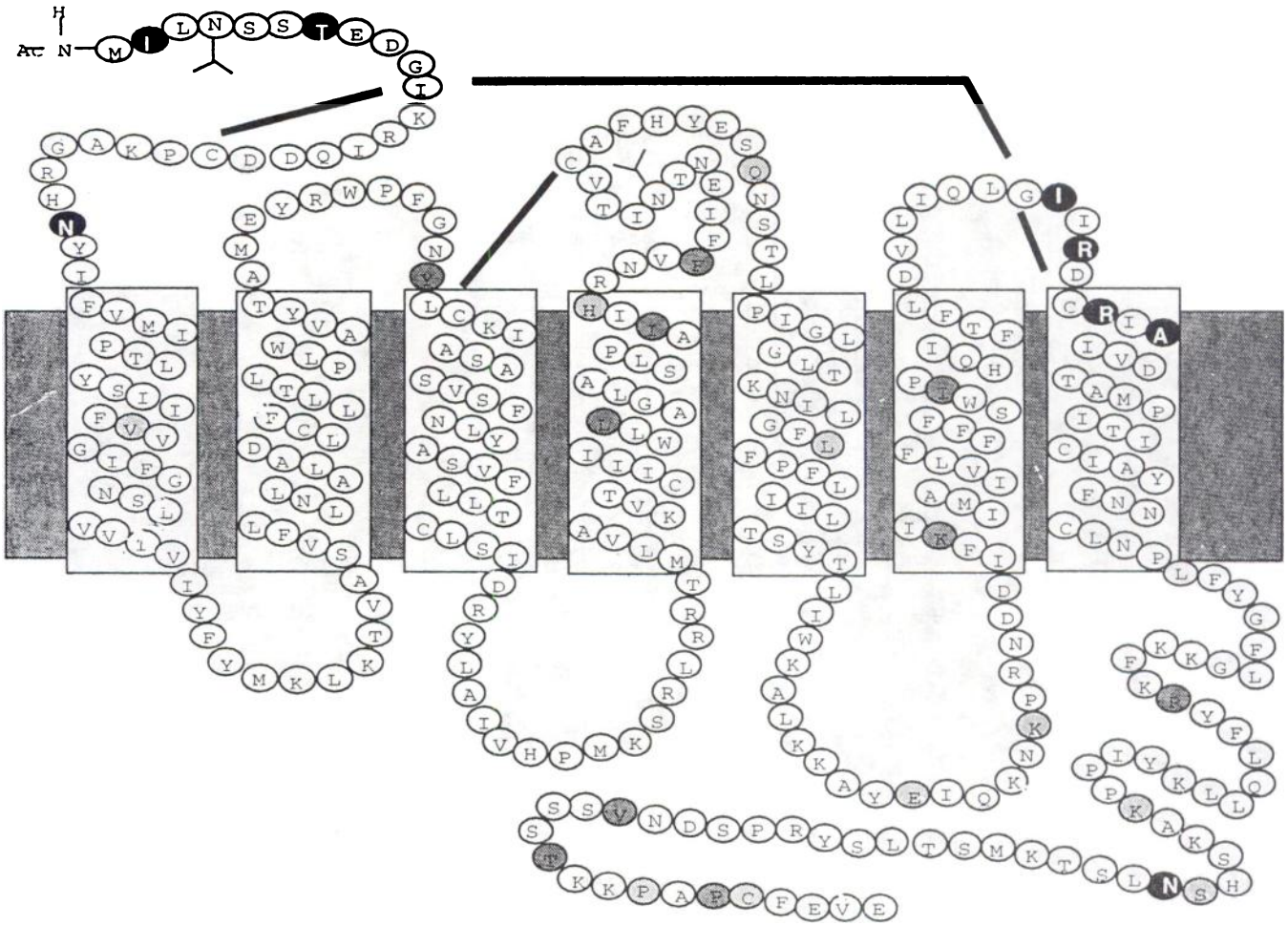


FIG. 8. Sequence of the human Ang type 1 receptor. The sequence is shown in the format standard for seven-transmembrane receptors. Potential transmembrane-spanning regions are indicated in boxes. Possible disulfide bridges in the extracellular domains are indicated by the bold lines. Possible sites of N-glycosylation are also indicated. Residues where differences are observed between the human and rat receptors are indicated by shaded ovals. The residues that also vary in the type 1B receptor are indicated by dark shading with white letters. Two positions (white lettering on a medium dark background) vary in both the human and type 1B receptors but show different substitutions. Light shading indicates positions where the human receptor differs uniquely from the type 1A receptor. Very light shading indicates positions where the human receptor differs from the type 1B receptor.

Receptor probes also allow for detailed study of expression patterns. In rat type 1, receptor RNA is found at highest levels in vascular smooth muscle, adrenal, and pituitary. The type 1A and 1B receptors have very similar expression patterns in rat, although differences in relative abundance may occur in different tissues. For example, the 1A receptor may predominate in vascular smooth muscle. The presence of two type 1 receptor loci also allows for differential regulation, and it has been reported that the rat 1B subtype is selectively responsive to estrogen in the anterior pituitary gland (Kakar et al., 1992a).

Finally, another class of Ang-binding protein has been described by Soffer and colleagues (Bandyopadhyay et al., 1988). This protein is intracellular in location and is structurally distinct from the seven-transmembrane class (Sugiura et al., 1991). This molecule has been cloned, thereby providing reagents for further functional analysis.

V. In Vitro Profile of an Angiotensin II Receptor Subtype 1-selective Antagonist

A. Affinity and Binding Characteristics for Angiotensin II Receptor Subtype 1 Receptor

Losartan exhibits high affinity for the specific Ang II-binding sites in various tissues including adrenal, brain, kidney, liver, vascular smooth muscle cells, heart, and many cell lines from rats, rabbits, dogs, mice, bovine, and human (Smith et al., 1992a; Munafo et al., 1992).

The apparent affinity of losartan depends on a number of experimental factors: (*a*) the ligand used, (*b*) the source of "receptor," and (*c*) the assay conditions. The affinity value of losartan ranges from 1.4 to 200 nM (table 6). The majority of values lie in the tens of nanomolar

TABLE 6
Affinity of losartan for AT_1 receptors

Radioligand	Species	Tissue	Losartan IC_{50} (nM)	Reference
^{125}I-Sar^1,Ile^8-Ang II		NG-108-C5 cells	40	Bryson et al., 1992
[^{3}H]Ang II	Murine	Anterior pituitary tumor	1.4	Crawford et al., 1992
^{125}I-Ang II	Rat	Astrocytic glial cells	60	Raizada et al., 1991
^{125}I-Ang II	Rat	Aortic smooth muscle cell	20	Chiu et al., 1990c
^{125}I-Ang II	Rat	Adrenal cortical	12	Chiu et al., 1990b
^{125}I-Ang II	Rat	Renal glomeruli membranes	19	Wolf et al., 1992
^{125}I-Ang II	Rat	Renal tubular membranes	19	
^{125}I-Ang II	Rat	Renal outer medullary membranes	34	
^{125}I-Ang II	Rat	Superior cervical ganglia	113	Stromberg et al., 1991
^{125}I-Ang II	Rat/human	Glomerular mesangial cells	30	Chansel et al., 1992b
^{125}I-Ang II	Bovine	Adrenal gene in COS-7 cells	200	Sasaki et al., 1991
^{125}I-Ang II	Human	Smooth muscle cell	26*	Criscione et al., 1990
^{125}I-Ang II	Human	Platelets	50	Burnier et al., 1991
[^{3}H]Ang II	Human	Hepatoma cells	17*	Wintersgill et al., 1992

* K_i (nM).

range. The reason for such variability may be protein binding, presence of heterogenous AT_1 or other AT receptor subtypes (Chiu et al., 1989a; Sasaki et al., 1991), type of radioligand used, and other interlaboratory technical variations (McQueen and Semple, 1991). It is important to note that the effect of protein binding may vary among compounds. The potency or affinity of peptidic compounds is unaffected, whereas DuP 532 affinity can be affected by the presence of nonreceptor protein (Chiu et al., 1991a). Losartan affinity is little affected by changes in the protein content of the assay (Chiu et al., 1991a). [^{3}H]Losartan ([butyl-1,2-^{3}H]losartan) is now commercially available and has the advantage of being more stable than peptide ligands. The specific activity of this material is 40 to 70 Ci/mmol and has a K_d of 6.4 nM in rat adrenal cortical microsomes (Chiu et al., 1990a). Ang I, II, and III all express similar affinities and orders of potency for the [^{3}H]losartan-binding site as they do for the [^{3}H]Ang II AT_1-binding site (Chiu et al., 1990a). With this labeled nonpeptide antagonist, it is possible to get a more accurate assessment of the AT_1 receptor characteristics and binding kinetics, e.g., [^{3}H]losartan displayed a calculated K_d of 0.173 (Chiu et al., 1990a). In a relatively purified Ang II receptor preparation, such as rat adrenal cortical microsomes or rat aortic smooth muscle cells, nonspecific binding of [^{3}H]losartan can be much higher than that of ^{125}I-Ang II (15% versus 5%).

B. Selectivity and Specificity

Losartan shows high selectivity for the AT_1 receptor subtype (30,000 more than for AT_2) (Chiu et al., 1990b). It is not certain whether it shows selectivity for AT_1 receptor subtypes (e.g., AT_{1A}, AT_{1B}, and AT_{1N}). Losartan, at concentrations of $<10^{-5}$ M, does not show affinity for other hormonal receptors, such as Ca^{2+} channels, α- and β-adrenergic, neurotensin, glycine, opioid, muscarinic, dopaminergic, and serotonergic receptors. On the functional level, losartan, up to 10^{-5} M, does not alter the contractile responses to potassium chloride and norepinephrine in the rabbit aorta or contractile responses to acetylcholine, serotonin, bradykinin, and histamine in the guinea pig ileum. The lack of effect on these systems indicates that losartan is a very specific agent for the Ang II AT_1 receptors.

C. Inhibition of Angiotensin II Second-Messenger Systems

Ang II elicits multiple cellular responses by interacting with specific receptors. The coupling of the receptors involves a number of second-messenger systems (fig. 9). Losartan blocks the actions of Ang II and the functional coupled AT_1-binding sites (table 7). Ang II stimulates phospholipase C (through a G-protein), the production of inositol 1,4,5-trisphosphate and diacylglycerol, and the mobilization of intracellular Ca^{2+} (Griendling et al., 1989). In aortic smooth muscle cells in culture (Chiu et al., 1990c), in bovine adrenal cells (Rasmussen-Ortega and Printz, 1991), and in rat liver cells (Bauer et al., 1991), the Ang II-induced calcium fluxes were inhibited by losartan (2×10^{-8} to 10^{-4} M). In bovine and rat adrenal cells (Rasmussen-Ortega and Printz, 1991;

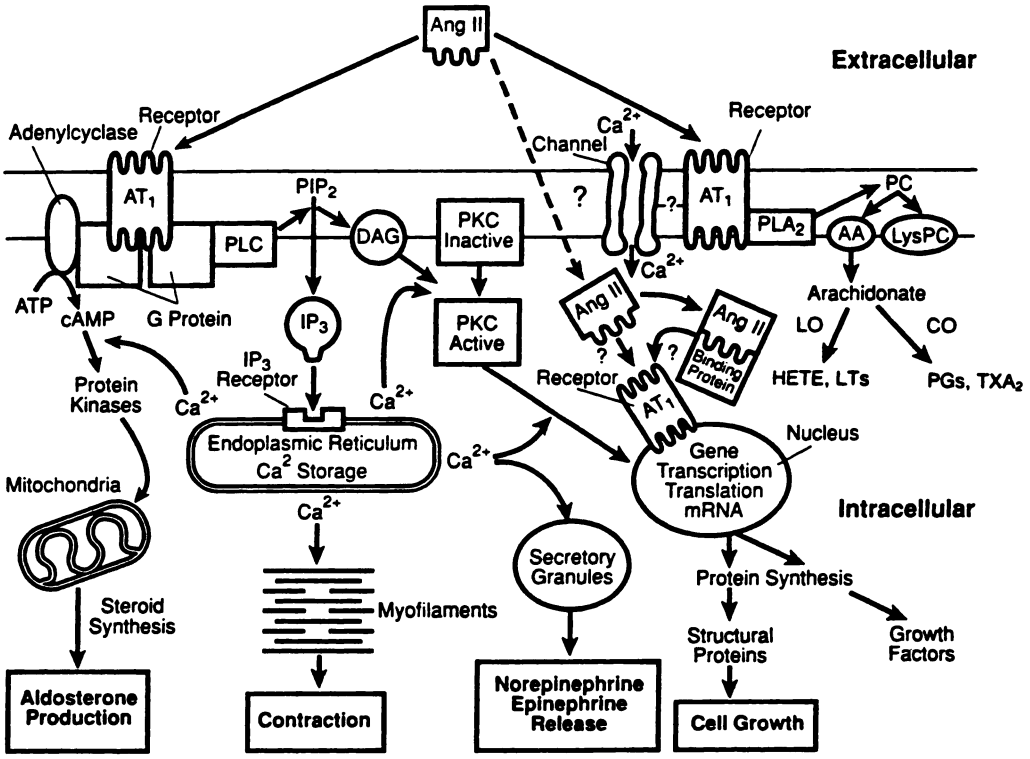


FIG. 9. Ang II receptor-cellular response coupling. PLC, phospholipase C; DAG, diacylglycerol; IP_3, inositol triphosphate; G protein, guanosine triphosphate-binding protein; PKC, protein kinase C; AA, arachidonic acid; PC, phosphatidylcholine; LysPC, lysophosphatidylcholine; LO, lipoxygenase; CO, cyclooxygenase; HETE, hydroxyeicostatetraenoic acid; TXA_2, thromboxane A_2; PGs, prostaglandins (e.g., prostaglandin E_2); Channel; calcium channel; PIP_2, phosphatidylinositol diphosphate; LTs, leukotrienes.

Hajnoczky et al., 1992), rat mesangial cells (Pfeilschifter, 1990), clone 9 rat liver-derived cells (Dudley et al., 1990), rat liver cells (Bauer et al., 1991), and in 7315c cells derived from murine pituitary tumor (Crawford et al., 1992), losartan (4×10^{-9} to 10^{-4} M) blocked the Ang II-induced increases in inositol 1,4,5-trisphosphate production. In 7315c cells, rat renal tubules, and rat hepatocytes, Ang II stimulated inositol 1,4,5-trisphosphate formation and inhibited adenylate cyclase activity (Bauer et al., 1991; Crawford et al., 1992). Both second-messenger responses to Ang II were attenuated by losartan.

Ang II also stimulates phospholipase A_2 or D leading to the release of arachidonate and its metabolic products (Peach, 1981; Ford and Gross, 1989). In an in situ rat cremaster preparation, inhibiting prostaglandin synthesis blocked the dilator, but not the constrictor, response to Ang II. By contrast, inhibiting lipoxygenase in rats reduced the in vitro and in vivo vascular effects of Ang II (Stern et al., 1989). In cultured human astrocytes, rat C6 glioma, and porcine smooth muscle cells, Ang II stimulated the release of prostaglandin E_2 and prostaglandin I_2 (Jaiswal et al., 1991a). Both responses were inhibited by losartan (10^{-7} M) in C6 glioma. Losartan, however, was found to produce a concentration-related (10^{-7} to 10^{-5} M) increase in prostaglandin I_2 release in the human astrocytes, C6 glioma, and porcine smooth muscle cells (Tallant et al., 1991). Such findings, however, were not reproduced by us or other investigators using cell cultures or isolated tissues (Leung et al., 1991a; Trachte et al., 1990).

D. Inhibition of Angiotensin II Tissue and Organ Responses

The role of Ang II as a mitogen stimulating the growth of smooth muscle (Campbell et al., 1991), cardiac (Morgan and Baker, 1991), and other cells (Schelling et al., 1991) in culture has been demonstrated by use of inhibitors of the Ang system. To date such findings have been confirmed with losartan. In cultured rat aortic smooth muscle cells, human SHSY54 neuroblastoma cells, rat mesangial cells, and murine proximal tubule cells, losartan blocked the Ang II-induced increase in protein and DNA synthesis as well as the hypertrophic response (Chiu et al., 1991c; Bakris et al., 1991; Chen and Re, 1991; Wolf et al., 1991a).

In isolated tissue, the nonpeptide Ang II receptor antagonist, losartan, selectively attenuates contractile responses of vascular and nonvascular smooth muscle and cardiac muscle to Ang II. Losartan blocked the actions of Ang II on turtle aorta, rat portal vein, stomach, and urinary bladder, rabbit jugular vein and aorta, and human colon, intestine, and urinary bladder (Chiu et al., 1990c; Wienen et al., 1990; Barbagiovanni et al., 1991; Rhaleb et al., 1991). The pA_2 values for losartan were similar, ranging from 8.19 to 8.66.

Other tissues have been utilized to characterize Ang II

TABLE 7

AT_1-mediated biochemical and second-messenger responses to Ang II (antagonized by losartan)

Response	Species	Organ/tissue	Losartan concentration	Reference
(+) Cyclic guanosine monophosphate	Murine	Neuroblastoma	10^{-9} M	Zarahn et al., 1992
(+) InP	Murine	Neuroblastoma N12–115 cells	10^{-9} M	Zarahn et al., 1992
	Rat (adult)	Astrocyte glial cells	10^{-6} M	Raizada et al., 1991
	Murine	Anterior pituitary tumor 7315c cells	1.8×10^{-7} M*	Crawford et al., 1992
Hypertrophy	Murine	Proximal tubular cells	10^{-6} M	Wolf et al., 1991b
	Rat	Aortic smooth muscle cell	10^{-6} M 5.8 nM	Sachinidis et al., 1991 Briand et al., 1992
(−) Adenyl cyclase	Murine	7315c cells	2.8×10^{-8} M*	Crawford et al., 1992
	Rat	Adrenal glomerulosa cells	10^{-5} M	Balla et al., 1991
(+) Intracellular [Ca^{2+}]	Rat	Aortic smooth muscle cell	10^{-8} M	Sachinidis et al., 1991
	Bovine	Adrenal glomerulosa cells	5×10^{-5} M	Ambroz and Catt, 1992
(+) c-*fos* expression	Rat	Hepatocytes	2.5×10^{-9} M	Gonzalez-Espinosa and Garcia-Sainz, 1992
(+) PLD	Rat	Renal mesangial cells	8 nM	Pfeilschifter et al., 1992
Prostaglandin E_2	Rabbit	Vas deferens	Not given	Jondreau et al., 1992
	Rat/human cells	Glomerular mesangial	10^{-7} M	Chansel et al., 1992b
Inositol 1,4,5-trisphosphate	Rat	Adrenal glomerulosa cells	10^{-5} M	Hajnoczky et al., 1992
	Rat	Superior cervical ganglia	10^{-6} M	Stromberg et al., 1991
Aldo		Adrenal glomerulosa cells	10^{-6} M	Hajnoczky et al., 1992 Balla et al., 1991
Aldo	Bovine	Adrenal glomerulosa cells	3×10^{-6} M 10^{-6} M	Boulay et al., 1992 Balla et al., 1991

* K_i (nM).

and its antagonists in vitro. Isolated perfused rat adrenal gland was used to show that losartan blocked the Ang II-induced release of epinephrine (Wong et al., 1990a). In rat kidney slices, Ang II-induced inhibition of renin release was blocked by losartan (Koepke et al., 1991). In the isolated perfused S_1 segment of the renal proximal convoluted tubule of the Munich-Wistar rat, Ang II markedly increased Na^+ reabsorption, and this was blocked by losartan. In isolated perfused hydronephrotic kidneys, the afferent and efferent arterioles were constricted by Ang II, and both were blocked by losartan (Loutzenhiser et al., 1991). In rat brain medullary slices, Ang II increased neuronal activity in 24 of 52 neurons tested, and this was blocked by losartan (McQueeney et al., 1992).

Losartan has no direct electrophysiological effects on the specialized conducting tissue of the heart (Brown et al., 1991). Losartan does, however, antagonize the Ang II-enhanced response to nerve stimulation in renal (Hegde et al., 1991; Rhee and Lee, 1991; Wong et al., 1991c) or mesenteric artery preparations (Jackson and Inagami, 1990; Cline and Stephenson, 1991). By contrast, in rabbit vas deferens, Ang II enhanced the "adrenergic" neurotransmission and reduced the "nonadrenergic" neurotransmission. Losartan blocked only the nonadrenergic component (Trachte et al., 1990). In specific areas of brain (e.g., PVN of the hypothalamus), Ang II raised levels of norepinephrine without affecting dopamine, 3,4-dehydroxyphenylethylene glycol, or DOPAC, and this effect was blocked by losartan (Veltmar et al., 1991a). In vitro field stimulation of rat brain striatal slices induced dopamine release as evidenced by increased DOPAC levels. Losartan (10^{-6} to 10^{-5} M) reduced this response (Jewell et al., 1991).

Losartan has become the prototypical tool used to determine the role of the Ang system in any biological system and is the reference standard for the AT_1 receptors.

VI. In Vivo Profile of an Angiotensin II Receptor Subtype 1-selective Antagonist

A. Selective Inhibition of Angiotensin II Pressor Responses in Pithed Rats

The pressor response to Ang II in rats is mediated by the activation of the AT_1 receptors (Wong et al., 1990a). Antagonism of the pressor response to Ang II, but not to other vasoconstrictor agents, in spinal pithed rats is

frequently used to determine the potency and selectivity of an Ang II receptor antagonist because direct effects of Ang II or other vasoconstrictors can be accurately assessed without the influence of baroreflex or other central reflex actions in this model (Wong et al., 1990e). Furthermore, the low basal blood pressure in spinal pithed rats allows full dose-pressor response curves for Ang II or other vasoconstrictors to be measured. Similar to the results obtained in isolated vascular and nonvascular tissues (Chiu et al., 1990c; Rhaleb et al., 1991), losartan is also a selective and competitive Ang II receptor antagonist, whereas saralasin exhibited a noncompetitive Ang II antagonism (Wong et al., 1990e). This was confirmed by Abdelrahman et al. (1992) who showed that losartan exerted a competitive antagonism of pressor responses to Ang II and Ang III in conscious rats with similar dissociation constants. EXP3174, an active carboxylic acid metabolite of losartan, inhibited the pressor responses to Ang II and Ang III selectively but noncompetitively, i.e., reducing the maximal responses to Ang II and Ang III (Wong et al., 1990c). A similar noncompetitive Ang II antagonism was also observed after administration of other carboxylic acid analogs of EXP3174, e.g., EXP3892 and DuP 532 (Wong et al., 1991a; Wong and Timmermans, 1991). The reduced maximal Ang II pressor response induced by EXP3892 was reversed by losartan (Wong and Timmermans, 1991), suggesting that the noncompetitive antagonism induced by EXP3892 is not likely due to irreversible binding to the Ang II receptors.

B. Selective Inhibition of Angiotensin II Pressor Responses in Experimental Animals

The inhibitory effect of losartan on the Ang II pressor response also was observed in conscious normotensive rats, myocardial infarcted rats, SHR, conscious normotensive dogs, and sodium-depleted dogs (Wong et al., 1990e,f; Raya et al., 1991; Wong et al., 1991b; Tofovic et al., 1991; MacFadyen et al., 1992; Abdelrahman et al., 1992). In conscious normal rats, losartan at 1 and 3 mg/kg, i.v., produced a biphasic inhibition of the pressor response to Ang II with a transient peak inhibition at 5 min, followed by a subsequent gradual increase in blockade of the Ang II pressor response over 3 h (Wong et al., 1990e), which is related to the formation of an active metabolite EXP3174 (Wong et al., 1990c). Inhibition of the Ang II pressor response was seen in normotensive rats following administration of other nonpeptide Ang II antagonists, including EXP3174 (Wong et al., 1990c), DuP 532 (Wong et al., 1991a), L-158,809 (Siegl et al., 1992), SK&F 108566 (Edwards et al., 1992b), GR117289 (Robertson et al., 1991), SR47436 (Cazaubon et al., 1992), SC-51895 (Olins et al., 1992a), ICI D8731 (Oldham et al., 1992), A-81282 (Lee et al., 1992), CGP48933 (Criscione et al., 1992), and TCV-116 (Shibouta et al., 1992) (table 2).

In anesthetized normotensive rats, losartan at effective Ang II inhibitory doses did not enhance the vasodepressor effect of bradykinin which was potentiated by captopril (Wong et al., 1990e). This agrees well with the in vitro data showing that losartan lacks ACE inhibitory activity (Chiu et al., 1990c; Wong et al., 1990b). Losartan at 10 mg/kg, i.v., also blocked the transient pressor response to bolus i.v. injection of saralasin in conscious normotensive rats, establishing that the pressor effect of saralasin is due to its Ang II receptor agonism (Wong et al., 1990b).

The increase in blood pressure produced by i.c.v. injections of Ang II in conscious normotensive rats and SHR was antagonized by i.c.v. administration of losartan (Wong et al., 1990e; Depasquale et al., 1992). Administration of losartan into the anterior hypothalamic area also blocked the pressor and bradycardiac responses to Ang II injected into the same area in NaCl-sensitive SHR (Yang et al., 1992b).

VII. Characterizing the Lack of Agonist Effect of Nonpeptide Antagonists (Cellular, Tissue, and Whole Animal Responses)

The full agonist effect of Ang II is related to its affinity for the Ang II receptor and its intrinsic activity to elicit the characteristic cellular or intracellular response. Peptide analogs of Ang II can bind to receptors, thereby acting as antagonists, and, in addition, elicit Ang II-like responses (Regoli, 1979). These peptide receptor antagonists are exemplified by saralasin ($Sar^1Val^5Ala^8$-Ang II) (Castellion and Fulton, 1979). The magnitude of the response to these "partial agonists" is peptide and tissue specific (Regoli, 1979). The agonist effects of saralasin were demonstrated both in vitro and in vivo in a variety of species including humans (Castellion and Fulton, 1979; Anderson et al., 1977).

The nonpeptide Ang II antagonists exemplified by losartan have high affinity for the Ang II receptor but do not generally elicit the characteristic cellular or intracellular responses. In the rat, peptide analogs of Ang II elicit marked agonist responses (Regoli, 1979). In the rat, saralasin 3 μg/kg, i.v., increases blood pressure 25 mm Hg, whereas losartan 10 mg/kg, i.v., has no effect. Furthermore, losartan pretreatment blocks all of the effects of saralasin (Wong et al., 1990a), thereby pinpointing its Ang II receptor site of action. In isolated vascular smooth muscle preparations (rabbit aorta and jugular vein, rat portal vein) and nonvascular smooth muscle (rat stomach and urinary bladder) losartan (0.10 to 5 M) exhibited no agonistic effect (Rhaleb et al., 1991). Likewise, in perfused mesenteric (Clark et al., 1992), renal (Clark et al., 1992), pulmonary (Gottliebson et al., 1992), and femoral (Osei and Kadowitz, 1992) circulation of the cat and in the renal (Chan et al., 1992) and coronary (Sudhir et al., 1992) circulation of the dog, losartan demonstrated no Ang II-like constrictor activity.

There have been a number of conflicting reports in isolated cells. In our laboratory, losartan and DuP 532 had no effect on the intracellular mobilization of calcium (fura 2 method) in rat or porcine smooth muscle cells in culture. Others have reported a 10% increase in the calcium transient by losartan (10^{-6} M) (Ko et al., 1992). In neonatal rat cardiomyocytes, losartan had no effect on cytosolic-free calcium (Kem et al., 1991). In human mesangial cells, losartan has been reported to increase the calcium transient 20% at 10^{-6} M (Ardaillou et al., 1992). In a preliminary report, losartan, PD123177, and CGP42112A all increased the calcium signal in rat renal mesangial cells (Madhun et al., 1992). The nature or importance of these observations is unknown. In both the rat smooth muscle cells and human mesangial cells, the increased calcium transient was not coupled to an increase in inositol metabolism (Ko et al., 1992; Ardaillou et al., 1992). In a rat vascular smooth muscle, losartan blocked the Ang II-induced efflux of calcium-45 but not the effect on basal release (Burnier et al., 1991).

The lack of Ang II-like effects in vivo, in isolated tissue, and in most cell culture preparations is the basis of the conclusion that losartan, and presumably the other nonpeptide antagonists, has no significant partial agonist activity. The possible effects on calcium transients observed at high concentrations should be explored further and considered in the design of cell culture experiments. In animals, including humans, however, losartan has no demonstrated agonist activity.

VIII. Inhibiting the Angiotensin System in Vivo

A. Acute Hemodynamic Effects

The Ang system plays a minimal role in the control of blood pressure in normotensive animals. For instance, in conscious normotensive rats with normal renin levels, acute i.v. administration of losartan or captopril did not lower blood pressure (Wong et al., 1990e). Even up to 100 mg/kg, i.v., losartan did not reduce or increase blood pressure in conscious rats. This result also suggests that losartan lacks Ang II receptor agonism. Similar results were also obtained with other Ang II antagonists including TCV-116 and L-158,809 (Steckelings et al., 1992b). However, chronic administration of 10 mg/kg/day of losartan for 4 weeks may produce a modest blood pressure lowering effect in normotensive rats (Wang et al., 1991). In anesthetized or conscious normotensive animals treated with furosemide or low salt diet, losartan lowered that component of blood pressure that is attributable to the elevation of PRA following sodium depletion (Wong et al., 1990e; Li and Zimmerman, 1990; Wood et al., 1990; Jover et al., 1991).

In conscious normotensive dogs with a normal plasma renin level, 1 to 10 mg/kg, i.v. bolus, of losartan caused a dose-dependent and transient decrease in blood pressure, which was accompanied by a decrease in heart rate (Wong et al., 1991b), whereas nonpeptide Ang II antagonists, including EXP3174 and DuP 532 (Rasmussen-Ortega and Printz, 1991), or ACE inhibitors, such as captopril, did not change blood pressure. Thus, the acute transient hypotensive effect of losartan given as an i.v. bolus in conscious dogs with normal plasma renin concentrations is probably unrelated to Ang II antagonism and is species dependent, because even up to 100 mg/kg of losartan, i.v., did not change blood pressure in conscious normotensive rats with normal renin levels (Wong et al., 1990e). However, i.v. infusion of 50 μg/kg/min of losartan did not lower blood pressure in anesthetized dogs but blocked the pressor response to Ang II (Chan et al., 1991), suggesting that i.v. infusion rather than i.v. bolus is a preferable method of administration of losartan in dogs.

The peripheral vasodilator effect of losartan has been well documented in several species. In anesthetized SHR, losartan, 10 mg/kg, i.v., lowered blood pressure and did not change cardiac output and heart rate, suggesting that losartan decreased blood pressure by peripheral vasodilation (Cody et al., 1991; Binkley et al., 1991). A similar peripheral vasodilation was also observed with losartan or EXP3174 in conscious salt-depleted dogs (MacFadyen et al., 1992) and anesthetized dogs (Richard et al., 1992b).

The influence of Ang II on vascular tone varies among vascular beds of which the kidney and the mesenteric vasculature are the most sensitive. In water-replete Brattleboro (vasopressin-deficient) rats, losartan did not alter blood pressure and hindquarter blood flow but slightly increased cardiac output and heart rate, as well as increased renal and mesenteric blood flow (Batin et al., 1991a,b). These results suggest that Ang II exerts a local vasoconstrictor influence in renal and mesenteric, but not in hindquarter, vascular beds, despite a normal plasma renin level (Batin et al., 1991a,b). In water-deprived Brattleboro rats, losartan decreased blood pressure, which is attributable to a more activated Ang system, and induced similar regional blood flow changes as in the water-replete Brattleboro rats (Batin et al., 1991a,b). An increase in RBF by losartan was also observed in euvolemic Munich-Wistar anesthetized rats (Fenoy et al., 1991b), anesthetized rabbits (Li and Zimmerman, 1990), and anesthetized dogs (Iwao, 1991; Wong et al., 1991b,c). In anesthetized furosemide-treated dogs, EXP3174 and enalaprilat (the active form of enalapril) caused similar decreases in blood pressure, total peripheral resistance, and coronary vascular resistance and did not alter heart rate, left ventricular dP/dt, cardiac output, or transmural regional myocardial blood flow (Richard et al., 1992b). These results suggest that endogenous Ang II does not regulate regional myocardial tissue perfusion (Richard et al., 1992b). Blockade of the influence of the sympathetic nervous system with α and β-adrenergic receptor blockers unmasked a mild negative inotropic effect of losartan (1.0 mg/kg, i.v.) in anesthetized

dogs, suggesting a positive inotropic effect of endogenous Ang II in this model (Moscucci et al., 1991b).

B. Effects on Renal Function

Ang II is a powerful vasoconstrictor and at physiological concentrations enhances proximal tubular sodium reabsorption (Hall, 1991). By blocking the renal effects of Ang II, ACE inhibitors or Ang II receptor antagonists produce prominent renal effects.

1. Renal vasoconstrictor effect of Angiotensin II. In the isolated perfused rat kidney, losartan did not have a direct vasoconstrictor effect but antagonized the vasoconstrictor effect of Ang II (Fontoura et al., 1991). In the isolated perfused hydronephrotic rat kidney, the vasoconstriction induced by Ang II at both the afferent and efferent arterioles was reversed by losartan (Loutzenhiser et al., 1991). The cortical and papilla blood flow response to Ang II was also blocked by losartan in inactin-anesthetized rats (Nobes et al., 1991).

2. Renal glomerular and tubular effects of angiotensin II. In anesthetized dogs, losartan, 5 mg/kg, i.v., and captopril, 1.5 mg/kg, i.v., caused similar renal vasodilation and natriuresis, which were more pronounced after sodium depletion (Wong et al., 1991b). In anesthetized dogs, losartan, 15 or 50 μg/kg/min, i.v., did not alter blood pressure or cardiac output but increased RBF, GFR, urine volume, sodium excretion, potassium excretion, and PRA (Chan et al., 1992; Iwao, 1991). Thus, losartan produces more prominent renal than systemic effects in dogs with a normal level of Ang II. However, others have reported that losartan, 5 mg/kg and 20 μg/kg/min, i.v., decreases GFR, urine flow, and sodium excretion in anesthetized dogs (Clark et al., 1991). This decrease in renal function is probably due to an acute i.v. bolus effect of losartan in dogs, which is not Ang II dependent (see section VIII.A).

In anesthetized Munich-Wistar rats, losartan, 10 mg/kg, i.v., decreased blood pressure, increased glomerular function, and inhibited renal sodium transport, especially in the S_1 subsegment of the proximal convoluted tubule (Xie et al., 1990). It was suggested that losartan may be the most potent diuretic currently known in the S_1 subsegment of the proximal convoluted tubule (Xie et al., 1990). This was consistent with the finding showing that losartan, but not enalaprilat, increased sodium excretion in conscious normotensive rats (Harton et al., 1991). The tubuloglomerular feedback response in anesthetized rats was reduced by losartan or ACE inhibitors (Braam et al., 1992).

3. Renal effect in animal models of human diseases. In conscious SHR, a new Ang II antagonist, L-158,809, 1 mg/kg, i.v., decreased blood pressure, increased renal plasma flow, GFR, urine volume, and sodium and potassium excretion (Kivlighn et al., 1991). By contrast, in volume-expanded SHR, losartan produced limited changes in renal function which is probably due to the suppression of the renal influence of the Ang system following volume expansion (Fenoy et al., 1991a). In spontaneously hypertensive dogs, losartan also produced beneficial renal hemodynamic effects and increased sodium excretion (Bovee et al., 1991). Increased urinary volume by losartan was also observed in rats with aorta-caval shunt-induced heart failure (Qing and Garcia, 1992).

In uninephrectomized-anesthetized dogs with acute renal ischemia (50% reduction in RBF), SK&F 108566, EXP3174, and captopril caused similar decreases in blood pressure, RBF, and GFR (Brooks et al., 1992). If the renal ischemia were moderate (20% reduction in renal perfusion), no changes in renal function were induced by L-158,809 or enalaprilat in anesthetized dogs (Kivlighn et al., 1992). In two-kidney one-clip renal hypertensive rats, losartan decreased GFR, RBF, and diuresis in both the clipped and contralateral normal kidneys with a greater effect in the clipped kidney (El Amrani et al., 1992). However, Lee and Blaufox (1991) observed that losartan increased RBF and GFR in the contralateral kidney and decreased RBF and GFR in the clipped kidney in two-kidney one-clip renal rats. This is supported by another study demonstrating that chronic treatment with losartan (20 mg/kg/day for 22 to 29 days) decreased GFR in the clipped kidney and did not affect the contralateral kidney (Imamura et al., 1992). It appears that the renal function of the clipped kidney is very sensitive to a decrease in blood pressure.

Ang II appears to play an important role in developing and maintaining normal renal function in neonatal rats, because chronic therapy with enalapril or losartan produced persistent abnormalities in fluid balance, i.e., increases in water intake and urine volume and a reduction in urine osmolality (Adams et al., 1992).

4. Mechanistic studies of renal effects of angiotensin-converting enzyme and nitric oxide inhibitors. Although ACE inhibitors have been studied extensively, the role of bradykinin in mediating the actions of ACE inhibitors is still not clear (Zusman, 1987; Ujhelyi et al., 1989). Pretreatment of renal artery-ligated hypertensive rats with a nonpeptide Ang II receptor antagonist such as EXP6803 or losartan or with KAA8, an Ang II monoclonal antibody, almost completely prevented the hypotensive effect of captopril (Wong et al., 1989, 1990d). Thus, the involvement of bradykinin in the antihypertensive effect of captopril in renal artery-ligated hypertensive rats is not likely (Wong et al., 1990d). A similar reduction in GFR was observed in dogs with renal artery stenosis following treatment with either captopril or the Ang II antagonists SK&F 108566 or EXP3174 (Brooks et al., 1992). These results suggest that bradykinin may not participate in the effect of captopril or renal function. However, renal effects of captopril may be mediated by blockade of the Ang II formation and bradykinin potentiation.

In euvolemic Munich-Wistar anesthetized rats, losartan blocked the increase in renal cortical blood flow but not the papillary blood flow after captopril administration (Fenoy et al., 1991b). The increase in papillary blood flow after captopril was blocked by a kinin receptor antagonist (Fenoy et al., 1991b). In anesthetized rabbits, combined administration of losartan and a kinin receptor antagonist, but not either antagonist alone, was necessary to prevent completely the renal vasodilatory effect of captopril (Hajj-Ali and Zimmerman, 1991a). Potentiation of endogenous bradykinin has also been implicated in some instances in the renal vasodilator effect of lisinopril in rabbits treated for 6 days (Hajj-Ali and Zimmerman, 1991b). However, losartan and MK521, an ACE inhibitor, produced similar renal hemodynamic changes, although the renin inhibitor RO 42–5892 caused greater changes in RBF and GFR (El Amrani et al., 1991). Results of these studies suggest that both the blockade of Ang II formation and bradykinin potentiation may participate in the renal hemodynamic effects of ACE inhibitors in some species.

NO (previously known as EDRF) produced by endothelial cells appears to play an important role in regulating vascular tone (Moncada et al., 1988) and may modulate the effects of endogenous vasoconstrictors such as Ang II. DeNicola et al. (1992a) reported that the decreased glomerular function induced by the NO synthase blocker NAME could be prevented by losartan pretreatment, suggesting that NO may counteract the renal effects of Ang II at both the glomerular and tubular level in anesthetized rats (DeNicola et al., 1992a). This was consistent with the study of Sigmon et al. who reported that losartan blocked the renal vasoconstrictor, but not systemic response, to NO inhibition (Sigmon et al., 1992). In anesthetized rabbits, NO inhibition partially blocked the renal vasodilator effect of losartan (Hajj-Ali and Zimmerman, 1992). However, losartan did not alter the systemic and renal response to NO inhibition in anesthetized dogs (Majid et al., 1992), suggesting that the renal effect of NO inhibition may vary from species to species.

5. Renal effect of angiotensin II receptor subtype 2-selective antagonist. In anesthetized rats, the AT_2-selective agent PD123177, at 60 to 120 mg/kg. i.v., caused diuresis and chloruresis, which were similar in magnitude to those elicited by maximal doses of losartan (Cogan et al., 1991). In a preliminary study, PD123177 also blocked the GFR but not the RBF response to Ang II in rats (Kost and Jackson, 1991). PD123319, but not losartan, has been shown to increase urine volume and free water clearance in anesthetized dogs without altering circulating vasopressin levels (Keiser et al., 1992). It is still unclear whether the renal responses to PD123177 or PD123319 are mediated by specific AT_2 receptors because the predominant Ang II receptor in the rat kidney is AT_1 (Fontoura et al., 1991; DeGasparo et al., 1990; Sechi et al., 1992).

C. Antihypertensive effects

The physiological utility of Ang II receptor antagonists, such as losartan, as antihypertensive agents has been demonstrated in genetic and experimentally induced hypertensive animal models (table 8) as well as in patients with human essential hypertensive (see Section X).

1. Renal hypertensive rats. In renal artery-ligated rats, a model of high renin renal hypertension (Cangiano et al., 1979), losartan is a potent and long-acting antihypertensive agent with i.v. and p.o. ED_{30} values of 0.78 and 0.59 mg/kg, respectively (Wong et al., 1990d). Despite a decrease in blood pressure, losartan did not cause tachycardia. Losartan achieved the same antihypertensive efficacy as captopril in this model. The mechanism of the antihypertensive effect of losartan in renal hypertensive rats is likely due to the blockade of the vasoconstrictor action of Ang II because captopril or saralasin, but not prazosin or indomethacin, abolished the antihypertensive effect of losartan (Wong et al., 1990d). This was further substantiated by significant correlations between the in vitro potencies in inhibiting the effects of Ang II (i.e., the specific binding of Ang II and the contractile effect of Ang II) and the i.v. antihypertensive potencies of a series of nonpeptide Ang II receptor antagonists, including losartan (Wong et al., 1990b). Akers et al. (1991) observed that losartan, 1 and 3 mg/kg, intraarterially, in the renal artery-ligated rats inhibited the Ang II pressor response maximally at 1 to 5 h but decreased blood pressure maximally at 5 to 7 and 3 to 7 h, respectively. Furthermore, there was a lack of correlation between the hypotensive effect and the Ang II antagonistic effect of losartan (Akers et al., 1991). We also observed that the maximal blockade of the Ang II pressor response by losartan occurred earlier, i.e., before the maximal decrease in blood pressure in SHR (Wong et al., 1990f). A possible explanation is that losartan may require a longer period to completely penetrate the vascular compartment to exert its maximal blockade of the endogenous Ang II-induced vasoconstriction.

Jaiswal et al. (1991a) reported that losartan stimulated prostacyclin and, to a lesser extent, prostaglandin E_2 release in rat C6 glioma, human astrocytes, and porcine aortic smooth muscle cells in culture, suggesting a possible involvement of vasodilating prostaglandins in the antihypertensive effect of losartan. This finding is not consistent with our data that showed that indomethacin did not affect the antihypertensive response to losartan in hypertensive rats (Wong et al., 1990d,f). Furthermore, Leung et al. (1991a) did not observe a prostacyclin-releasing effect of losartan, whereas Ang II stimulated prostacyclin release in porcine aortic smooth muscle cells. Losartan also did not affect the increased prosta-

TABLE 8
Antihypertensive effects of losartan

Species	Preparation	Losartan dose (mg/kg)	Duration	Route	Baseline (mm Hg)	Maximum response (mm Hg)	Reference
Rats	2K1L	0.1–10	Acute	i.v.	i.v. ED_{30} = 0.78 mg/kg		Wong et al., 1990d
Rats	2K1L	1–100	Acute	p.o.	p.o. ED_{30} = 0.59 mg/kg		Wong et al., 1990d
Rats	2K1L	1, 3	Acute	intraarterial	155, 151	99, 87	Akers et al., 1991
Rats	2K1C (anesthetized)	10	5–9 days	p.o.(?)	142 ± 6	123 ± 2	Braam et al., 1992
Rats	2K1C	125 mg/liter in drinking water	5 days	p.o.	166 ± 6	103 ± 3	DeNicola et al., 1992c
Rats	2K1C (anesthetized)	0.8	Acute	i.v.	161 ± 6	148 ± 6	Lee and Blaufox, 1991
		1.5	Acute	i.v.	180 ± 6	148 ± 3	
Rats	2K1C	20	22–29 days	p.o.	169	124	Imamura et al., 1992
Rats	Aortic-coarcted	1	Acute	p.o.	~180	~115	Steckelings et al., 1992b
Rats	⅝ Nephrectomy (high salt)	3*	Acute	i.v.	Prevent hypertension		Kanagy and Fink, 1992
Rats	Partial renal infarct	30	8 weeks	p.o.	185 ± 5	128 ± 5	Dzielak et al., 1992
SHR	Conscious	10	Acute	i.v.	159 ± 5	112 ± 6	Wong et al., 1990f
SHR	Conscious	10	15 days	p.o.	Prevent hypertension		Wong et al., 1991d
SHR	Conscious	10	7 days	i.v.	149 ± 7	124 ± 12	Bunkenburg et al., 1991
Wistar-Kyoto rat	Conscious	10	7 days	i.v.	114 ± 11	99 ± 5	
SHR	Conscious (3 wks.)	15	4, 10 weeks	p.o.	Prevent hypertension		Morton et al., 1991
Rats	TGR(mREN2)27	10	4.5 weeks	p.o.	200	110 (day 4)	Bader et al., 1992
Rats	TGR(mREN2)27	10	7 days	p.o.	194 ± 8	150 ± 4	Moriguchi et al., 1992
Rats	TGR(hAO) + hrenin	10	Acute	i.v.	~190–200	−46	Wagner et al., 1992
	TGR(hAO) + rrenin	10	Acute	i.v.	~190–200	−50	
Rats	Deoxycorticosterone acetate-salt (high salt)	10	Acute	i.v.	No effect		Wong et al., 1990d
Rats	Endothelin-infused (high salt)	3	Acute	i.v.	Decreased blood pressure		Mortensen and Fink, 1992
Rats	Cold-induced	56–112	2 weeks	p.o.	Prevent hypertension		VanBergen et al., 1992
Monkey	2K1C	1–10	Acute	p.o.	129 ± 7	−24 ± 3	Keiser et al., 1991

* Total dose.

glandin E_2 secretion in anesthetized dogs (Imanishi et al., 1992). A similar lack of effect of losartan on prostaglandin release was reported by Trachte et al. (1990) in the isolated rabbit vas deferens.

Antihypertensive activities of losartan or other Ang II antagonists in other hypertensive models, including two-kidney one-clip, one-kidney one-clip, and reduced renal mass aortic-coarctation renal hypertensive rats (Lee and Blaufox, 1991; Mantlo et al., 1991; Wang et al., 1991; Braam et al., 1992; Dzielak et al., 1992; DeNicola et al., 1992c; Kanagy and Fink, 1992; Gabel et al., 1992), as well as renal hypertensive cynomologus monkeys (Keiser et al., 1991) and dogs (Brooks et al., 1992), have also been reported.

2. *Deoxycorticosterone acetate-salt hypertensive rats.* Losartan, 10 mg/kg, i.v., did not lower blood pressure in deoxycorticosterone acetate-salt hypertensive rats with a low PRA (Wong et al., 1990d). This dose of losartan was effective in decreasing blood pressure to a normotensive level in renal hypertensive rats (see section VIII.C.1). Thus, the acute antihypertensive effect of losartan in rats appears to depend on the pretreatment level of PRA. A new Ang II antagonist TCV-116, 10 mg/kg, p.o., also did not lower blood pressure in deoxycorticosterone acetate-salt hypertensive rats, whereas it lowered blood pressure in two-kidney one-clip renal hypertensive rats following a p.o. ED_{25} dose of 0.03 mg/kg (Wada et al., 1992).

3. *Spontaneously hypertensive rats.* Losartan, given either p.o. or i.v., elicited an acute antihypertensive effect in conscious SHR (Inagami et al., 1991; Wong et al., 1990f; Wood et al., 1990). No tolerance to its antihypertensive effect was noted after repeated daily p.o. dosing of 10 mg/kg (Wong et al., 1991d). Losartan and captopril have minimal influences on blood pressure in Wistar-Kyoto normotensive rats (Wong et al., 1990f). In our early study, we observed that the acute antihypertensive efficacy of i.v. captopril was less than that of i.v. losartan in sodium-replete SHR but not in sodium-depleted (furosemide-treated) SHR (Wong et al., 1990f). In contrast, Bunkenburg et al. (1991) reported that losartan infused i.v. at 10 or 30 mg/kg/day reduced blood pressure to the same extent as did benzaprilat, an ACE inhibitor, infused i.v. at 3 or 10 mg/kg/day during a 48-h period in SHR. Similarly, we also observed that chronic p.o. administration of captopril and losartan at 10 mg/kg/day for 15 days produced a similar decrease in blood pressure in sodium-replete SHR (Wong et al., 1991d). The discrep-

ancy between these studies may be related to differences in methods of drug administration (i.v. bolus versus i.v. infusion or p.o.) or experimental conditions (Bunkenburg et al., 1991). Losartan (15 mg/kg/day) and captopril (100 mg/kg/day) given in the drinking water for 10 weeks caused the development of hypertension and vascular hypertrophy in young SHR. The hypotensive effects of losartan and captopril were still observed even at 17 weeks after treatment was stopped, suggesting that Ang II may be involved in the early onset of hypertension in SHR (Morton et al., 1992).

Because PRA is normal in SHR and an Ang II monoclonal antibody against Ang II, which neutralizes circulating Ang II, did not lower blood pressure in SHR (Wong et al., 1990g), it has been suggested that the high blood pressure in SHR is maintained by Ang II generated locally in the vasculature. Because losartan did not lower blood pressure in 24-h bilateral nephrectomized SHR (Inagami et al., 1991; Wong et al., 1990f), it was speculated that vascular renin derived from the kidney contributes to the local generation of Ang II for the maintenance of the vascular tone in SHR (Wong et al., 1990f). This agrees well with the observation that a specific anti-rat renin antibody lowered blood pressure in SHR, which indicates that extracellular renin is responsible for the vascular generation of Ang II (Inagami et al., 1991). The demonstration that the hypotensive effect of losartan or ACE inhibitors is not lost until 24 h after bilateral nephrectomy in SHR further suggests the importance of vascular renin derived from the kidney in the maintenance of blood pressure in SHR (Inagami et al., 1991).

Although it appears that the peripheral renin-Ang system may participate in the control of blood pressure in SHR, other investigators have also examined the role of the brain Ang system. DePasquale et al. (1992) reported that i.c.v. injection of 10 μg of losartan blocked the pressor response to i.c.v. administered Ang II but did not lower blood pressure in conscious SHR. This result suggests that losartan decreases blood pressure by blocking peripheral but not central Ang II receptors. However, in a preliminary study, i.c.v. injection of L-158,809, but not 10 μg of EXP3174, lowered blood pressure in young SHR (15 to 18 weeks of age) (Pare et al., 1992). Unexpectedly, 10 μg of losartan, i.c.v., lowered blood pressure 18 h postdosing, which lasted for up to 2 weeks, suggesting the formation of a non-EXP3174 metabolite (Pare et al., 1992). However, these results are preliminary and should be interpreted cautiously. In salt-sensitive SHR, but not in Wistar-Kyoto rats, injection of 20 and 40 μg of losartan into the anterior hypothalamus (but not posterior hypothalamus) lowered blood pressure dose dependently for at least 1 h (Yang et al., 1991b). The selective AT_2 antagonist PD123391 injected into the anterior hypothalamus did not lower blood pressure in this model (Yang et al., 1992b). In a follow-up study, the same laboratory demonstrated that the hypotensive effect of i.c.v. losartan is enhanced in salt-sensitive SHR fed a diet high in salt (Yang et al., 1992a). These data suggest that Ang II in the anterior hypothalamus regulates blood pressure via the activation of AT_1 receptors in the salt-sensitive SHR but not in Wistar-Kyoto rats (Yang et al., 1992a). High salt intake in salt-sensitive SHR caused a further increase in blood pressure, which may be due to an enhanced activation of AT_1 in the anterior hypothalamus (Yang et al., 1991a).

Peripheral administration of other Ang II antagonists such as DuP 532 (Wong et al., 1991a), L-158,809 (Kivlighn et al., 1991), SC-51895 (Olins et al., 1992a), and TCV-116 (Wada et al., 1992) also lowered blood pressure in conscious SHR.

4. Transgenic hypertensive rats. Introducing an additional renin gene, the murine *Ren-2* gene, into the germ line of rats resulted in a transgenic hypertensive rat strain, TGR(mREN2)27, with an overexpression of renin in the adrenal but not in the kidney (Bader et al., 1992). Although the plasma renin and Ang II levels were normal in these animals, losartan, 10 mg/kg/day for 4.5 weeks, lowered blood pressure to a normotensive level with increases in plasma renin and Ang II (Bader et al., 1992). These results suggest that TGR (mREN2)27 may represent a useful model for studying the roles of local adrenal Ang system in hypertension.

5. Other hypertensive models. In genetic Penn hypertensive dogs, losartan, 1 to 30 mg/kg, i.v., caused a moderate, but dose-dependent, decrease in blood pressure (Bovee et al., 1991). Losartan reversed the Ang II-induced hypertension in rats fed a high sodium diet (Gorbea-Oppliger et al., 1992). Losartan significantly decreased blood pressure within 5 min of administration, probably because of blockade of the direct vasoconstrictor effect of Ang II. Interestingly, the time required to reach the maximum depressor effect increased progressively with increased duration of Ang II infusion, probably related to the slow pressor effect of Ang II (Gorbea-Oppliger et al., 1992), which may operate through a central nervous system mechanism (Smits et al., 1991). Losartan decreased blood pressure in endothelin-infused hypertensive rats (Mortensen and Fink, 1992). Because endothelin elevates PRA in vivo and stimulates ACE activity in vitro (Rubanyi and Parker Botelho, 1991), the increased Ang II may contribute to the high blood pressure in endothelin-infused rats, which may account for the observed antihypertensive effect of losartan in this model. In a preliminary study, losartan also enhanced the hypotensive effect of prazosin in rats with chronic NO synthesis inhibition (Samsell et al., 1992). This may be related to the fact that blockade of the vasodilator influence of NO may unmask or enhance the vasoconstrictor tone of Ang II.

D. Effects on Neurotransmitter Release

Interactions between the renin-Ang system and the sympathetic nervous system have been demonstrated in

vitro and in vivo. Ang II may act on the brain to increase sympathetic outflow, on the sympathetic ganglia and adrenal medulla to increase catecholamine release, and at sympathetic nerve terminals to facilitate the release of norepinephrine (Reid, 1992). The pressor response to i.c.v. Ang II in animals is mediated mainly by increased efferent sympathetic activity (Peach, 1977). The central blood pressure effect of Ang II antagonists and their inhibitory effects on the central Ang II pressor response have been discussed (see sections VIII.C.3).

Functional Ang II receptors have been demonstrated in the sympathetic ganglia and adrenal medulla (Peach, 1977). Previously, Knape and VanZwieten (1988) showed that the Ang II-induced tachycardiac response in the pithed normotensive rat is attributable indirectly to its stimulation of the sympathetic ganglia because ganglionectomy greatly reduced the tachycardiac response to Ang II. In the pithed rat, losartan, but not PD123177, inhibited the tachycardiac response to Ang II, suggesting that the Ang II receptor in the sympathetic ganglia is most likely of the AT_1 subtype. This is consistent with the binding study showing AT_1 receptors in rat superior cervical ganglia (Stromberg et al., 1991) and the in vitro functional study showing that the Ang II-induced depolarization of rat superior cervical ganglion was blocked by losartan but not by PD123177 (Hawcock et al., 1992).

In the isolated perfused rat adrenal, Ang II induced a dose-dependent increase in adrenal epinephrine secretion but was much less efficacious than acetylcholine (Wong et al., 1990a). We showed that PD123177, even at 10^{-3} M, did not alter the Ang II-induced adrenal epinephrine secretion. On the contrary, losartan, which only inhibited 10% of the total Ang II binding in the rat adrenal medulla (Chiu et al., 1989a), abolished the Ang II-induced adrenal epinephrine secretion. This result suggests that the Ang II-induced adrenal epinephrine secretion may be mediated by a small population of the AT_1-binding sites. The function of the AT_2-binding sites in the rat adrenal medulla remains unknown.

In anesthetized dogs, losartan and its active metabolite EXP3174 (see section II.B), but not PD123177, blocked the renal vasoconstrictor response to renal nerve stimulation but not to norepinephrine, suggesting a presynaptic action of losartan (Wong et al., 1991c). This was confirmed by the observation that losartan reduced sympathetic nerve stimulation-evoked overflow of norepinephrine in canine gracilis muscle in vivo (Schwieler et al., 1992). The sympathetic actions of ACE inhibitors may be more complex, because benzaprilat has been reported to modulate sympathetic function prejunctionally by reduced Ang II production, enhanced bradykinin production, and increased prostaglandin production (Schwieler et al., 1992).

The vasoconstrictor response to sympathetic nerve stimulation (electrical stimulation of spinal cord), but not to i.v. norepinephrine, was reduced by losartan in the pithed rat (Wong et al., 1992a). A similar result was also obtained in pithed SHR (Richer et al., 1992). By contrast, Ohlstein et al. (1992) showed that losartan did not reduce the pressor response to spinal cord stimulation. Reasons for the discrepancies between these studies may be related to a low i.v. dose of losartan used (1 mg/kg). In Wistar-Kyoto rats, but not in SHR, Ang II potentiated noradrenergic neurotransmission in the mesenteric vascular bed, which was blocked by losartan but not by PD123177 (Tofovic et al., 1991). Taken together, these results suggest that endogenous Ang II enhances renal adrenergic function at the prejunctional AT_1 receptors. A postsynaptic effect of losartan has also been shown by a study in isolated perfused rat kidney. In this study, Ang II potentiated the renal vasoconstrictor response to renal nerve stimulation postjunctionally which was blocked by losartan, but not by PD123177 (Hegde et al., 1991).

Although a physiological role for Ang II in the vas deferens is not known, this tissue has been used for the study of selective subtype Ang II receptor agonists or antagonists (Trachte et al., 1990). In the rabbit isolated vas deferens, Ang II depressed the nonadrenergic (ATP-mediated) and augmented adrenergic contractile contractions in response to electrical stimulation, probably by a prejunctional mechanism affecting neurotransmitter release (Trachte et al., 1990). Losartan eliminated the nonadrenergic, but not the adrenergic, neuromodulatory effects of Ang II, whereas an opposite effect was observed with saralasin, suggesting subtype receptors for Ang II in this tissue (Trachte et al., 1990).

E. Blocking Cardiac Hypertrophy and Failure

Ang II can influence cardiac function by actions on myocytes (contraction, growth, metabolism), conduction tissue, fibroblasts (matrix deposition), coronary artery smooth muscle cells (constriction, dilation, hypertrophy), coronary artery endothelium (release of vasoactive peptides, altered permeability), and sympathetic nerve endings (norepinephrine release which could indirectly affect contractile state, conduction, growth, coronary resistance, and metabolic state) (Lindpainter and Ganten, 1991; Dietz et al., 1991; Morgan and Baker, 1991; Weber and Brilla, 1992).

Interfering with the synthesis of Ang II has proven effective in preventing or reversing cardiac hypertrophy in hypertension-induced (load-induced) cardiac hypertrophy (Nagano et al., 1991; Weber and Brilla, 1992), in high volume output failure models (Gay, 1990), and in coronary ligation-induced failure in rats (Thollon et al., 1989; Litwin et al., 1991). In addition, ACE inhibitors have been shown to decrease mortality in both animal models (Pfeffer et al., 1985; Sweet et al., 1987) and in clinical trials of patients with congestive heart failure (Kjekshus et al., 1992; The SOLVD Investigators, 1991). Although the uniqueness of the cardioprotective poten-

tial of the ACE inhibitors has been questioned (McMurray et al., 1991), these inhibitors of the Ang system are widely used as an adjunct to treatment of cardiac hypertrophy and failure with digitalis and diuretics (Liebson, 1990; Francis, 1991). The site of the beneficial effect of ACE inhibitors involves reduction in blood pressure (afterload) and the other sites of the actions of Ang II cited above.

In rats with aortic banding, a low dose of ramipril prevented the development of cardiac hypertrophy and improved hemodynamics without lowering blood pressure, suggesting that other mechanisms are involved (Linz et al., 1991). For example, in homogenates of human cardiac tissue, only 4 to 11% of the Ang II formed in vitro from labeled Ang I is inhibited by ACE inhibitors. It was suggested that ACE inhibitors do not interfere with the positive inotropic support provided by Ang II (Urata et al., 1990). The inotropic effect of Ang II in human heart has not been studied extensively, but the magnitude and the importance of this action of Ang II remains to be determined.

Blocking the effects of Ang II at its specific receptor sites with losartan and other nonpeptide receptor antagonists that lack agonist activity should be helpful in confirming that the efficacy of ACE inhibitors in cardiac hypertrophy and in cardiac failure is due to decreasing Ang II availability and not due to potentiation of bradykinin through inhibition of kininase II. In isolated rat myocytes, Ang II can increase protein accumulation, and this effect is blocked by losartan (Kessler-Icekson et al., 1992; Murphy et al., 1992). In endothelial cells cultured from rat hearts, losartan blocked the Ang II-induced increase in endothelin-1 mRNA which is thought to be a mediator of cardiac hypertrophy (Chua et al., 1992b). In fibroblasts cultured from rat heart, Ang II inhibits trypsin-activated collagenase activity. Matsubara et al. (1992) found that this response was not inhibited by losartan but was inhibited by PD123177. However, the same group showed that collagen synthesis ([^{3}H]proline incorporation) in adult rat heart fibroblasts is stimulated by high doses of Ang II (10^{-4} M), and this effect is blocked by both losartan and PD123177 (Brilla et al., 1992). In isolated canine ventricular myocytes, losartan blocked the Ang II-enhanced L-type calcium current (Moscucci et al., 1991a). Losartan had no effect on transmembrane action potentials of isolated Purkinje fibers from the dog (Brown et al., 1991).

In the SHR, losartan lowered blood pressure and reduced cardiac hypertrophy. Losartan administered to SHR, 10 days (Kirby et al., 1992) or 21 days (Morton et al., 1991) of age, produced long-lasting reductions in blood pressure and reduced heart weight. Likewise, in 24- to 35-day-old SHR, losartan normalized blood pressure and reduced cardiac hypertrophy (Soltis and Newman, 1992; Oddie et al., 1992). In 77-day-old SHR, 10 mg/kg/day losartan for 14 days significantly reduced blood pressure, left ventricular weight, and cardiac levels of Ang II (Mizuno et al., 1992). In Dahl-S hypertensive rats, 30 mg/kg/day losartan for 7 weeks significantly lowered blood pressure only after 6 weeks but decreased the heart weight to body weight ratio (Sugimoto et al., 1992).

In hypertension induced by aortic banding, 3 mg/kg/day losartan was compared with 10 μg/kg/day of the ACE inhibitor, ramipril. When dosed from 0 to 6 weeks after banding ("prevention") or 6 to 12 weeks after banding ("regression"), losartan lowered blood pressure more, but heart weight less, than ramipril (Linz et al., 1991). This suggests that the bradykinin-potentiating effects of ramipril are involved in the hypertrophy in this model.

In hypertension induced by exposure to cold (5°F) for 1 to 3 weeks, losartan blocked the increase in pressure but did not block the norepinephrine-related increases in cardiac hypertrophy, renal hypertrophy, or brown fat (VanBergen et al., 1992). In rats chronically infused with a low (nonpressor) dose of Ang II (1.8 μg/h) for 7 to 14 days, a 19 to 21% increase in the left ventricular weight to body weight ratio was observed along with a decrease in angiotensinogen mRNA. Both of these effects were blocked by AT_1 receptor blockade with losartan (Baker et al., 1991).

In chronic heart failure induced by coronary artery ligation in rats, ACE inhibitors have been shown to improve hemodynamics and increase survival (Pfeffer et al., 1985). A mortality study with losartan in coronary artery-ligated rats has not yet been completed. In more acute experiments, Ang II receptor blockade with losartan was shown to lower left ventricular pressure, and there was a trend toward reduced heart weight (Raya et al., 1991; Smits et al., 1992). In one of these studies, however, losartan, 15 mg/kg/day, s.c. by osmotic pump, did not preserve cardiac function as assessed at the end of the dosing period (either days 1 to 25 or days 21 to 35 after coronary ligation) (Smits et al., 1992). By contrast, captopril, but not benazepril, was effective in preserving cardiac function (Smits et al., 1992). Whether this apparent difference between losartan (or other ACE inhibitors like benazepril) and captopril is related to the doses of the agents used or involves different mechanisms is not known.

In pacing-induced failure in dogs and sheep, inhibitors of Ang II synthesis have been shown to improve acute hemodynamics (Fitzpatrick et al., 1992; Matsubara et al., 1992). In conscious dogs, losartan and captopril lowered blood pressure and increased RBF on day 5 (compensated phase) and on day 15 of pacing (decompensated phase). Neither losartan nor captopril increased cardiac output on day 5 and only captopril increased cardiac output on day 15 (Matsubara et al., 1992). The nature of this observed difference between the two inhibitors of the Ang system is unknown. In pacing-induced failure in

sheep, losartan, like captopril, lowered left atrial pressure and increased cardiac output (Fitzpatrick et al., 1992). Both compounds increased PRA and decreased plasma aldosterone and ANF levels when administered after 7 days of rapid ventricular pacing (Fitzpatrick et al., 1992).

In a high-output model of heart failure produced by aorta-caval shunts, losartan (10 mg/kg twice daily by gavage) and captopril (1 g/liter in the drinking water for 7 to 8 days starting 3 weeks after surgery) lowered mean arterial pressure and left ventricular end-diastolic pressure to control levels, reversed ventricular hypertrophy, reduced ANF levels, and increased urinary water excretion (Qing and Garcia, 1992). Similar effects were seen in aorta-caval rats dosed with losartan starting 3 days before and for 1 week after surgery (Ruzicka and Leenen, 1993). The returned responsiveness to ANF and an increase in sodium excretion was also noted in aorta-caval rats treated with losartan (Abassi et al., 1992).

In the mouse, losartan, like captopril and enalapril, reduced the cardiac hypertrophy, inflammation, and necrosis associated with viral myocarditis (Araki et al., 1992). Inhibitors of the Ang system have been evaluated for their cardiac effects in a number of other experimental settings. Losartan, enalaprilat, and the rat renin inhibitor CGP44099A given before global ischemia in isolated perfused rat hearts reduced the median duration of fibrillation (e.g., from 14 to approximately 6 min). However, none of the compounds affected creatine phosphokinase release, coronary resistance, or left ventricular diastolic pressure (Fleetwood et al., 1991). In vivo, losartan (50 μg/kg/min beginning 5 min before occlusion and continued throughout) reduced ventricular arrhythmias, creatine kinase, and aldosterone levels (Matsuo et al., 1992). In a dog "stunned myocardium" model, losartan significantly improved recovery from ischemia with or without control of pressure with an aortic balloon (Zheng et al., 1992). Infarct size was not affected by either EXP3174 or enalapril given 30 min before circumflex coronary artery ligation in dogs (Richard et al., 1992a). In rabbits, however, ramipril, but not losartan, was reported to decrease infarct size (Hullinger et al., 1992).

It can be concluded that Ang II has complex effects on cardiac hypertrophy and may play an important role in various stages of cardiac failure. Ang II receptor antagonists, like ACE inhibitors, block virtually all of the effects of Ang II in isolated tissue and in most in vivo situations. However, in some studies, differences between losartan and ACE inhibitors have been reported (Oddie et al., 1992; Smits et al., 1992; Murakami et al., 1991; Hullinger et al., 1992) and warrant further study to determine whether these differences relate to noncomparative doses or other technical factors or represent real differences in the mechanism of action of the two classes of Ang system antagonists.

F. Renal Protective Effects

ACE inhibitors have been proven to be beneficial over other antihypertensive agents in animal models of progressive renal disease, including diabetic nephropathy and other intrinsic renal diseases (Anderson and Brenner, 1990a,b). It is speculated that intrarenal generation of Ang II constricts the renal efferent arteriole and causes an increase in glomerular hydraulic pressure (Anderson and Brenner, 1990a,b). Glomerular hyperfiltration, hyperperfusion, and/or hypertension may then initiate and induce glomerular lesions. Blockade of the intrarenal formation of Ang II by ACE inhibitors may, therefore, retard the deterioration of renal failure.

Similarly, beneficial effects of losartan or other Ang II antagonists have been shown in animals with renal failure. In rats subjected to renal ablation, losartan decreased urinary protein excretion (Lafayette et al., 1992). In rats with 25% reduced renal mass hypertensive and streptozotocin-induced diabetes, losartan decreased blood pressure without altering urine output (Yeun et al., 1992). In SHR with 5/6 nephrectomy, a more severe renal failure model, losartan also decreased urinary protein excretion to the same extent as enalapril (Kohzuki et al., 1992). In streptozotocin-induced diabetic rats, chronic therapy with losartan for 3 to 4 weeks decreased blood pressure, glomerular capillary pressure, and efferent arteriolar resistance as well as increased capillary plasma flow without altering single nephron glomerular filtration (Anderson and Ingelfinger, 1991), confirming the role of Ang II in regulating systemic vascular and glomerular vascular tones. In rats with 25% reduced renal mass hypertensive and streptozotocin-induced diabetes, a more severe renal failure model, losartan did not alter urine output despite a decrease in blood pressure (Yeun et al., 1992). Chronic therapy with losartan (30 mg/kg/day from 8.9 to 19 weeks of age) provided renal protection against stroke and hypertensive renal damage and decreased cerebral lesions in saline-loaded stroke-prone SHR (Stier et al., 1991).

Protein feeding or amino acid infusion (e.g., glycine) produces glomerular hyperfiltration in animals and humans. In a glycine-induced glomerular hyperfiltration rat model, the Ang II antagonist SK&F 108566 or the ACE inhibitor enalapril reduced the glycine-induced elevated GFR and renal plasma flow (Wang and Brooks, 1992). In contrast, the increased GFR response to glycine infusion in rats with renal diseases, such as streptozotocin-induced diabetic rats (DeNicola et al., 1992b) and Goldblatt hypertension (DeNicola et al., 1992c), was blocked by captopril but not by losartan, suggesting that other effects of ACE inhibitors, such as bradykinin or NO, may contribute to the increased GFR response to glycine infusion in rats with renal diseases but not in normal rats. In rats with renal diseases, significant decreases in proximal tubular reabsorption during glycine infusion were prevented by captopril or losartan (DeNicola et al., 1992b,c). These results suggest that Ang II exerts an inhibitory effect on proximal reabsorption during glycine infusion.

G. Inhibiting the Response to Vascular Injury

The role of Ang II in the development of myointimal hyperplasia following endothelial denudation and vascular injury was suggested by the findings that the ACE inhibitor, cilazapril, decreased neointimal formation by approximately 80% in rats 14 days after carotid artery balloon injury (Powell et al., 1989). Other antihypertensives were significantly less active (e.g., verapamil, 0%; minoxidil, 4%; and hydralazine, 34%), suggesting that Ang II played a critical role (Powell et al., 1991). Although the initial clinical experience with ACE inhibitors given as adjuncts to angioplasty has been unimpressive (Semuys and Hermans, 1992), efforts continue to understand the role of Ang II as a growth factor in this response to vascular injury. Inhibitors of the Ang system are also being studied for their effect on vascular grafts. ACE inhibition reduced intimal hyperplasia in experimental vein grafts in rabbits (O'Donohoe et al., 1991) but had little effect on injured arteries and vascular grafts in baboons (Hanson et al., 1991). Species differences, the dose and duration of dosing, and the type and extent of injury may explain the inconsistent findings.

Ang II receptor blockade with the peptide inhibitor saralasin (Pan et al., 1992) or with the nonpeptide receptor antagonist losartan inhibited the injury-induced hyperplasia in rats (Laporte et al., 1991; Osterrieder et al., 1991; Prescott et al., 1991). Losartan (10 to 20 mg/kg/day) significantly reduced the proliferative response equivalent to ACE inhibitors (Laporte et al., 1991; Osterrieder et al., 1991). In one study in rats, losartan, 10 mg/kg/day, reduced the proliferative response more than did benazeprilat, 3 mg/kg/day (e.g., 49% reduction for losartan and 35% for benazeprilat). In that study, losartan reduced DNA synthesis in medial smooth muscle cells by 53% (Prescott et al., 1991). Ramipril has been reported to have a greater effect than losartan. Interestingly, the bradykinin antagonist Hoe140, when administered concurrently with ramipril, reduced the inhibition to that seen with losartan, suggesting that bradykinin plays a role in the proliferative response (Farhy et al., 1992). Whether the apparent lesser effect of losartan is a dosing problem or reflects specific AT_1 receptor blockade is not known. In rabbits, losartan, 10 mg/kg/day in the drinking water, reduced the myointimal response by >50% (Azuma et al., 1992).

These data suggest that Ang II is involved in the intimal response to vascular injury in rats and rabbits. They do not exclude a role for bradykinin or the involvement of AT_2 subtype receptors nor do they address the apparent lack of effect of Ang system inhibitors in pigs, baboons, and humans.

H. Antimitogenic Activity

The role of Ang II as a growth factor or growth modulator has been demonstrated in a number of fibroblast (Ganten et al., 1975), adrenal cortical (Gill et al., 1977), vascular smooth muscle (Owens, 1985), cardiac (Khairallah et al., 1972), and tumor (Chen et al., 1991) cells, and, in each case, peptide Ang II receptor antagonists or ACE inhibitors inhibited the responses to Ang II (for reviews see Schelling et al., 1991; Heagerty, 1991). Ang II appears to require the presence of other growth factors to elicit growth and may act indirectly through release of growth factors, such as endothelin and platelet-derived growth factor A-chain (Hahn et al., 1991). The implications of these growth effects of Ang II in hypertension (Paquet et al., 1990), angiogenesis (LeNoble et al., 1991), cardiac, and vascular hypertrophy (Schelling et al., 1991) in the vascular response to injury (Dzau et al., 1991) and in tumorogenesis (Fernandez et al., 1985) have led to designating the growth promoter effect as the "third paradigm" (first paradigm: Ang II as a pressor; second paradigm: Ang II as a regulator of cellular function) (Katz, 1992).

The "growth" response to Ang II in isolated vascular smooth muscle cells appears to be mediated by AT_1 subtype receptors. In rat aortic smooth muscle cells, administration of Ang II (10^{-7} M) to quiescent cells increased protein synthesis (hypertrophy) by 45% and DNA synthesis (hyperplasia) by 56% after a 24-h incubation. Both responses were blocked by losartan (10^{-5} M) and saralasin (10^{-5} M) (Chiu et al., 1991c). Losartan, sarcosin 1-isoleucin-8 Ang II, and DTT, but not CGP42112A (AT_2-selective peptide), blocked the 4-fold increase in [^{3}H]thymidine incorporation induced by Ang II (but not by platelet-derived growth factor-BB or platelet-derived growth factor-AA) in cultured vascular smooth muscle cells (Briand et al., 1992). Likewise, losartan inhibited the Ang II-induced increase in cell protein synthesis ($ED_{50} = 6.2 \times 10^{-8}$ M) and had no effect on the response to platelet-derived growth factor (Ko et al., 1992). Ang II-induced protein and DNA synthesis in vascular smooth muscle cells was enhanced by glucose and blocked by losartan but not by PD123177 (Natarajan et al., 1992).

Ang II-enhanced, glucose-induced cellular hypertrophy in rat renal proximal tubule cells was blocked by losartan, suggesting that this response was AT_1 mediated. The Ang II-induced (in the presence of insulin) increase in thymidine incorporation and endothelin production in human mesangial cells was also blocked by losartan and captopril (Bakris et al., 1991). By contrast, the Ang II-induced increase in insulin-dependent thymidine incorporation in human SHSY5Y neuroblastoma cells was blocked to a similar extent by both losartan and PD-123177 (66% versus 59%, respectively). In rat mesenteric artery preparations, losartan, but not PD 123319, blocked the Ang II increased protooncogene expression (c-*fos*, c-*myc*, and c-*jun* mRNA levels) (Lyall et al., 1992). Similarly, losartan, but not PD123177, blocked the Ang-induced accumulations of c-*fos* mRNA

in rat hepatocytes (Gonzalez-Espinosa and Garcia-Sainz, 1992).

In vivo the vascular hypertrophic response to Ang II was demonstrated by morphometric analysis of vessels or microvascular beds after chronic infusions of low-dose Ang II (Lever et al., 1992) or treatment with ACE inhibitors (Wang and Prewitt, 1990). Captopril decreased aortic and arteriolar dimensions and rarefaction (decrease in microvascular density) in the cremaster muscle in the rat which was independent of the arterial pressure (Wang and Prewitt, 1990). In similar experiments, a dose of losartan (10 mg/kg/day) did not normalize pressure in renal hypertensive rats (one kidney, one clip) or reverse hypertrophy of the aortic wall but did decrease the number of small vessels (Wang et al., 1991). Likewise, an even smaller dose (4.6 mg/kg/day) did not block the increase in blood pressure in rats receiving a high salt diet and infused with Ang II (5 ng/kg/min) nor did it reduce microvascular density (Hernandez et al., 1991). Although the response to exogenous Ang II was blocked by this dose of losartan (Hernandez et al., 1991), others have shown that a 5-mg/kg/day dose was ineffective, but 15 mg/kg/day was very effective in reducing the vascular response to balloon injury in rats (Kauffman et al., 1991a). In chronic Ang II infusion studies in rats, losartan reversed the Ang II-induced hypertension in conscious rats (Gorbea-Oppliger et al., 1992), but no analysis of the microvasculature was performed. In the SHR, chronic treatment with AT_1-selective blockade normalized blood pressure and vascular hypertrophy (Morton et al., 1992; Oddie et al., 1992; Soltis and Newman, 1992).

Ang II appears to have an angiogenic effect expressed in the rabbit cornea (Fernandez, 1990), the chorioallantoic membrane of the chick embryo (LeNoble et al., 1991), and the rat sponge implant model (Fan and Hu, 1992). In the chorioallantoic membrane assay, Ang II increased microvascular density involving both larger (40 to 100 μm) and smaller (10 to 40 μm) vessels. Losartan blocked the larger and CGP42112A blocked both responses, suggesting that the receptor is atypical AT_1 or AT_2. The natural ligand for the chick, however, is Val^5-Ang II instead of the Ile^5-Ang II for mammals (Khosla, 1985) used in this study (LeNoble et al., 1992). In rats implanted with a polyether sponge, neovascularization was assessed with a ^{133}Xe clearance technique. Daily Ang II treatments for 14 days increased blood flow approximately 10%. This response was totally abolished by losartan but not by CGP42112A, suggesting the involvement of the AT_1 receptor subtypes (Fan and Hu, 1992).

I. Effects on Nonvascular Smooth Muscle

The effects of Ang II receptor blockade in vivo on nonvascular smooth muscle have not been published. Some data are available concerning the effects of Ang II receptor blockade on in vitro preparations of rat urinary bladder and uterus, guinea pig ileum, duodenum, and gastric smooth muscle, both longitudinal and circular, and guinea pig esophageal muscularis mucosae (Tanabe et al., 1992; Schinke et al., 1991; Yang et al., 1992c; Eglen et al., 1991; Regoli et al., 1991; Koepke et al., 1991; Barnes et al., 1991a; Rhaleb et al., 1991; Panek et al., 1990; Wong et al., 1990b). Uterine smooth muscle contracts in a dose-dependent fashion to increasing concentrations of Ang II (Barnes et al., 1991a; Panek et al., 1990). These contractions are competitively antagonized by losartan but are not affected by the AT_2-selective antagonists, PD123319 or EXP655 (Barnes et al., 1991a; Panek et al., 1990). Interestingly, DTT (1 mM) was unable to antagonize uterine contractions induced by Ang II (Barnes et al., 1991a). The physiological implications of Ang II-induced contractions of the uterus are unknown but suggest that a local Ang system may be involved in parturition.

Ang II has been implicated as a contractile agonist of the smooth muscle of the gastrointestinal tract (Rhaleb et al., 1991; Schinke et al., 1991; Eglen et al., 1991; Yang et al., 1992c). In the longitudinal and circular smooth muscle of guinea pig stomach, Ang II produces dose-dependent contractions that can be prevented by losartan (Yang et al., 1992c; Eglen et al., 1991). However, Ang II-induced contractions of gastric longitudinal muscle can also be abolished by indomethacin and tyrosine kinase inhibitors, whereas Ang II-induced contractions of gastric circular muscle are only partially inhibited by tyrosine kinase inhibitors and are resistant to indomethacin (Yang et al., 1992c). Thus, Ang II may influence the contractile activity of the stomach via both direct and indirect actions.

Isolated rat ileum and duodenum and guinea pig ileum have been used in vitro to evaluate the effects of Ang II on contraction (Eglen et al., 1991; Schinke et al., 1991; Wong et al., 1990b). Ang II-produced dose-dependent contractions in longitudinal smooth muscle of the ileum were 96% of the methacholine contraction, whereas Ang II-induced contractions of the circular muscle of the ileum were only 16% of the methacholine contractions (Schinke et al., 1991). Contractions induced by Ang II were abolished in circular muscle by preincubating the smooth muscle with losartan; losartan shifted the dose-response curve for longitudinal muscle in a competitive fashion (Schinke et al., 1991). Duodenal smooth muscle responded to Ang II with only 24% of the methacholine contraction, and this was abolished by losartan; however, duodenal circular muscle responded only weakly to methacholine and not at all to Ang II (Schinke et al., 1991). In guinea pig ileum, Ang II-induced contractions were attenuated by atropine and tetrodotoxin for both maximal response and potency (Eglen et al., 1991). Losartan antagonized the contractile response of Ang II in guinea pig ileum by shifting the dose-response curve and by depressing the maximal response (Eglen et al., 1991).

Therefore, Ang II may exert a direct effect on gastrointestinal motility.

Ang II has also been shown to produce in vitro contractions in smooth muscle strips from rat urinary bladder (Tanabe et al., 1992). The contractions are dose dependent; they are weakly inhibited by PD123319 and potently inhibited by losartan (Tanabe et al., 1992). These data suggest a possible role for Ang II in micturition.

J. Effects on Reproductive Organs

Ang II receptors have been identified on a number of structures in the ovary. However, the classification of receptor subtypes seems to differ among species. Ang II receptors on follicular granulosa cells of the rat have been shown to be exclusively of the AT_2 subtype in vivo and in vitro (Pucell et al., 1991). Biochemical characterization of the AT_2 receptors on the rat ovarian granulosa cells indicated that none of the traditional second-messenger systems, e.g., polyphosphoinositide turnover, intracellular calcium levels, and cAMP or cyclic guanosine monophosphate, are associated with this receptor (Pucell et al., 1991). Ang II receptors on other ovarian structures such as the thecal cell layers of the follicle, interstitium, corpus luteum, blood vessels and the surface epithelium are the AT_1 subtype (Pucell et al., 1991). In a similar study by Brunswig-Spickenheier and Mukhopadhyay (1992), Ang II receptors on the thecal, granulosa, and luteal cells from bovine ovary revealed that such receptors were present only on thecal cells. The Ang II receptor subtype was the AT_2; no AT_1 receptors were identified on any of these cells (Brunswig-Spickenheier and Mukhopadhyay, 1992). These Ang II receptors were upregulated on cultured thecal cells by exposure of the cells to luteinizing hormone (0.3 to 100 ng/ml) or 8-bromo-cAMP (0.1 to 1.0 mM) in a dose-dependent fashion (Brunswig-Spickenheier and Mukhopadhyay, 1992). This receptor upregulation was found to be prevented by the protein synthesis inhibitor, cycloheximide (Brunswig-Spickenheier and Mukhopadhyay, 1992).

Ang II binding to receptors after luteinizing hormone treatment was displaced by PD123319, an AT_2 receptor-selective antagonist, at nanomolar concentrations but required micromolar concentrations of losartan to displace ^{125}I-Ang II binding (Brunswig-Spickenheier and Mukhopadhyay, 1992). Although Ang II receptor subtypes have been characterized on rat and bovine ovarian cells, the physiological effects of Ang II on bovine thecal or rat ovarian cells have yet to be established (Pucell et al., 1991; Brunswig-Spickenheier and Mukhopadhyay, 1992). There is increasing evidence in the literature implicating Ang II in the regulation of ovulation (Andrade-Gordon et al., 1991) which would be consistent with the upregulation of AT_2 Ang II receptors by luteinizing hormone in bovine thecal cells. Saralasin has been shown to attenuate germinal vesicle breakdown and ovulation induced by human chorionic gonatrophin in gonadotrophin-stimulated rats by approximately 60% (Pellicer et al., 1988). Simultaneous administration of excess Ang II restored normal rates of germinal vesicle breakdown and ovulation (Pellicer et al., 1988). In addition, saralasin has also been demonstrated to inhibit ovulation in an in vitro perfused rat ovary system (Andrade-Gordon et al., 1991). Other investigators have been able to induce ovulation in the in vitro perfused rabbit ovary with Ang II in the absence of gonadotrophin, and this was blocked by saralasin (Andrade-Gordon et al., 1991). Therefore, Ang II appears to play a role in the process of ovulation, either directly or via stimulation of steroidogenesis (Andrade-Gordon et al., 1991).

Dudley et al. (1990) demonstrated that the rabbit uterus contains both Ang II receptor subtypes, with a predominance of the AT_2 subtype. Contractile responses to 3 nM Ang II of isolated rabbit uterus, however, were partially inhibited by losartan (59%) but not by PD123319 (Dudley et al., 1990). The significance of these receptor subtypes in the uterus is unknown.

In the male reproductive system, the presence of Ang II receptor subtypes has been demonstrated on the vasa deferentia of the rabbit based on bioassay (Trachte et al., 1990). Electrical stimulation of the isolated rabbit vas deferens reveals both adrenergic and nonadrenergic components (Trachte et al., 1990). Ang II increased adrenergic neurotransmission and prostaglandin E_2 synthesis in a concentration-dependent manner while depressing nonadrenergic neurotransmission (Trachte et al., 1990). Saralasin preferentially antagonized the adrenergic neuromodulatory effects of Ang II, whereas the nonadrenergic neuromodulatory and prostaglandin E_2-releasing effects were suppressed to a lesser extent (Trachte et al., 1990). Losartan produced the opposite selectivity by eliminating the depression of nonadrenergic neurotransmission without significantly altering the potentiation of the adrenergic neurotransmission caused by Ang II (Trachte et al., 1990). Losartan also abolished the Ang II-induced stimulation of prostaglandin E_2 synthesis (Trachte et al., 1990). The authors concluded that these actions were mediated by two Ang II receptor subtypes (Trachte et al., 1990).

K. Central Effects

1. Vasopressin release. Administration of Ang II i.c.v. causes an increase in blood pressure, arginine vasopressin release, natriuresis, salt appetite, and drinking (Hogarty et al., 1992). The AT_1 receptor subtypes in the brain are associated with the regions that control the pressor and dipsogenic responses (Hogarty et al., 1992). These regions that control cardiovascular functions include the subfornical area, organum vasculosum of lamina terminalis, PVN, and the nucleus tractus solitarius (Hogarty et al., 1992). AT_2 receptor subtypes have been detected in the cerebellum and the superior olivary nu-

cleus, areas that are not associated with cardiovascular effects (Hogarty et al., 1992). Vasopressin release has been demonstrated to affect the pressor response of i.c.v. Ang II (Hogarty et al., 1992; Hogarty and Phillips, 1991). Arginine vasopressin release in response to i.c.v. administration of Ang II (50 ng) caused a peak plasma concentration 1 min after Ang II application of 24.2 ± 4.0 pg/ml which was significantly attenuated by pretreatment with 0.7 μg, i.c.v., losartan to 14.4 ± 3.3 pg/ml (Hogarty et al., 1992; Hogarty and Phillips, 1991).

In vitro and in vivo experiments have demonstrated indirect evidence that central Ang II stimulation of vasopressin release may involve a catecholaminergic pathway in the brain (Stadler et al., 1992). Stadler et al. (1992) used a novel technique of brain microdialysis coupled with high-performance liquid chromatography and electrochemical detection in conscious rats to investigate whether the stimulation of paraventricular Ang II receptors engenders a release of catecholamines from the PVN. Central administration of 1 and 100 ng Ang II increased, in a concentration-dependent manner, the release of norepinephrine in the PVN (Stadler et al., 1992; Veltmar et al., 1991b). Ang II had no effect on dopamine, DOPAC, or 3,4-dihydroxyphenylethylene glycol at any dose (Stadler et al., 1992; Veltmar et al., 1991b).

Administration of losartan i.c.v. (5 μg) 5 min prior to a 100-ng Ang II dose abolished the release of norepinephrine from the PVN (Stadler et al., 1992; Veltmar et al., 1991b). In some experiments, norepinephrine release was measured from a dorsal hypothalamic region near the PVN; no effect of Ang II was seen on release of norepinephrine in this region (Stadler et al., 1992). In additional studies, the release of vasopressin by Ang II was found to be due to release of norepinephrine and activation of α_1 receptors in the PVN (Veltmar et al., 1992). The same microdialysis technique was used to measure catecholamine release in the supraoptic nucleus in response to i.c.v. Ang II (100 pg to 100 ng supraoptic nucleus) (Qadri et al., 1992). Ang II elicited a selective dose-dependent increase in the release of norepinephrine from the supraoptic nucleus which was sensitive to i.c.v. pretreatment with losartan (Qadri et al., 1992). In the supraoptic nucleus, vasopressin release in response to i.c.v. Ang II was inhibited 50% by prazosin but not by β_1- or β_2 antagonists (Qadri et al., 1992). Thus, the data suggest that vasopressin release in response to Ang II in both the PVN and supraoptic nucleus are mediated by Ang II-stimulated release of norepinephrine and activation of α_1-receptors in these nuclei (Veltmar et al., 1992; Qadri et al., 1992).

2. *Drinking/thirst.* Antagonism of Ang II-induced water intake has been examined extensively in rats. Pretreatment with s.c. losartan at 10 mg/kg, but not PD123177 at 100 mg/kg, abolishes s.c. Ang II-induced drinking, suggesting that the drinking response to Ang II is mediated by AT_1 receptors (Wong et al., 1990a). The dipsogenic response to i.c.v. administered Ang II (50 ng) was shown to be significantly decreased by losartan pretreatment (0.7 μg) and by PD123177 (0.7 μg). These data suggest that drinking is centrally mediated by both AT_1 and AT_2 receptor subtypes (Hogarty et al., 1992). In contrast, a similar study by Stephenson and Steele (1992) showed that water intake was significantly inhibited by losartan, 1.0 μg, i.c.v., whereas PD123177 i.c.v. at the same concentration had no effect. The difference in doses of Ang II (50 versus 1 ng) used in these studies could explain these results. However, the data from Stephenson and Steele are in agreement with those of Dourish et al. (1992) who demonstrated that Ang II (0.1 to 1.0 mg/kg, s.c.) caused a dose-dependent increase in water intake in water-replete rats (Dourish et al., 1992). This effect was dose dependently blocked by losartan but not by an AT_2-selective antagonist even at doses of 100 mg/kg, s.c. (Dourish et al., 1992).

In a study in severely dehydrated cows (43.5 h of water restriction), neither ramipril (3 mg) nor mannitol (270 mM. i.c.v.) significantly decreased water consumption (Blair-West et al., 1991). However, a 3-mg i.c.v. infusion of losartan significantly lowered the quantity of water consumed in severely dehydrated cows, suggesting that the synthetic pathway for Ang II in this area of the brain may not be via converting enzyme (Blair-West et al., 1991). Thus, the data suggest that Ang II-induced drinking is mediated by AT_1 receptor subtypes.

In a series of experiments by Fregly and Rowland (Fregly and Rowland, 1991, 1992; Blair-West et al., 1991), the effects of Ang peptides on drinking was shown to be via the AT_1 receptor. Losartan at 5, 10, and 20 mg/kg, s.c., 15 min before Ang II was administered dose dependently decreased drinking induced by Ang II (150 μg/kg, s.c.) as compared with Ang II alone (Fregly and Rowland, 1991). Losartan, 10 mg/kg, also attenuated the drinking response to 200 μg Ang I and 500 μg Ang III 0.5, 1.0, and 2.0 h later (Fregly and Rowland, 1991). Injection of losartan, 3 or 10 mg/kg, s.c., 45 min prior to 200 μg/kg Ang II (s.c.) significantly attenuated the drinking response to this dose of Ang II (Fregly and Rowland, 1991). Interestingly, when i.c.v. Ang II (10 ng) was tested against losartan (10 mg/kg, s.c.), potent inhibition of the Ang II drinking response occurred; however, when the Ang II was administered i.v. and the losartan was given i.c.v., no inhibitory effect of losartan was observed (Fregly and Rowland, 1991). This study was later extended to determine whether s.c. losartan would reach the area of the brain responsible for salt appetite in sodium-depleted rats (Lasix and 24-h Na-free conditions) (Rowland et al., 1992). The results showed that s.c. administration of losartan was unable to antagonize sodium appetite in sodium-depleted rats; however, i.c.v. administration of losartan effectively decreased (by 75%) sodium intake in sodium-depleted rats (Rowland et al.,

1992). These results indicate that salt appetite, as well as drinking, are mediated by the AT_1 receptor subtype, but route of administration and duration of action may play a role in the central inhibition of Ang II effects on drinking and salt appetite (Rowland et al., 1992; Fregly and Rowland, 1991, 1992). Phillips and coworkers (1992) examined the effects of p.o. administration (in drinking water) of 3 mg/kg losartan on the drinking response to i.c.v. injected Ang II (50 ng) to determine whether losartan crosses the blood-brain barrier (Bui et al., 1992). After 3 days of losartan (3 mg/kg), no amelioration of water intake was observed in response to i.c.v. administered Ang II, suggesting that for this dosing regimen losartan does not cross the blood-brain barrier (Bui et al., 1992).

Fregly and Rowland (1992) also tested the effects of blockade of the renin-Ang system with losartan on attenuation of the dipsogenic response of other agents hypothesized to induce drinking via stimulation of renin release and subsequent Ang II formation. In these experiments, the effects of losartan (5, 10, and 20 mg/kg) on the dipsogenic responses to isoproterenol, serotonin, the serotonin precursor, 5-hydroxytryptophan, and the hypertonic agent, polyethylene glycol were measured. Subcutaneous administration of losartan or Sar^1-Ile^8-Ang II prior to isoproterenol failed to reduce water intake at any of the times measured (0.5, 1.0, 2.0 h) (Fregly and Rowland, 1992). However, both propranolol (1.0 mg/kg, i.p.; all times) and captopril (60 mg/kg, s.c.; 0.5 and 1.0 h only) blocked the effects of isoproterenol on water intake (Fregly and Rowland, 1992). The lack of effect of losartan versus the attenuation by captopril (approximately 50%) may be dose related, because lower doses of captopril had no effect and the doses of losartan are much lower than that of captopril. Losartan, at the doses tested, had no effect on drinking induced by any of the other agents, suggesting that another mechanism (other than renin release) may be involved (Fregly and Rowland, 1992).

3. Behavior. In the last several years the effects of converting enzyme inhibitors on behavior have come under scrutiny. Although inhibition of the renin-Ang system by converting enzyme inhibitors is specific, converting enzyme itself is a nonspecific dipeptidyl carboxypeptidase (Garrison and Peach, 1990). Converting enzyme inhibitors can enhance cognitive performance and can inhibit suppressed behaviors, consistent with compounds that have anxiolytic activity (Barnes et al., 1989; Costall et al., 1989, 1990). With the advent of specific nonpeptide Ang II receptor antagonists, it can be determined whether the effects of converting enzyme inhibitors on cognition and behavior are due to inhibition of a central renin-Ang system or is a nonspecific effect.

Reports of a number of studies have been published recently concerning the effects of losartan on the activities of mice or rats in a behavioral paradigm which is predictive of anxiolytic potential. In these studies, the light aversion test was used in which a mouse is placed in the middle of a brightly lit area of a box; the other side of the box has a door leading to a dimly lit black room (Barnes et al., 1990b, 1991,c,d; Chansel et al., 1992a). The time required for the mouse to find the door and go inside the dark area is measured in the absence or presence of drug (Barnes et al., 1990b; Chansel et al., 1992a; Barnes et al., 1991c,d). A normal mouse will go into the dark area as quickly as he finds it (Barnes et al., 1990b, 1991b,c,d; Chansel et al., 1992a). Losartan (0.1, 1.0, 10, 100, 1000 μg/kg, p.o., 45 min before the test) was found to have a positive effect on the amount of time spent on the white side which is associated with anxiolytic drugs, such as diazepam (Barnes et al., 1990b, 1991c). In addition, there were significantly increased line crossings on the white side of the box, whereas total line crossings remained the same between losartan-treated and vehicle-treated mice, indicating that losartan had no sedative effect (Barnes et al., 1990b, 1991c). When mice were given an AT_2-selective receptor antagonist or PD123177 (Barnes et al., 1991c,d) in this same behavioral paradigm, no anxiolytic potential was observed.

Measurement of dopamine and the dopamine metabolites, DOPAC and homovanillic acid, and 5-hydroxytryptamine 45 min after treatment of mice with 1 μg/kg of losartan revealed a decrease in dopamine in the amygdala and entorhinal cortex, a decrease in the levels of DOPAC in the entorhinal cortex, and a decrease in the levels of homovanillic acid in the amygdala (Barnes et al., 1991d,e). There was an increase in the levels of 5-hydroxytryptamine in the amygdala and striatum (Barnes et al., 1991d,e). Therefore, centrally synthesized Ang II may play a role in the development of anxiety through the AT_1 receptors. This may be associated with changes in some of the neurotransmitters of the amygdala and entorhinal cortex. It should be noted, however, that these behavioral changes observed with losartan are all below the antihypertensive threshold for the drug (Barnes et al., 1990b, 1991c,d,e; Chansel et al., 1992a).

The effects of losartan on cognition have been studied in mice (Barnes et al., 1990a, 1991d,e,f), rats (Dennes et al., 1992), and common marmosets (Carey et al., 1991). Cognition studies in mice have utilized the mouse habituation paradigm (Barnes et al., 1990a, 1991d,e,f). The black/white aversion test is repeated for several days to determine how quickly the mice learn to find the door to the black area (Barnes et al., 1990a, 1991d,e,f). Cognition is enhanced by drug treatment if a treated mouse finds the door significantly faster than a vehicle-treated mouse. Because the average mouse learns this behavior after 3 to 5 days, enhancement is usually seen on days 2 to 4 (Barnes et al., 1990a, 1991d,e,f; Martin, 1991). In the mouse habituation test, daily administration of losartan (10 ng/kg, p.o.) or PD123177 (10 ng/kg, i.p.) significantly reduced the latency to the dark area com-

pared to vehicle on days 2 and 3 but not on days 4 to 6 (Barnes et al., 1990a, 1991d,e,f). When scopolamine (0.25 mg/kg, i.p.) was administered to mice on days 4 and 6, it significantly impaired the learned behavior, increasing the latency to dark to times observed upon first exposure to the box (Barnes et al., 1990a, 1991e,f). Daily administration of losartan (10 ng/kg, p.o.) (Barnes et al., 1991e,f) or PD123177 (10 ng/kg, i.p.) (Barnes et al., 1991e,f) prevented the increased latency seen with scopolamine in the vehicle control group.

Similar results were demonstrated in a model of cognitive impairment in marmosets (Carey et al., 1991). Scopolamine caused an impairment in the acquisition of an object discrimination task, such that the number of trials required to reach criterion and the mean errors/task were increased significantly (Carey et al., 1991). Treatment of the marmosets with 10 ng/kg, s.c., losartan prevented the scopolamine-induced deficits; however, losartan, 1 ng/kg, s.c., caused a scopolamine-like performance impairment in saline-treated marmosets (Carey et al., 1991), which was not observed in the mouse habituation paradigm (Barnes et al., 1990a, 1991e,f). Enhancement of cognition by losartan in the mouse habituation paradigm and the acquisition of an object discrimination task in marmosets occurs at doses that are inadequate to produce the anxiolytic effects (Barnes et al., 1991c,d,e,f; Carey et al., 1991), whereas PD123177 exhibits only cognitive enhancement properties (Barnes et al., 1991d,f). These data suggest, if confirmed, that cognition can be enhanced by both AT_1- and AT_2-selective receptor antagonists.

In an assessment of cognitive performance in the radial maze and in a delayed nonmatching to position task in the rat, losartan (10 mg/kg, s.c., administered 18 and 2 h before testing) partially reversed the scopolamine-induced performance deficit in the radial arm maze ($P < 0.05$) and improved performance above vehicle in the delayed nonmatching to position task, particularly at the 8- and 16-s delays (day 7, $P < 0.05$) (Dennes et al., 1992). In this study, higher doses of losartan were used, and the results suggest that more studies are needed to determine the role Ang II plays in cognition and reduction of anxiety.

Other studies of the effects of losartan on various aspects of mental function have been performed. The learned helplessness model is highly sensitive to antidepressant drugs and was used to investigate the effects of losartan alone or in coadministration with antidepressant drugs (Martin, 1991). The results indicate that daily injections of losartan (0.5 to 2 mg/kg) significantly reverse the behavioral deficit induced by inescapable shock, and at inactive doses, losartan significantly potentiates the antidepressant effects of imipramine (4 to 8 mg/kg/day) (Martin, 1991). Additionally, Ang II receptor antagonists have been examined in the prevention of apomorphine-induced (dopamine agonist-induced) stereotypy in the rat (Banks and Dourish, 1991). Both losartan (30 and 100 mg/kg, p.o.) and an AT_2-selective antagonist (10 and 30 mg/kg, p.o.) administered 45 min before s.c. injection of apomorphine partially or completely blocked the apomorphine-induced p.o. stereotypy (Banks and Dourish, 1991). In addition, losartan (100 mg/kg) caused immobility, vacuous mouth movements, penile grooming, and yawning, whereas WL19 (30 mg/kg) caused only some of these effects (Banks and Dourish, 1991). Therefore, it appears that attenuation of the apomorphine-induced behavior is effected by both AT_1 and AT_2 Ang II receptor antagonists.

In another study, the effects of losartan were evaluated on hyperlocomotion induced by the D_1 receptor agonist, SKF82958, and the D_2 agonist, (+)-PHNO, in the rat (Banks et al., 1991). Hyperlocomotion was dose dependently induced by SKF82958 (30, 100, and 300 μg/kg, s.c.) and (+)-PHNO (10, 30, 100, and 300 μg/kg, s.c.). Losartan (30 and 100 mg/kg) blocked locomotor activity induced by SKF82958 (0.1 mg/kg); however, locomotion induced by (+)-PHNO (10 and 30 μg/kg) was potentiated by 100 mg/kg losartan (Banks et al., 1991). The interactions between the Ang II receptors and dopamine receptors are complex because losartan blocked the D_1-induced locomotor activity and enhanced the D_2-induced locomotor activity (Banks et al., 1991). Losartan, 1 μg/kg p.o., 45 min prior to testing was shown to reduce dopamine turnover in the amygdala and the entorhinal cortex (Barnes et al., 1991c). In addition, losartan (10 mg/kg, s.c., 18 h before analysis) caused a significant decrease in striatal dopamine, but no change in DOPAC was observed. However, chronic administration (21 days) of losartan at the same dose did not affect striatal levels of dopamine but significantly decreased levels of DOPAC (Dwoskin et al., 1992).

The effects of losartan on cocaine toxicity have been examined in conscious, instrumented rats administered an acute, lethal dose of cocaine (65 mg/kg, i.p.) (Latour et al., 1992). Losartan, 25 mg/kg, intraarterially, alone increased survival time and, in conjunction with diazepam (0.5 mg/kg), controlled hypertension (losartan) and convulsions (diazepam) (Latour et al., 1992).

L. Interactions with Other Peptide Systems

1. Nitric oxide/endothelium-derived relaxing factor. Recently, there have been a series of studies investigating the interactions of the Ang system and other peptide systems. One of the most studied to date is the interaction of the Ang system and EDRF, because these systems represent opposite effects in vivo, e.g., vasoconstriction for the Ang system and vasodilation for EDRF. The interaction of the Ang system and EDRF in renal function experiments have been examined in many of these studies, with contradictory results (Sigmon et al., 1991; Majid et al., 1992; Sigmon et al., 1992; Hajj-Ali and

Zimmerman, 1992; Baylis et al., 1992; DeNicola et al., 1992a).

In a study in conscious rats, an inhibitor of EDRF synthesis, NAME, was administered systemically either alone (10 mg/kg) or in the presence of losartan (3 mg/kg) or losartan plus enalaprilat (2 mg/kg) (Baylis et al., 1992). Arterial blood pressure increased in all treatment groups to the same extent and was not affected by losartan or losartan plus enalaprilat (Baylis et al., 1992). This is in agreement with a study by Sigmon et al. (1991) who showed similar results in anesthetized rats, indicating that Ang II does not mediate the hypertensive effect of EDRF synthesis blockade. In conscious rats, NAME increased RVR and filtration fraction and decreased renal plasma flow (Baylis et al., 1992). Combining losartan with NAME did not alter any of these responses significantly, although a combination of NAME, losartan, and enalaprilat elicited a significantly smaller increase in RVR, a smaller decline in renal plasma flow, and a smaller increase in the filtration fraction when compared to the group that received NAME and losartan (Baylis et al., 1992). The authors concluded that endogenous Ang II in the kidney does not mediate the renal hemodynamic effects of acute EDRF blockade in the basal state and that EDRF plays a major regulatory role in the control of renal hemodynamics (Baylis et al., 1992).

Similar conclusions were drawn from experiments performed in denervated kidneys of anesthetized, sodium-replete dogs treated with intrarenally administered losartan (50 μg/kg/min). Intrarenal administration of losartan caused a significant decrease in systemic mean arterial pressure and RVR and a significant increase in RBF without significant changes in GFR, urine volume, or sodium excretion (Majid et al., 1992). Treatment of the losartan-treated dogs with NAME (50 μg/kg/min) resulted in significant increases in arterial pressure (9.2 ± 3.3%) and RVR (45.1 ± 6.8%) and significant decreases in RBF (23.5 ± 3.4%), urine flow (48.0 ± 9.0%), and sodium excretion (74.6 ± 3.6%) without significant changes in GFR (Majid et al., 1992). These data from anesthetized dogs and conscious rats suggest that endogenous Ang II in the kidney does not modulate the local, endogenous EDRF response to kidney vasculature.

In similar studies performed by Sigmon et al. (1991, 1992) using anesthetized rats, changes in mean arterial pressure, RVR, and RBF were measured. When rats were treated with NAME alone (10 mg/kg) or in combination with enalaprilat (320 μg/kg) or enalaprilat + DuP 753 (10 mg/kg), arterial pressure increased significantly in all three groups (Sigmon et al., 1991). However, RBF was decreased significantly only in rats treated with NAME alone; NAME in combination with enalaprilat or enalaprilat + losartan exhibited no change in RBF (Sigmon et al., 1991). RVR was significantly increased in all three groups, but the magnitude of the increase was different. RVR increased 85% with NAME alone, 52% for NAME + enalaprilat, and only 27% for NAME + enalaprilat + losartan (Sigmon et al., 1991). The authors concluded that inhibition of EDRF synthesis produces a decrease in RBF that is mediated in part by Ang II (Sigmon et al., 1991). The results suggest that EDRF buffers the vasoconstrictor effects of endogenous Ang II in the kidney to regulate RBF (Sigmon et al., 1991). The authors postulated that the degree of vasoconstriction following NAME is related to PRA (Sigmon et al., 1992). To test this hypothesis, rats were chronically subjected to either a low sodium diet or deoxycorticosterone acetate + 1% sodium chloride for drinking to manipulate PRA by diet (Sigmon et al., 1992). The rats with high PRA responded to NAME with an increase in arterial pressure and RVR (67%) and a decrease in RBF (27%), whereas the rats with low PRA exhibited a similar increase in arterial pressure. RVR increased only 30% and RBF was unchanged (Sigmon et al., 1992). Rats with high PRA treated with losartan + NAME showed the same increase in arterial pressure as the other groups; however, RVR increased only 30% and RBF was unchanged (same as rats with low PRA) (Sigmon et al., 1992). Therefore, chronically inhibiting Ang II by dietary manipulations or acutely by pharmacological manipulations did not reduce the systemic pressor response but greatly attenuated the renal response to NAME (Sigmon et al., 1992), corroborating the earlier results. Similar investigations performed in anesthetized rabbits yielded the same results as above, further confirming the interaction of EDRF and endogenous Ang II in regulating RBF (Hajj-Ali and Zimmerman, 1992).

The discrepancies in the data presented above are probably due to differences in experimental protocols, anesthetized versus conscious rats, 10 versus 3 mg/kg losartan, and 320 μg/kg versus 2 mg/kg enalaprilat (Sigmon et al., 1991, 1992; Baylis et al., 1992). Additional data support the interaction of endogenous EDRF and the Ang system intrarenally. The regulation of juxtamedullary afferent and efferent arteriolar diameters were studied using the in vitro blood perfused juxtamedullary nephron technique (Ye and Healy, 1992). Afferent and efferent arterioles were visualized during perfusion at a renal pressure of 110 mm Hg, and diameters were measured in response to increasing doses of NAME (1 to 1000 μM) in kidneys from rats pretreated with either saline (losartan vehicle) or losartan (3 mg, i.v.) (Ye and Healy, 1992). Both afferent and efferent arterioles exhibited a dose-dependent decrease in diameter in response to NAME that was significant at all doses, with afferent arterioles showing slightly greater sensitivity (Ye and Healy, 1992). In kidneys from rats pretreated with losartan, vasoconstriction of both afferent and efferent arterioles was significantly attenuated at all doses of NAME (Ye and Healy, 1992). These data indicate that tonic EDRF synthesis influences both afferent and efferent

arteriole resistance and that the effect of NAME is attenuated by Ang II receptor blockade, suggesting an interaction between EDRF and Ang II in afferent and efferent arteriolar resistance (Ye and Healy, 1992).

NO has been postulated to modulate basal renal hemodynamics Bayliss et al., 1992). Additionally, NO has been implicated in the renal response to protein or amino acid load (Tolins and Raij, 1991; King et al., 1991). In a study by DeNicola et al. (1992a), the effects of NO synthesis blockade on the glomerular and tubular response to continuous glycine infusion were analyzed. Additionally, these investigators were interested in determining whether Ang II and NO interacted to modulate the responses to glycine infusion and whether there exists an interaction between NO and Ang II influencing the basal state of glomerular and tubular function (DeNicola et al., 1992a). In control rats, glycine increased single nephron GFR and plasma flow with no change in absolute proximal tubular reabsorption (DeNicola et al., 1992a). NAME (0.5 mg/kg/min) abolished the vasodilation induced by glycine; pretreatment with losartan (10 mg/kg, i.v. bolus) normalized the response to glycine at both glomerular and tubular levels (DeNicola et al., 1992a). In the absence of glycine, NAME produced glomerular arteriolar constriction, decreased the glomerular ultrafiltration coefficient, and reduced single nephron GFR, which was associated with a dramatic decrease in absolute proximal tubular reabsorption (DeNicola et al., 1992a). Losartan prevented both glomerular and tubular changes induced by NAME, indicating that NO is a physiological antagonist of Ang II at both the glomerular and tubular level (DeNicola et al., 1992a). Therefore, the interaction between endogenous EDRF and Ang II intrarenally may be a critical factor in the control of basal renal function and in the tubular and glomerular response to glycine (DeNicola et al., 1992a).

Experimental evidence has consistently shown that acute treatment of normal rats or dogs with NAME causes an increase in arterial pressure that is insensitive to converting enzyme inhibitors or losartan. However, one study in which renin-dependent hypertensive transgenic rats were used showed that chronic administration (7 days) of losartan (10 mg/kg/day) or lisinopril (20 mg/kg/day) could attenuate the effects of NAME infusion on systolic blood pressure (Moriguchi et al., 1992). The degree of attenuation of the NAME response was correlated with the antihypertensive effects of losartan or lisinopril (Moriguchi et al., 1992). Whether this effect will be substantiated in other models of renin-dependent hypertension is unknown.

In summary, there appears to be adequate evidence to support the hypothesis that endogenous EDRF and Ang II interact to regulate RBF and control basal glomerular and tubular function and the glomerular response to glycine infusion. Additionally, preliminary evidence in renin-dependent hypertensive transgenic rats suggests that pathological activation of the Ang system may alter the ability of endogenous EDRF to modulate systemic arterial pressure.

2. *Atrial Natriuretic Factor.* Several studies have been performed to assess the relationship between the Ang system and ANF (Qing and Garcia, 1992; Camargo et al., 1991; Chua et al., 1992a). Camargo et al. (1991) determined that, in stroke-prone SHR fed 4% sodium chloride, PRA is not appropriately suppressed and increases progressively in association with the development of renal vascular injury. They studied PRA and ANF responses in stroke-prone SHR receiving a 4% sodium chloride diet that were either treated with losartan or vehicle (Camargo et al., 1991). In vehicle-treated, rats PRA was stable for the first 4 weeks and increased progressively after 6 weeks with ANF showing a concomitant increase (Camargo et al., 1991). In animals treated with losartan, PRA showed an increase at 2 weeks but returned to baseline after 6 weeks; ANF exhibited the appropriate reciprocal relationship with PRA throughout the 12-week study (Camargo et al., 1991). Losartan-treated rats had lower blood pressure and produced greater urine volume throughout the study (Camargo et al., 1991). In addition, losartan-treated animals had less renal damage at 8 and 12 weeks and did not have cardiac hypertrophy at 12 weeks as did the controls (Camargo et al., 1991). These data indicate that in sodium chloride-fed stroke-prone SHR, Ang II antagonism results in a normal relationship between the cardiac and renal volume-regulating/-sensing hormones, renin and ANF (Camargo et al., 1991).

The interaction between ANF and the Ang system has been studied in an aorta-caval shunt model of moderate cardiac failure. Rats were treated for 7 days with either losartan (10 mg/kg, p.o., twice daily) or captopril (1 g/liter in drinking water) or vehicle (controls) beginning 3 weeks after surgery; another group of rats was surgically opened and closed without receiving the aorta-caval shunt (shams) (Qing and Garcia, 1992). Mean arterial pressures were lower and right atrial pressures (RAP) and left ventricular end-diastolic pressures were higher in aorta-caval shunts compared to shams; captopril and losartan decreased mean arterial pressure compared to untreated aorta-caval shunts (Qing and Garcia, 1992). Left ventricular end-diastolic pressures were significantly lower in captopril-treated rats and tended to be lower in losartan-treated rats; both suppressed left ventricular end-diastolic pressure elevations observed with aorta-caval shunts to values similar to shams (Qing and Garcia, 1992). Captopril and losartan significantly decreased absolute and relative heart weights compared to untreated aorta-caval shunt animals, which demonstrated marked cardiac hypertrophy (Qing and Garcia, 1992). Aorta-caval shunt animals had decreased right atrial total ANF concentration and increased ventricular ANF content and concentration (Qing and Garcia, 1992).

Plasma carboxy- and amino-terminal ANF levels were elevated, and hematocrit was lower in aorta-caval shunt animals than in sham controls (Qing and Garcia, 1992). Captopril or losartan treatment resulted in a clear regression of cardiac hypertrophy that was associated with plasma ANF levels similar to those in sham-operated rats (Qing and Garcia, 1992). Losartan-treated rats had decreased plasma carboxy-terminal ANF levels with increased urinary volume and hematocrit (Qing and Garcia, 1992). These data demonstrate that chronic treatment with captopril or losartan in rats with aorta-caval shunts improved hemodynamic parameters, diminished water retention, reversed cardiac hypertrophy, and restored plasma and tissue ANF to somewhat normal levels, indicating that losartan and captopril are effective in the treatment of chronic heart failure (Qing and Garcia, 1992). In ovine heart failure (produced by rapid ventricular pacing), losartan decreased blood pressure, left atrial pressure, and plasma ANF levels (Fitzpatrick et al., 1992).

A potential mechanism for the positive effects of losartan treatment in chronic heart failure is suggested from a study by Chua et al. (1992a). They reported that 24 h after aortic constriction mRNAs for both atrial natriuretic peptide and brain natriuretic factor in the left ventricles were significantly elevated (Chua et al., 1992a). Treatment with losartan after aortic constriction resulted in lower levels of atrial natriuretic peptide and brain natriuretic factor compared to untreated animals (Chua et al., 1992a). This suggests that Ang II may play a role in the increase in both atrial natriuretic peptide and brain natriuretic factor mRNAs in the left ventricle after aortic constriction (Chua et al., 1992a). This may also explain the increases in plasma ANF levels that occurred in the untreated aorta-caval shunt animals and the decreases in plasma ANF levels seen in aorta-caval shunt animals treated with captopril and losartan. Therefore, these data indicate direct interaction of the Ang system with the ANF system.

IX. Bioavailability and Pharmacokinetic Considerations

As the first nonpeptide Ang II receptor antagonist, losartan is being utilized in many different experimental preparations. In rats, losartan is active and is converted to an active metabolite designated EXP3174 (Christ et al., 1990). The $t_{1/2}$ following a single 3-mg/kg, i.v., dose in the rat was 5.7 ± 2.0 and 6.0 ± 0.8 h for losartan and EXP3174, respectively, and 4.7 ± 0.4 and 5.0 ± 0.29 h, respectively, for a single 10-mg/kg p.o. dose (Christ et al., 1990). In dogs, losartan is not converted to an active metabolite to any significant extent. The $t_{1/2}$ is 0.5 and 2.3 h following i.v. and p.o. dosing, respectively (D. Christ, personal communication). In humans, losartan is converted to the active metabolite EXP3174. The $t_{1/2}$ for the 40-mg dose of losartan was 2.2 h and the $t_{1/2}$ for the EXP3174 that was formed was 6.7 h (Koppers and Hauswirth, 1992). The levels of losartan and EXP3174 in plasma and urine can be determined simultaneously by high-performance liquid chromatography (Furtek and Lo, 1992).

The metabolism of losartan has been studied in liver slices from rat, monkey, and humans. In rat, the primary route of metabolism is oxidative, leading to the free carboxylic acid (EXP3174), whereas in the monkey, the primary route is glucuronidation of the tetrazole moiety. In humans, approximately equal amounts of the oxidation and glucuronic acid-conjugated metabolites are formed (Stearns et al., 1992). Losartan is not a prodrug like enalapril, which is inactive itself and must be converted to the active enalaprilat. Losartan is active itself, and in some species, such as the rat and humans, it is converted to an active metabolite. The Ang II receptor blockade in these two species is, therefore, a net effect of the actions of losartan and EXP3174. Interestingly, the newly described nonpeptide antagonist TCV-116 is an ester prodrug that is inactive and must be converted to CV-11974.

Losartan is being widely used to define AT_1 function and to determine the Ang II component of previously observed actions of ACE inhibitors. Unfortunately, many studies utilize only one dose and assume that inhibition of a single dose of Ang II indicates AT_1 receptor blockade. In the dog, steady state Ang II receptor blockade can best be achieved by constant infusion of 30 to 50 μg/kg/min. Single i.v. doses are complicated by possible nonspecific blood pressure effects (Wong et al., 1991f). In rats, p.o. doses of 30 to 40 mg/kg/day have the expected comparable effects with ACE inhibitors, presumably due to more complete receptor blockade. In rats, for example, a 40-mg/kg/day dose of losartan produced a two-log shift in the Ang II dose-response curve (Raya et al., 1991). Lower doses (5 to 15 mg/kg/day) have yielded inconsistent results (Smits et al., 1992; Kauffman et al., 1991b).

X. Demonstrating Angiotensin II Antagonism and Antihypertensive Efficacy in Humans

The first Ang II antagonist to be studied clinically is losartan. The tolerance and inhibitory effects of losartan on the pressor action of exogenous Ang I and Ang II were studied in normal volunteers (Christen et al., 1991a,b). A dose-dependent inhibition of the systolic blood pressure response to exogenous Ang I was seen following single p.o. doses of losartan from 2.5 to 40 mg. The inhibitory effect after the 40-mg dose was still present 24 h after dosing. Dosing of losartan (5, 10, 20, or 40 mg, p.o., daily) for 8 days produced a dose-dependent inhibition of the pressor response to Ang II. Six hours after the 40-mg dose, the response to Ang II was reduced by approximately 60%. There was still a blocking effect detectable 24 h after the 40-mg dose. There was also a dose-related compensatory increase in PRA and

immunoreactive Ang II that was greater on day 8 than on day 1.

In subsequent studies (Munafo et al., 1992; Burnier et al., 1992b), the inhibitory effects of 40, 80, and 120 mg (p.o., daily) losartan on the pressor response to Ang II were studied. The 80-mg dose produced a maximum inhibition of Ang II action (94%) that could not be differentiated from the effect of 120 mg. The fact that inhibition of the pressor response to Ang II persists well beyond the time when plasma concentrations of losartan ($t_{1/4}$ 1.5 to 2 h) are nil (Christen et al., 1991b) suggested the presence of an active metabolite (EXP3174).

The time course of plasma concentrations of losartan and EXP3174 have been studied (Christen et al., 1991b; Munafo et al., 1992; Shum et al., 1991). From this work, it is clear that the prolonged duration of effect of losartan is due to the presence of EXP3174 which has a $t_{1/4}$ of 6 to 7 h.

Healthy male volunteers were examined to assess the safety, tolerance, and pharmacokinetics of single p.o. doses ranging from 10 to 300 mg. Losartan was well tolerated (Shum et al., 1991). Peak plasma concentrations of losartan were reached in 0.25 to 2 h and declined rapidly with a mean $t_{1/4}$ of 1.3 h. Mean maximum plasma concentration ranged from 21.1 to 2870.3 ng/ml for the 10- to 30-mg dose.

Nelson et al. (1991) reported the antihypertensive effects of 50, 100, and 150 mg (p.o., daily) losartan compared to placebo and 10 mg (p.o., daily) enalapril in 98 mildly to moderately hypertensive patients. After 5 days of dosing, there was a significant decrease in both systolic and diastolic blood pressures when compared to the placebo group. There was no difference in the magnitude of response between the three doses of losartan or the 10-mg dose of enalapril. It would appear that the selected doses of losartan are at the high end of the dose-response curve because 100 and 150 mg had no additional effect on blood pressure. Further studies are in progress to define the dose-response relationships of losartan with regard to blood pressure lowering.

Hagino et al. (1992) studied the chronic effects (2 to 4 weeks) of losartan on 24-h ambulatory blood pressure in eight hospitalized patients with essential hypertension receiving a normal sodium diet. These authors concluded that losartan exerted a long-acting hypotensive effect without changing the circadian rhythm or the variability of blood pressure in essential hypertension. Urinary excretion of creatinine was not altered. They also reported a favorable effect on uric acid metabolism. Losartan has been reported to increase urinary secretion of uric acid (Nakashima et al., 1992). In this study of single p.o. doses of 25, 50, 100, or 200 mg losartan, the drug produced a dose-dependent decrease in serum uric acid 4 h after dosing, which was accompanied by a corresponding increase in urinary excretion of uric acid. Similar results were seen in the multiple-dose study in that serum uric acid was decreased along with an increase in urinary uric acid secretion 4 h after dosing on days 1 and 7 of dosing. Although the mechanism of this effect is not known, the authors suggested the possibility that losartan may reduce the reabsorption of uric acid in renal tubules, thereby causing an increase in uric acid secretion (Nakashima et al., 1992). Because hyperuricemia occurs in up to 33% of patients with untreated mild hypertension, this potentially clinically useful effect of losartan should be further studied.

The effect of AT_1 receptor antagonism has also been evaluated in special subject/patient populations such as with salt depletion (Doig et al., 1992; Burnier et al., 1992a), nondiabetic renal disease (Madhun et al., 1992), and congestive heart failure. The results to date suggest that Ang II receptor antagonism gives results similar to those observed with ACE inhibitors. This conclusion will be validated by the large number of clinical trials now under way with losartan, TCV-116 (Ogihara et al., 1992), and other AT_1-selective nonpeptide Ang II antagonists.

XI. Future Perspectives

Ang II and the Ang system which encompasses the substrate angiotensinogen, the synthesizing enzymes renin and ACE, and the family of Ang peptides have emerged as critical elements of normal physiological control of the cardiovascular system and have been implicated as mediators in the pathogenesis of cardiovascular disease, especially hypertension and heart failure. The cloning and sequencing of the Ang II receptor in several species, including humans, now provide the possibility of assessing not only its three-dimensional structure but also the design of new generations of novel small molecules that can selectively interfere with the receptor-cellular response coupling. The functional importance of the AT_1 receptor subtypes (AT_{1A}/AT_{1B}) characterized in rodents is not known nor is whether such subtypes exist in humans. With reconstituted synthetic "receptors" or genetically altered receptors, the importance of the small differences in amino acid sequence should be understood. Ang II appears to share signal transduction pathways that are common with other hormones and growth factors. Knowing the structure of the intracellular domain of the Ang II receptor may allow us to understand how the mitogenic effects of Ang II are expressed alone and in combination with other growth factors.

The definition of a functional Ang II receptor versus and Ang II-binding site has not been established. The current wealth of data generated with the nonpeptide Ang II receptor antagonists, losartan and PD123177, have confirmed the concept of Ang II receptor heterogeneity but have added a new "type" designated AT_2. They have provided little evidence for subtypes of functionally coupled receptors, e.g., losartan blocks virtually all of the known effects of Ang II. The AT_2 receptor has not yet been cloned, but its structure my lead the way to

a functional definition. It is clear that much more work is needed to understand why the AT_2 site is so interestingly found in fetal tissue and in discrete regions of brain.

Does inhibiting the Ang system with ACE inhibitors and Ang II receptor antagonists give comparable results? With the early peptide antagonists such as saralasin, this was a difficult question. With losartan and the new nonpeptide receptor antagonists, this question is being studied, but certainly has not been resolved. From a mechanistic point of view, it is important to understand the biology of Ang II, and from a therapeutic point of view it may be critical to saving lives from cardiovascular and other diseases in which Ang II is involved. If these comparisons are to be made, however, full dose-response studies must by evaluated because many of the differences, or lack of differences, to date have been based on single-dose studies that may not be comparable in inhibiting the Ang system.

The full "efficacy" of current inhibitors of the Ang system may be masked by compensatory increases in PRA, Ang I, or Ang II. With ACE inhibitors, renin inhibitors, and peptide and nonpeptide Ang II receptor antagonists, these compensatory changes may be modifying the inhibitory responses. Not until the Ang system can be turned off, e.g., at the gene expression level, will the full role of the Ang system and of its inhibitors be known.

The clinical evaluation of losartan will allow the clinical scientist to explore in humans the many questions raised by the myriad of preclinical studies presently being carried out with this drug. Whether losartan is as good as an ACE inhibitor or whether it is the "ideal" Ang II receptor antagonist cannot be evaluated at this time, but, as the first of a new class of agents, it will serve as a bench mark for future compounds.

The pharmacology of nonpeptide Ang II receptor antagonists, which have a high specificity for the AT_1 receptor and which lack Ang II-like agonist activity, has been defined by losartan. New compounds that are either nonselective and block both AT_1 and AT_2 receptor types or selectively block one of the AT_1 receptor subtypes are likely to be developed and will support an even broader role for Ang II in cardiovascular and other diseases. Furthermore, the individual physical and receptor characteristics of these compounds are likely to expand the therapeutic application of inhibitors of the Ang system.

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