Br. J. Pharmacol. (1993), **110**, 761–771

Pharmacological profile of valsartan: a potent, orally active, nonpeptide antagonist of the angiotensin II AT_1-receptor subtype

[1]Leoluca Criscione, Marc de Gasparo, Peter Bühlmayer, Steven Whitebread, Hans-peter R. Ramjoué & Jeanette Wood

Cardiovascular Research Department, Pharmaceuticals Division, CIBA-GEIGY Limited, CH-4002 Basel, Switzerland.

1 The pharmacological profile of valsartan, (S)-N-valeryl-*N*-{[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]-methyl}-valine, a potent, highly selective, and orally active antagonist at the angiotensin II (AII) AT_1-receptor, was studied *in vitro* and *in vivo*.

2 Valsartan competed with [^{125}I]-AII at its specific binding sites in rat aortic smooth muscle cell membranes (AT_1-receptor subtype) with a K_i of 2.38 nM, but was about 30,000 times less active in human myometrial membranes (AT_2-receptor subtype).

3 In rabbit aortic rings incubated for 5 min with valsartan, at concentrations of 2, 20 and 200 nM, the concentration-response curve of AII was displaced to the right and the maximum response was reduced by 33%, 36% and 40%, respectively. Prolongation of the incubation time with valsartan to 1 h or 3 h, further reduced the maximum response by 48% or 59% (after 20 nM) and by 59% or 60% (after 200 nM) respectively. After 3 h incubation an apparent pK_B value of 9.26 was calculated. Contractions induced by noradrenaline, 5-hydroxytryptamine, or potassium chloride were not affected by valsartan. No agonistic effects were observed in the rabbit aorta at concentrations of valsartan up to 2 μM.

4 In bovine adrenal glomerulosa, valsartan inhibited AII-stimulated aldosterone release without affecting the maximum response (pA_2 8.4).

5 In the pithed rat, oral administration of valsartan (10 mg kg^{-1}) shifted the AII-induced pressor response curves to the right, without affecting responses induced by the electrical stimulation of the sympathetic outflow or by noradrenaline. Animals treated with valsartan 24 h before pithing also showed significant inhibition of the response to AII.

6 In conscious, two-kidney, one-clip renal hypertensive rats (2K1C), valsartan decreased blood pressure in a dose-dependent manner after single i.v. or oral administration. The respective ED_{30} values were 0.06 mg kg^{-1} (i.v.) and 1.4 mg kg^{-1} (p.o.). The antihypertensive effect lasted for at least 24 h after either route of administration. After repeated oral administration for 4 days (3 and 10 mg kg^{-1} daily), in 2K1C renal hypertensive rats, systolic blood pressure was consistently decreased, but heart rate was not significantly affected.

7 In conscious, normotensive, sodium-depleted marmosets, valsartan decreased mean arterial pressure, measured by telemetry, after oral doses of 1–30 mg kg^{-1}. The hypotensive effect persisted up to 12 h after 3 and 10 mg kg^{-1} and up to 24 h after 30 mg kg^{-1}.

8 In sodium-depleted marmosets, the hypotensive effect of valsartan lasted longer than that of losartan (DuP 753). In renal hypertensive rats, both agents had a similar duration (24 h), but a different onset of action (valsartan at 1 h, losartan between 2 h and 24 h).

9 These results demonstrate that valsartan is a potent, specific, highly selective antagonist of AII at the AT_1-receptor subtype and does not possess agonistic activity. Furthermore, it is an efficacious, orally active, blood pressure-lowering agent in conscious renal hypertensive rats and in conscious normotensive, sodium-depleted primates.

Keywords: Aldosterone; AT_1-receptor; human myometrium; hypertension; losartan; marmoset; pithed rat; rabbit aorta; renal hypertensive rats; telemetry; valsartan

Introduction

The octapeptide, angiotensin II (AII) which is formed from its precursor, the decapeptide angiotensin I, by the proteolytic action of the angiotensin-converting enzyme (ACE), has been shown to play a key role in the regulation of blood pressure and fluid and electrolyte homeostasis (for review see Hofbauer & Wood, 1986). Prevention of the formation of AII, via inhibition of ACE, has provided a powerful strategy for the treatment of hypertension and congestive heart failure (Ondetti *et al.*, 1977; Kramer *et al.*, 1983; CONSENSUS trial, 1987; Ondetti, 1991). Attempts to develop therapeutic agents capable of blocking AII at its receptor failed in the past, due to the antagonists being peptides that lacked oral activity (Pals *et al.*, 1979). In addition, saralasin, the most extensively investigated compound in this class, displayed unwanted agonistic properties (Pals *et al.*, 1971; Hofbauer *et al.*, 1976). More recently, starting from imidazole derivatives first described by Furukawa *et al.* (1982), it has been possible to identify and characterize specific, nonpeptide, AII-receptor antagonists (Duncia *et al.*, 1990; Carini *et al.*, 1990; Weinstock *et al.*, 1991; Bühlmayer *et al.*, 1991; Mantlo *et al.*, 1991).

Whitebread *et al.* (1989) first reported the existence of one subtype of AII receptor in vascular smooth-muscle cells, and a second in human uterus, while both subtypes were found in the rat and human adrenal glomerulosa, in rat uterus and

[1] Author for correspondence at: Ciba-Geigy Ltd, K 125.9.01, CH-4002 Basel, Switzerland.

human renal artery. Similar results, also relating to other tissues and species, have subsequently been reported (Chiu *et al.*, 1989; see also Smith *et al.*, 1992 for review). An international committee (Bumpus *et al.*, 1991) has proposed the designations AT_1 for the receptor subtype sensitive to DuP 753 (losartan), and AT_2 for the subtype sensitive to PD 123177 (Blankley *et al.*, 1991) and CGP 42 112 A (Whitebread *et al.*, 1989; 1991). The AT_1-receptor subtype is the one responsible for the well-known effects of AII, such as vasoconstriction, aldosterone and adrenaline release, water intake, and cellular proliferation (Criscione *et al.*, 1990; Herblin *et al.*, 1991; Timmermans *et al.*, 1991a; Chiu *et al.*, 1991c; Smith *et al.*, 1992), whereas the pathophysiological role of the AT_2-receptor subtype is not yet well understood (Criscione *et al.*, 1990; Herblin *et al.*, 1991; Dudley *et al.*, 1991; Timmermans *et al.*, 1991c). Recent reports, however, indicate that the AT_2 receptor does not interact with guanine-nucleotide-binding protein (Bottari *et al.*, 1991), but may stimulate tyrosine phosphatase activity (Bottari *et al.*, 1992).

Losartan (initially described as DuP 753), was the first orally active antagonist of the AT_1-receptor subtype (Timmermans *et al.*, 1990; Wong *et al.*, 1990d; Smith *et al.*, 1992). This compound is an efficacious antihypertensive agent in animals (Wong *et al.*, 1990a,d) and is also active in man (Christen *et al.*, 1991). The blood pressure-lowering effect of losartan in rats, however, depends partly on its conversion to the active, hepatically generated carboxylic acid metabolite, EXP3174, which is about 20 times more potent than the parent compound as an AT_1-receptor antagonist (Wong *et al.*, 1990c; Timmermans *et al.*, 1991b). The formation of an active metabolite in the liver is seen as a potential problem in predicting dosages of losartan for patients with impaired liver function (Wong *et al.*, 1991a). Moreover, the metabolism of losartan seems to differ in rats, monkeys, and man (Stearns *et al.*, 1992), and there are species-specific differences in its pharmacokinetics (Smith *et al.*, 1992).

The present paper describes the *in vitro* and *in vivo* pharmacological profile of valsartan, (known as CGP 48933), (S)-N-valeryl-*N*-{[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]-methyl}-valine, (Figure 1). The novelty of this structure resides in the replacement of the heterocycle imidazole of losartan with a nonplanar, acylated amino acid. In some experiments *in vivo*, the effects of valsartan have been compared with those of losartan. A preliminary account of this work has been published as an abstract (Criscione *et al.*, 1992).

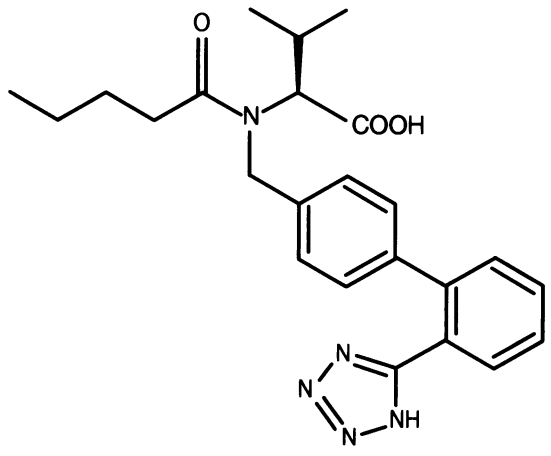

Figure 1 Chemical structure of valsartan, (S)-N-valeryl-*N*-{[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]-methyl}-valine.

Methods

Binding of valsartan to the AII receptor of smooth muscle cells from rat aorta and human myometrium

A primary culture of rat aortic smooth muscle cells (SMC) was grown on Dulbecco's Minimum Essential Medium (DMEM) containing 4.5 g l^{-1} glucose and supplemented with 4 mM L-glutamine, 15% foetal calf serum and penicillin-streptomycin, 200 iu–200 μg ml^{-1}. At confluence, the cells were washed twice with phosphate-buffered saline (PBS) and harvested with a rubber policeman. They were homogenized (Polytron setting 8, 1 × 8 s) in 20 mM sodium bicarbonate and centrifuged for 30 min at 60,000 *g* in a Beckman centrifuge (L7-35). The pellet was resuspended in 50 mM Tris-HCl buffer, pH 7.4, containing 125 mM NaCl, 6.5 mM $MgCl_2$, 1 mM EDTA, and a cocktail of peptidase inhibitors (antipain, phosphoramidon, leupeptin, pepstatin, bestatin, amastatin, each at 1.25 μg ml^{-1}) with a tight-fitting teflon-pestle homogenizer. The membrane preparations were kept in aliquots at – 80°C until used; no apparent loss of AII-binding activity was seen under these conditions. Protein concentration was measured according to the method of Bradford (1976) using bovine serum albumin (BSA) as standard.

Human uteri were obtained from informed and consenting patients undergoing hysterectomy. The tissue was cut in small pieces, immediately frozen on dry ice and kept at – 80°C until required. It was homogenized in 20 mM sodium bicarbonate (Polytron setting 8, 3 × 8 s) and centrifuged at 600 *g* for 20 min at 4°C. The pellet was resuspended and treated similarly once more. The pooled supernatants were then centrifuged at 60,000 *g*, and the pellet was resuspended in buffer, as described above.

The experiments were performed using an automatic pipetting and filtration device (Filter-Prep 101, Ismatec, Zurich, Switzerland). Briefly, 20–30 μg protein was incubated at 25°C for 60 min with [^{125}I]-AII (175 pM) and varying concentrations of unlabelled competitors, dissolved in dimethylsulphoxide (DMSO); the final concentration of the latter in the incubation was 1%. The binding experiments were performed in the presence or absence of BSA (Chiu *et al.*, 1991a). The reaction was terminated by the addition of 2 ml ice-cold buffer. Bound and free radioactivities were separated by immediate filtration through Whatman GF/F filters, pretreated with 0.2% BSA in PBS, which were washed 3 times with 2 ml cold PBS. The radioactivity trapped on the filter was measured in a gamma counter (Pharmacia-LKB, Uppsala, Sweden) at 70% efficiency. Nonspecific binding was determined in the presence of 1 μM unlabelled AII. Degradation of the radioligands during the incubation was always less than 10%, as determined by thin layer chromatography, using the method described by Whitebread *et al.* (1991).

Functional antagonism, specificity, and potency of valsartan in rabbit isolated aortic rings

Rabbits (2–2.5 kg, Chinchilla, male, Dr K. Thomae, Germany) were killed by a blow to the neck and the descending thoracic aorta quickly removed. From each aorta, rings of 2–3 mm width were prepared and mounted between two parallel hooks under an initial resting tension of 2.5 g. Thereafter, rings were immersed in a 20 ml tissue bath containing a modified Krebs-Henseleit solution of the following composition (mM): NaCl 119, KCl 4.8, $CaCl_2$ 2.53, $NaHCO_3$ 24.8, Mg_2SO_4 1.2, KH_2PO_4 1.2 and glucose 10; at 37°C, gassed with 95% O_2 and 5% CO_2. Each preparation was allowed to equilibrate for at least 1 h. Isometric responses were measured with a force transducer (K30, Hugo Sachs Electronics, Freiburg, Germany) coupled to a tissue-bath data-acquisition system (Buxco Electronics, Inc., Sharon, CT, U.S.A.). Data were analyzed with the Buxco digital computer

and a software package (Branch Technology, Dexter, MI, U.S.A.).

In a first series of experiments, contractions of rabbit aortic rings were induced with graded, cumulative concentrations of AII (Hypertensin CIBA), noradrenaline, 5-hydroxytryptamine (5-HT) or potassium chloride. Control rings were incubated with appropriate concentrations of the vehicle (DMSO). Valsartan or solvent was added to the organ-bath 5 min before the dose-response curves of the various vasoconstrictor agents were determined.

In a second series of experiments, contractions induced by graded, cumulative concentrations of AII were tested after the rings had been incubated with valsartan or solvent for 1 h or 3 h respectively.

Effects of valsartan on AII-induced aldosterone release in bovine adrenal glomerulosa cells

Bovine adrenal glands were obtained from a local slaughterhouse and processed within 45–60 min. Adrenals from 5–7 animals were collected and placed in ice-cold, potassium-free Medium 199 (Amimed, Muttenz, Switzerland). After removal of adherent fat, 0.5 mm-thick slices of capsular tissue with adherent glomerulosa tissue were cut with a Staddie-Riggs microtome. Glomerulosa tissue slices (about 8 g of tissue) were washed in three volumes of ice-cold, potassium-free Medium 199 with 0.4% BSA. The tissue was minced with scissors and incubated for 45 min at 37°C in 25 ml of potassium-free Medium 199, pH 7.3, containing 0.4% BSA, 10 mg ml^{-1} dispase and 25 $\mu g\ ml^{-1}$ DNase from bovine pancreas (Boehringer Grade II, Mannheim), under constant agitation at 150 r.p.m. in an atmosphere of 95% O_2/5% CO_2. Cells were dispersed by aspirating and expelling tissue 10–20 times with a narrow-bore 10 ml pipette every 15 min during the incubation period. The cell suspension was centrifuged (350 *g*, 8 min, 4°C), the pellet resuspended in 25 ml enzyme solution, and the enzymatic procedure repeated for 30 min. Following dissociation, the cell suspension was filtered through a 70 μm nylon filter, and the cells washed 3 times in potassium-free Medium 199 containing 0.4% BSA. The cell pellet was resuspended in Medium 199 containing 5.5 mM potassium and 0.1% BSA. The final cell suspension was filtered through a 100 μm nylon filter. Cells were counted in a haemocytometer and cell viability (usually >90%) was assessed by trypan blue exclusion. The yield from 10–14 adrenals was $6–8 \times 10^7$ cells. For use in a subsequent incubation, one half of the cell preparation was stored at 4°C in DMEM supplemented with 10% foetal calf serum. These cells were found to be responsive to AII for at least 12–16 h.

For aldosterone release, adrenal cells were suspended in Medium 199 containing 5.5 mM potassium and 0.1% BSA, at a concentration of 5.21×10^5 cells ml^{-1}. Aliquots of 960 μl (5×10^5 cells) were distributed into 35-mm, six-well, tissue-culture plates. AII, with or without test compound, was added in 40 μl of Medium 199 at the indicated concentrations. The cells were incubated for 2 h at 37°C under 95% O_2/5% CO_2 with constant shaking at 70 cycles min^{-1} and then centrifuged at 1200 *g* at 4°C for 10 min. Aldosterone in the supernatant was measured directly with a radioimmunoassay kit (Coat-a-Count) from Diagnostic Products Corporation (Buehlmann Laboratories).

Functional antagonism, specificity, and potency of valsartan in vivo *in pithed rats*

Male rats of the Sprague-Dawley-derived strain of *Rattus norvegicus* (Tif: RAIf) (280–350 g) were obtained from Tierfarm AG, Sisseln, Switzerland. They were pithed under Pentothal anaesthesia, according to the technique of Gillespie & Muir (1967). Immediately after pithing, the rats were ventilated with room air, enriched with 33% O_2, using a respiratory pump (Harvard Model 683, South Natick, Mass, USA, at 50 cycles min^{-1}, 10 ml kg^{-1}). The body temperature of the rats was maintained at 37°C by a thermostatically controlled heating lamp and monitored with a rectal thermometer (Systag TCU-82, Rüschlikon, Switzerland). The left common carotid artery was cannulated for mean arterial pressure (MAP) measurement (Isotec transducer, Miamisbourg, OH, U.S.A.; Hellige recorder, Freiburg im Breisgau, Germany). Drugs were administered through a cannula placed in the right jugular vein. Atropine (0.3 mg kg^{-1}) and tubocurarine (2 mg kg^{-1}) were given i.v., 10 min apart, after pithing. Thereafter, rats were challenged with AII (0.03 to 100 $\mu g\ kg^{-1}$), the electrical stimulation of the sympathetic outflow (0.1 to 10 Hz, 40mA, 1 ms, for 10 s), or noradrenaline (0.03 to 30 $\mu g\ kg^{-1}$).

Rats were given orally 10 mg kg^{-1} of valsartan 2 h ($n = 7$), 4 h ($n = 8$) or 24 h ($n = 5$) prior to the challenge with the pressor agents. Control rats ($n = 7$), received 2 ml kg^{-1} of the solvent.

Antihypertensive effect of valsartan in renal hypertensive rats (2K1C)

Male normotensive (WKY) rats, aged 5–6 weeks (IFFA, CREDO, L'Arberesle, France), were made hypertensive by constriction (0.2 mm silver clip) of one renal artery under light ether anaesthesia (Goldblatt 2K1C). Rats were used 5–7 weeks after clipping. Systolic blood pressure (SBP) and heart rate (HR) were measured indirectly in the tail arteries of conscious restrained rats with an inflatable cuff and a piezoelectric detector attached to a pen recorder (W + W Electronics blood-pressure recorder, Model 8005, Comerio, Italy). The rats were placed in individual restraining tubes, transferred to an oven preheated to 30°C, and left there for 1 h for the arteries in the tail to dilate. SBP and HR were measured weekly after the renal clip was implanted. Only rats with a SBP higher than 220 mmHg were used.

Intravenous administration An on-line computerized system was used for continuous intra-arterial measurements of MAP and HR in unrestrained rats, as described previously (Bunkenburg *et al.*, 1991). Catheters were implanted in a femoral vein and an artery under halothane anaesthesia at least 48 h before an experiment. Throughout the experimental procedure, the rats were kept in individual cages where they could move freely with access to food and water.

Valsartan was given by single bolus i.v. injection in doses ranging from 0.01 to 10 mg kg^{-1}. Each animal received only one dose. One group received the ACE inhibitor, enalaprilat (3 mg kg^{-1} i.v.). MAP and HR were measured continuously throughout the experiment.

In another group of rats, the nonselective β-adrenoceptor antagonist, propranolol (1 mg kg^{-1}, i.v.) was administered 30 min before an i.v. bolus injection of valsartan (3 mg kg^{-1}). MAP and HR were measured continuously as described above.

Single oral administration Rats were treated with single doses (1, 3 and 10 mg kg^{-1}) of valsartan, given by gavage. SBP and HR were measured in conscious restrained rats (by the tail-cuff method described above) before and 2, 4 or 24 h after administration of valsartan. The rats were returned to their cages between measurements. In a separate study, losartan was given orally at a dose of 10 mg kg^{-1} p.o.

Repeated oral administration Rats were treated once daily for 4 days with 3 or 10 mg kg^{-1} of valsartan. SBP and HR were measured by the tail-cuff method before and 2, 4 or 24 h after each administration. The rats were restrained for the measurement, but were returned to their cages between measurements.

In all experiments *in vivo*, the compounds were dissolved by addition of NaOH (0.1 M) and the pH adjusted to about 8 with HCl (0.1 M). Control rats received equivalent volumes of the solvent mixture.

Hypotensive effect of valsartan in conscious, normotensive sodium-depleted marmosets

Experiments were performed in conscious freely moving marmosets (250–350 g in weight, aged 1–3 years) with an implanted transmitter unit (AM Unit, model TA11PA-C40, Data Sciences, Inc., St. Paul, Minnesota, U.S.A.), for the measurement of blood pressure by telemetry (Schnell & Wood, 1993). The pressure transmitters were implanted into the peritoneal cavity under aseptic conditions and light anaesthesia (combination of alfaxalonum (10 mg kg^{-1}), atropine (0.15 mg kg^{-1}), and diazepam (0.75 mg kg^{-1}) i.m.). With the aid of a microscope, the ascending aorta was exposed through a midline incision in the abdomen and the sensor catheter was placed in the aorta below the renal artery pointing upstream. The transmitter was sutured to the inner abdominal wall with a continuous suture. Immediately after surgery the marmosets were given penicillin and piroxicam (5000 iu and 2 mg per animal, respectively). The marmosets were allowed to recover for at least 4 weeks before any experiment was started. One week before beginning an experiment, the marmosets were maintained on a low sodium diet consisting of laboratory chow (NAFAG 9627, Gossau, Switzerland) supplemented with fruit. They received furosemide (approximately 5 mg day^{-1}) in their drinking water (0.2 mg ml^{-1}) for 48 h before drug administration.

MAP and HR were measured continuously while the marmosets were freely moving in their normal cages commencing 48 h before drug administration. The compounds were given by gavage. MAP was monitored for a further 24 h. The marmosets received either vehicle (0.9% saline, 1 ml kg^{-1}), valsartan or losartan in doses of 1, 3, 10 and 30 mg kg^{-1}. Values for MAP and HR were averaged over a 1 h period and changes calculated using each marmoset as its own control; each post-administration value was subtracted from the value for the corresponding period of time in the pretreatment period.

Drugs

The drugs used were obtained from commercial suppliers: ^{125}I-labelled AII and Sar^{1}Ile^8 AII (2200 Ci $mmol^{-1}$) (Anawa; Wangen, Switzerland); unlabelled AII, Sar^{1}Ile^8 AII, Sar^{1}Ala^8 AII (Bachem; Bubendorf, Switzerland); peptidase inhibitors (Novabiochem; Läufelfingen, Switzerland); culture media (Amimed; AG, Muttenz, Switzerland); 5-HT and noradrenaline (Fluka, Switzerland); Hypertensin CIBA (CIBA-GEIGY, Basel, Switzerland); heparin and diazepam (Roche, Basel, Switzerland); halothane (Hoechst, Zurich, Switzerland); atropine (Siegfried A.G., Zofingen, Switzerland); tubocurarine (Wellcome AG, Reinach, Switzerland); Pentothal (Abbott AG, Cham, Switzerland); saffan (Glaxovet Ltd., Uxbridge, U.K.); penicillin (Duplocilline LA, (Veterinaria AG), Zurich, Switzerland); piroxicam (Piroxicam-mepha, Mepha Pharma AG, Aesch, Switzerland). Enalapril was kindly supplied by MSD. Valsartan, losartan and propranol were synthesized in the Chemistry Department of Ciba-Geigy.

Statistical analysis

Dose-response curves were analyzed using the four-parameter logistic method of De Lean *et al.* (1978) to estimate IC_{50} or EC_{50}. Inhibition constants (K_i) were calculated from competitive binding experiments according to the formula $K_i = IC_{50}/(1 + L/K_d)$, where L is the concentration of radioactive ligand and K_d its dissociation constant. K_i was calculated from the reciprocal analysis of [^{125}I]-Sar^{1}Ile^8-AII binding in the presence and absence of valsartan, using the formula $K_i = [\text{valsartan}]/((K_{d'}/K_d)-1)$, where $K_{d'}$ is the apparent K_d in the presence of [valsartan]. K_d was calculated using the LIGAND programme (Munson & Rodbard, 1980). In the isolated aortic rings, the conventional Schild analysis for estimation of potency could not be used because valsartan reduced the maximum response to AII. Instead, apparent pK_B values were derived by using a double-reciprocal regression plot as described by Kenakin (1984) and Robertson *et al.* (1992). In adrenal glomerulosa cells (inhibition of aldosterone biosynthesis), the maximum response to AII was not affected by valsartan, thus a pA_2 value was calculated according to Wiest *et al.* (1991).

MAP, SBP and HR are presented as means of absolute or changes over the time period of interest and expressed as mean ± s.e.mean. The ED_{30} of valsartan, i.e. dose which decreased either MAP or SBP by 30 mmHg, was derived by linear regression. Data were analyzed by analysis of variance followed by Bonferroni's method or Student's unpaired *t* test at time points of interest. Significance level was taken as $P<0.05$.

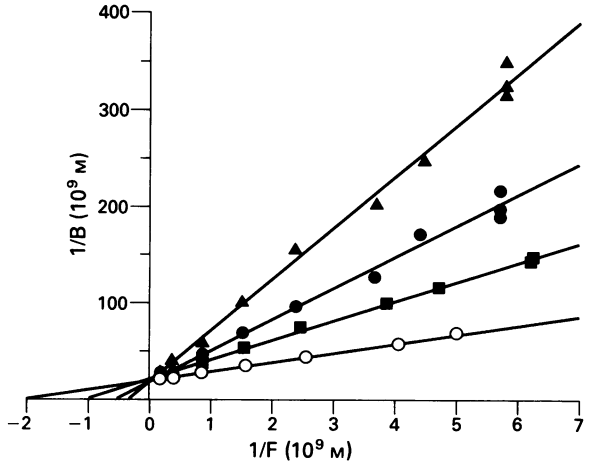

Figure 2 Reciprocal analysis of [^{125}I]-Sar^{1}Ile^8-AII binding to rat aortic smooth muscle cell membranes in the presence and absence (control ○) of three concentrations of valsartan (■ 1, ● 2, ▲ 4 nM). Radioligand (0.175 nM) was incubated for 60 min at 25°C with unlabelled Sar^{1}Ile^8-AII (0.05–5 nM). The K_d values for the control was 0.50 nM. The K_i values at the three concentrations of valsartan were 0.87, 0.85 and 0.88 nM. B = bound Sar^{1}Ile^8-AII; F = unbound Sar^{1}Ile^8-AII.

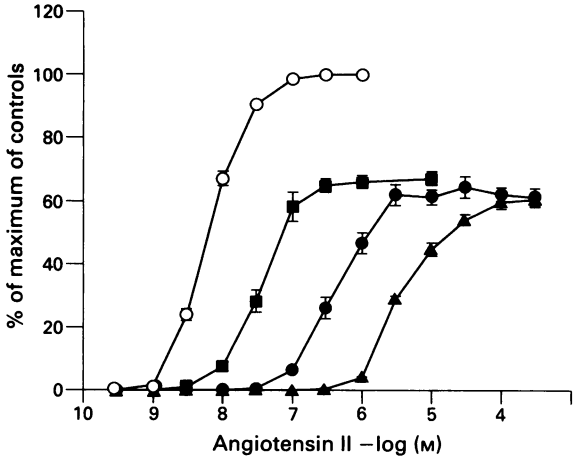

Figure 3 Inhibitory effects of valsartan (■ 2, ● 20, ▲ 200 nM, incubated for 5 min) on the contractions induced by angiotensin II (AII) in rabbit aortic rings. Results are expressed as percentage of the maximum response obtained with AII in rings treated with appropriate concentrations of the solvent (○). Values represent the mean ± s.e.mean of 12–14 experiments.

Results

Binding of valsartan to the AII receptor of smooth muscle cells (SMC) from rat aorta (AT_1) and human myometrium (AT_2)

Valsartan competed for [^{125}I]-AII binding to membranes from SMC and human myometrium with K_i values of 2.38 ± 0.31 nM (mean ± s.e.mean; $n = 5$) and 57.7 ± 9.4 μM ($n = 5$), respectively, giving a selectivity factor of more than 30,000. By contrast, the peptide antagonist, Sar^{1}Ile^8-AII, bound with high affinity to both receptor subtypes (K_i 0.85 ± 0.07 nM and 0.23 ± 0.04 nM, $n = 4$, respectively). The corresponding K_d values for AII were 1.28 ± 0.16 nM ($n = 11$) and 0.44 ± 0.035 nM ($n = 10$). A reciprocal analysis of [^{125}I]Sar^{1}Ile^8-AII binding to SMC in the absence or presence of 1, 2 or 4 nM valsartan demonstrated that this compound is a competitive inhibitor at the receptor level, as in the presence of inhibitor, the B_{max} (y-intersect) remained unchanged (Figure 2).

The results quoted above were all obtained in the absence of BSA in the incubation medium. In vascular SMC, valsartan had a 3 fold lower affinity in the presence of BSA (K_i 7.06 ± 0.63 nM, $n = 9$; K_d for AII: 0.67 ± 0.09 nM, $n = 7$). In human myometrium, valsartan inhibited binding by 39 ± 1.8%, $n = 5$, at 100 μM.

Functional antagonism, specificity, and potency of valsartan in rabbit isolated aortic rings

Valsartan, when incubated for 5 min at concentrations of 2, 20 or 200 nM, displaced the concentration-response curve of AII to the right and reduced the maximum contractile response to AII by 33%, 36% and 40%, respectively (Figure 3). When the rings were incubated for 1 h or 3 h with 20 nM valsartan, the AII concentration-response curve was further displaced to the right and the maximum response was decreased by 48% and 59% respectively. After 200 nM, the maximum response to AII was similarly reduced after 1 h (by 59%) or after 3 h (by 60%), and the EC_{50} values for AII were not significantly affected (6.5 and 8.9 μM respectively). After 3 h incubation, an apparent pK_B value of 9.26 was calculated. In Figure 4, the effects of the three different incubation times (5 min, 1 h or 3 h) with 20 nM valsartan are given. In this experiment, the calculated EC_{50} for AII was 15 nM (control) and 410 nM after incubation for 5 min with 20 nM valsartan. The EC_{50} for AII increased 2 fold after

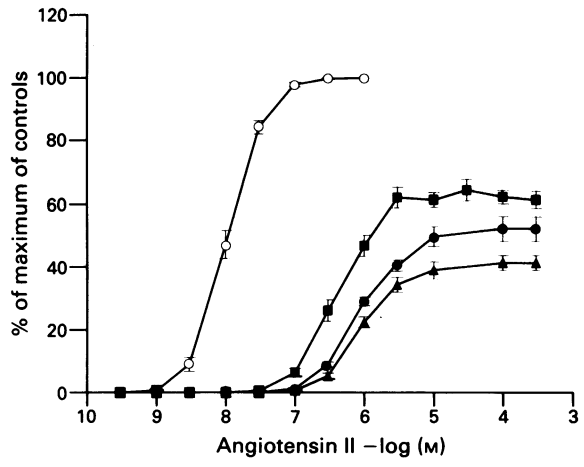

Figure 4 Inhibitory effects of 20 nM valsartan incubated for 5 min (■), 1 h (●), or 3 h (▲), on the contractions induced by angiotensin II (AII) in rabbit aortic rings. Results are expressed as percentage of the maximum response obtained with AII in rings treated with appropriate concentrations of the solvent (○). Values represent the means ± s.e.mean of 4–14 experiments.

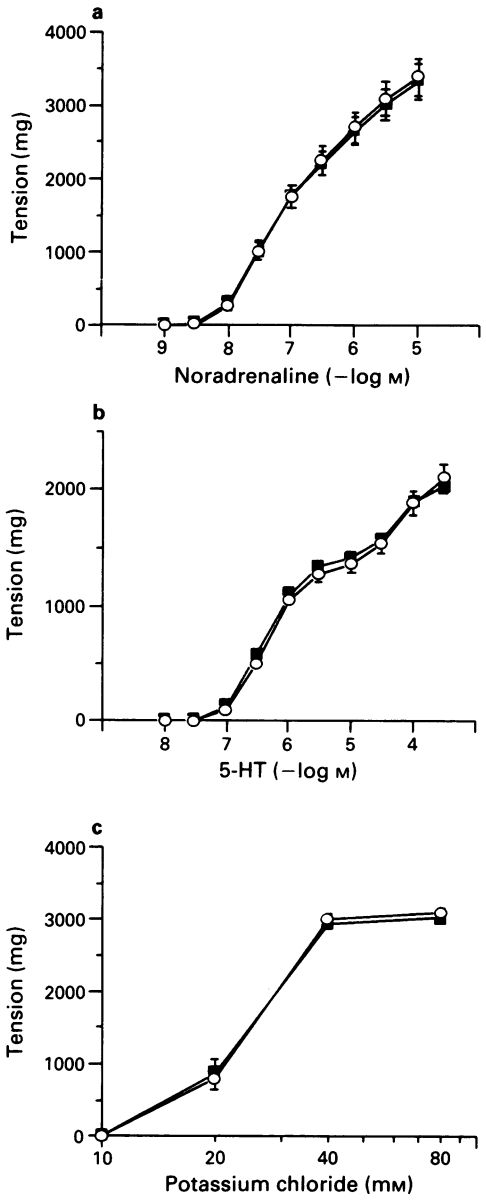

Figure 5 Effects of vehicle (○) or valsartan (■ 2 μM) on the concentration-response curves of rabbit aortic rings for contractions induced by noradrenaline (a), 5-hydroxytryptamine (5-HT) (b) and potassium chloride (c). Values represent the means ± s.e.mean of 8 experiments in each group.

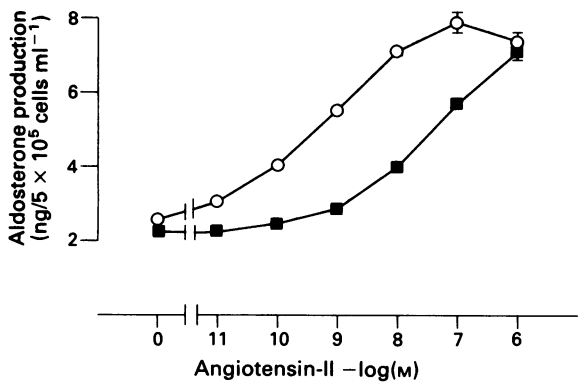

Figure 6 Effects of vehicle (○) or valsartan (■ 300 nM) on angiotensin II-stimulated aldosterone production in dispersed bovine adrenal glomerulosa cells. Values represent means ± s.e.mean of six determinations. Error bars smaller than the symbols were omitted.

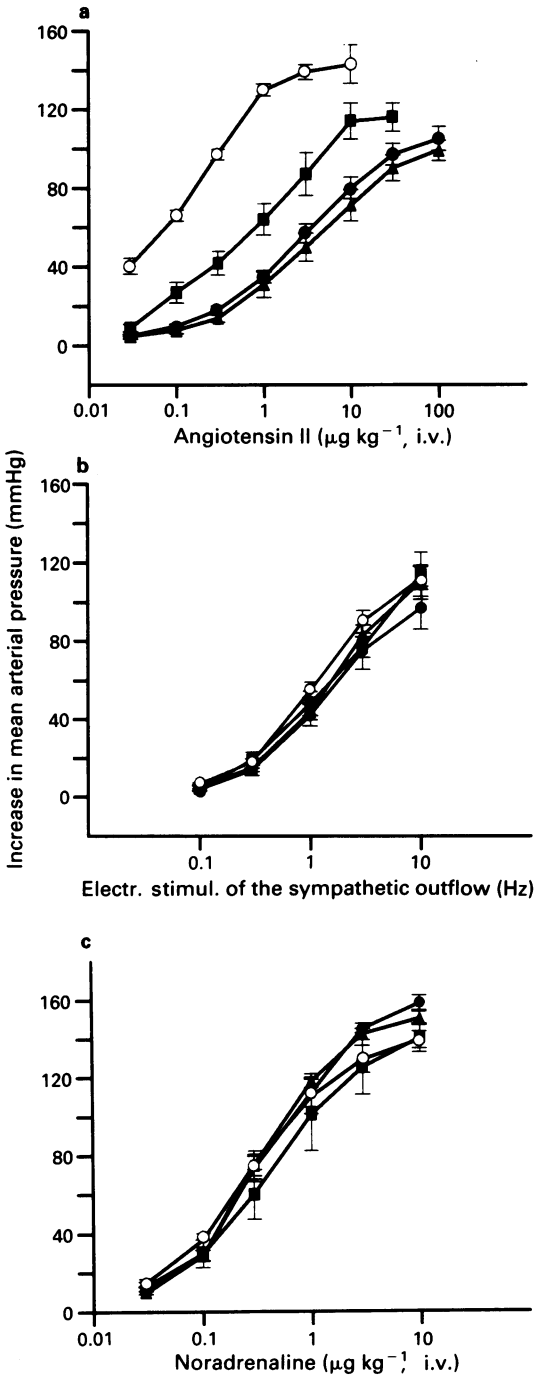

Figure 7 Effects of oral application of vehicle (○) or valsartan 10 mg kg^{-1} on the pressor-response curves to angiotensin II (a), electrical stimulation of the sympathetic outflow (b) and noradrenaline (c) in pithed rats. Animals were treated with valsartan 2 h (▲), 4 h (●) and 24 h (■) prior to challenge with the three pressor agents. Values represent the means ± s.e.mean of 5–8 experiments in each group. In (a), all three treatment regimes were significantly different fron controls, and the 24 h regime was significantly different from the 2 and 4 h regimes respectively.

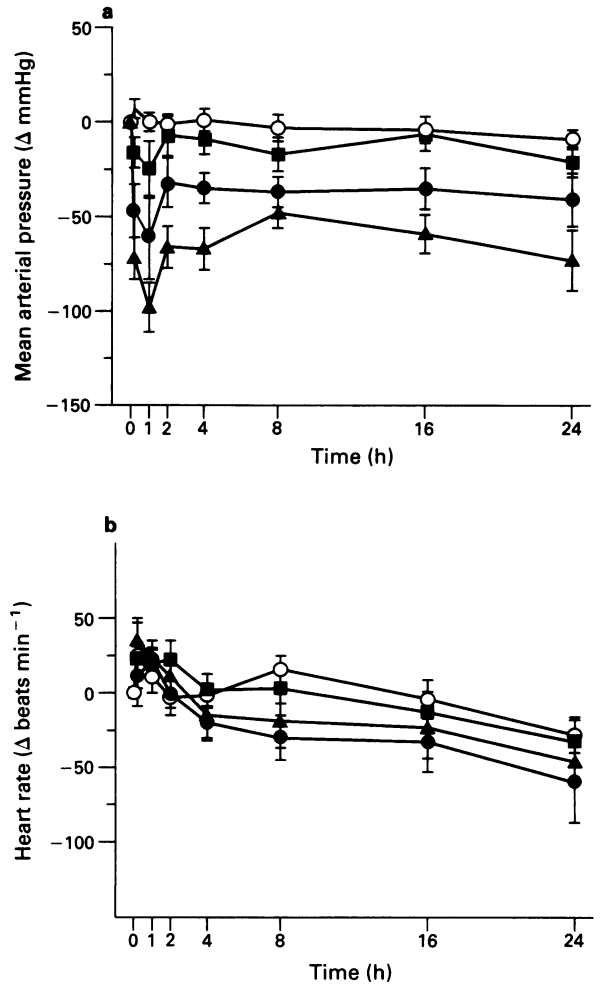

Figure 8 Effects of intravenous administration of vehicle (○) or valsartan (■ 0.03, ● 0.1, ▲ 1 mg kg^{-1}) on mean arterial pressure (a) and heart rate (b) in conscious, renal hypertensive rats (2K1C). Values represent the means ± s.e.mean of 5–7 animals per group.

incubation for 1 h (920 nM) or 3 h (930 nM). At a concentration of 2 μM, the compound had no effect on contractions induced by noradrenaline, 5-HT, or potassium chloride (Figure 5). No agonistic effects were observed up to a concentration of 2 μM.

Effects of valsartan on AII-induced aldosterone release in bovine adrenal glomerulosa cells

The addition of increasing concentrations of AII (10 pM to 10 μM) to dispersed adrenal glomerulosa cells results in a concentration-dependent stimulation of aldosterone production. As shown in Figure 6, the maximum AII-induced aldosterone response was more than 3 times the basal aldosterone values. The addition of valsartan (300 nM) to this system inhibited aldosterone production, as indicated by the parallel shift to the right of the AII concentration-response curve (Figure 6). The calculated ED_{50} values for AII in the presence and absence of the antagonist were 49 nM and 0.64 nM, respectively. The pA_2 value was calculated to be 8.41 (K_B = 3.9 nM). Valsartan did not affect the aldosterone release induced by the addition of 5.5 mM potassium (results not shown).

Functional antagonism, specificity and duration of action of valsartan in vivo *in pithed rats*

Valsartan at a dose of 10 mg kg^{-1} p.o. did not significantly affect basal MAP values in any of the three time regimes. Vehicle-treated rats had an initial MAP of 57 ± 3.6 mmHg, (n = 7); the 2 h group, 51 ± 5.2 mmHg, (n = 6); the 4 h group, 51 ± 3.1 mmHg; (n = 8); and the 24 h group, 47 ± 3.4 mmHg, (n = 5). The pressor-response curves to AII were significantly shifted to the right after all three time regimes. The effects at 24 h, however, were significantly less pronounced than after 2 h and 4 h respectively (Figure 7a). The pressor-response curves to electrical stimulation of the sympathetic outflow or to noradrenaline were not affected by the compound at any time regime (Figure 7,b,c).

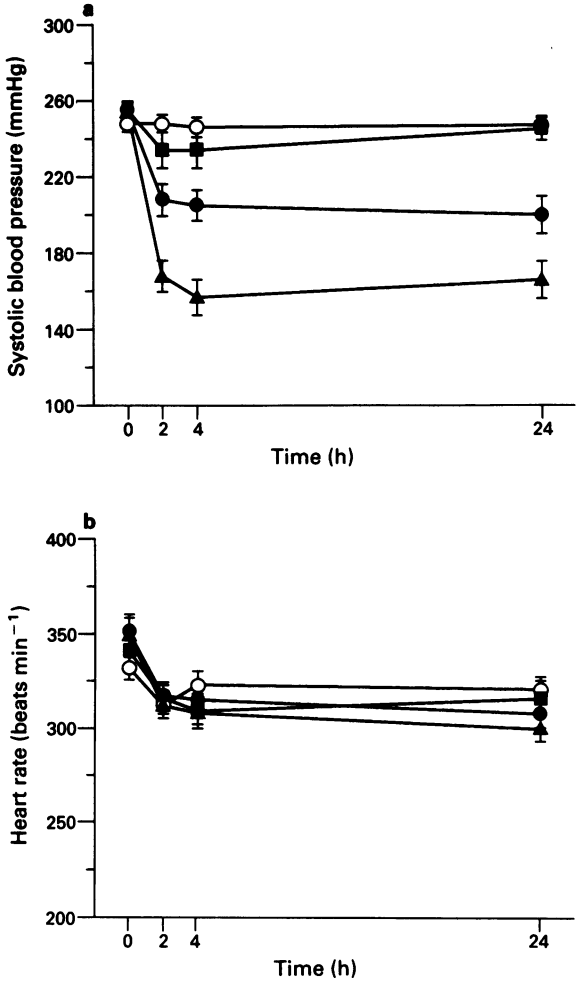

Figure 9 Effects of oral administration of vehicle (○) and valsartan (■ 1, ■ 3, ▲ 10 mg kg^{-1}) on systolic blood pressure (a) and heart rate (b) in conscious renal hypertensive rats (2K1C). Values represent means ± s.e.mean of 16–29 animals per group.

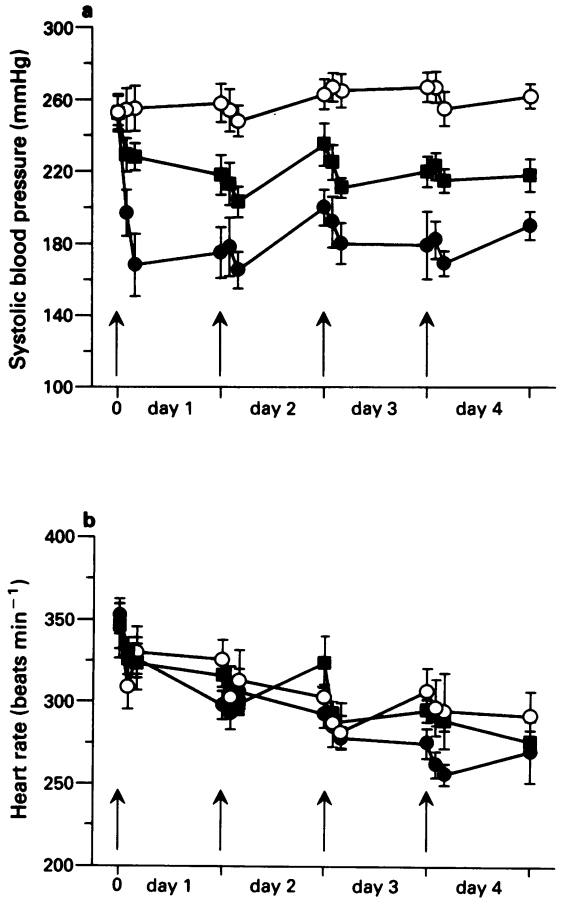

Figure 11 Effects of repeated oral administration of vehicle (○) or valsartan (■ 3 mg kg^{-1}) and (● 10 mg kg^{-1}) on systolic blood pressure (a) and heart rate (b) in conscious renal hypertensive rats (2K1C). Rats were treated once daily for 4 days at time points indicated by the arrows. Measurements were performed 2, 4 and 24 h after each administration. Values represent the means ± s.e.mean of 6 rats in each group.

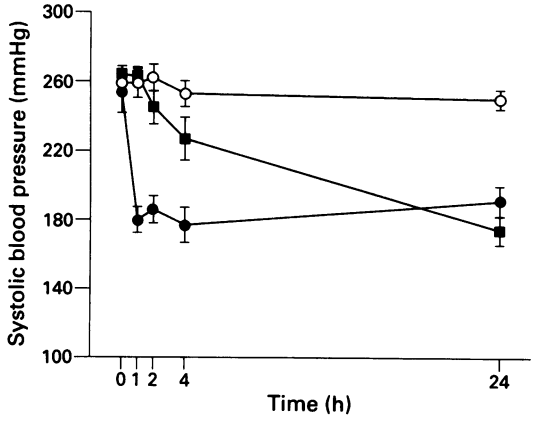

Figure 10 Effects of oral administration of vehicle (○), losartan (■ 10 mg kg^{-1}), and valsartan (● 10 mg kg^{-1}) on systolic blood pressure in conscious, renal hypertensive rats (2K1C). Measurements were performed 1, 2, 4 and 24 h after a single administration. Values represent means ± s.e.mean of 5–12 animals per group.

Antihypertensive effect of valsartan in conscious renal hypertensive rats (2K1C)

Intravenous administration Valsartan (0.03–1 mg kg^{-1}, n = 5–7) decreased MAP in a dose-dependent manner (Figure 8a). The 3 mg kg^{-1} and 10 mg kg^{-1} doses did not have any additional antihypertensive effect (data not shown). The dose which produced a decrease of 30 mmHg (ED_{30}) was calculated to be 0.06 mg kg^{-1}. The initial immediate decrease in MAP was accompanied by a transient increase in HR in the first 30–60 min after injection. The antihypertensive effects of doses of 0.1 mg kg^{-1} and above persisted for up to 24 h (Figure 8a). The maximum response induced by 3 mg kg^{-1} of valsartan (− 105 ± 7 mmHg) at 60 min was similar to that induced by the same dose of the ACE inhibitor enalaprilat (− 106 ± 8 mmHg) at 60 min. The transient increase in HR observed after i.v. administration of valsartan was prevented by pretreatment with propranolol 1 mg kg^{-1} (30 min before) (data not shown).

Single oral administration Valsartan in oral doses of 1, 3 or 10 mg kg^{-1}, decreased SBP in a dose-dependent manner. The maximal decreases were of − 20.9 ± 8.6 (n = 16), − 49.7 ± 8.1, (n = 29) and − 97.4 ± 11.4 (n = 17) mmHg respectively. The dose which produced a decrease of 30 mmHg (ED_{30}) was calculated to be 1.4 mg kg^{-1}. With the threshold dose of 1 mg kg^{-1}, the antihypertensive response persisted for less

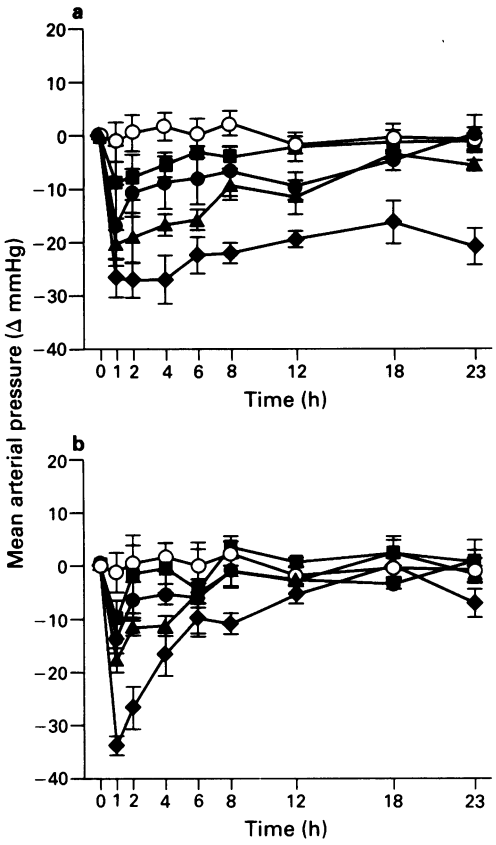

Figure 12 Effect of oral administration of vehicle (○) or valsartan (a) or vehicle and losartan (b) on mean arterial pressure in conscious, freely moving and sodium-depleted marmosets. The doses for both agents were 1 (■), 3 (●), 10 (▲) and 30 (◆) mg kg^{-1}. Values represent means ± s.e.mean of 4–6 animals per group.

than 24 h, whereas with the doses of 3 and 10 mg kg^{-1} the effect lasted for at least 24 h (Figure 9a). HR tended to decrease during the experiment in all groups and there was no significant effect of valsartan (Figure 9b). Compared with losartan, valsartan had a more rapid onset of action, whereas the effect of both compounds 24 h after administration was similar (Figure 10).

Repeated oral administration On the first day of administration of doses of 3 or 10 mg kg^{-1} (Figure 11a), the blood pressure-lowering effect was similar to that seen in the single-dose experiment (Figure 9a). The antihypertensive effects persisted for 24 h after the first dose. After the administration of the second, third and fourth dose, a small, non significant additional fall in SBP was observed. SBP remained consistently decreased during the treatment period. HR, was not significantly affected by valsartan when compared to the vehicle control group (Figure 11b).

Hypotensive effect of valsartan in conscious, normotensive, sodium-depleted marmosets

Valsartan induced dose-dependent reductions in MAP in conscious freely-moving sodium-depleted marmosets (Figure 12a). The threshold dose was about 1 mg kg^{-1}. With doses of 3 mg kg^{-1} and above, the maximum response developed within 1 h of administration. The response persisted for up to 12 h after valsartan at 3 and 10 mg kg^{-1} and for more than 24 h after the 30 mg kg^{-1} dose. Losartan also induced dose-dependent reductions in MAP in this model, but the duration of the hypotensive response was much shorter than with valsartan. MAP had recovered 10–12 h after the 30 mg kg^{-1} p.o. dose of losartan (Figure 12b). Neither of the AII antagonists had a significant effect on HR in the dose-range tested when compared with the vehicle-treated marmosets.

Discussion

In this paper, we present evidence that valsartan is a potent, highly specific, and orally active nonpeptide receptor antagonist of the AT_1 receptor subtype. Moreover, these data clearly demonstrate that valsartan is an efficacious and long-acting antihypertensive agent in renal hypertensive rats, and also lowers blood pressure in normotensive sodium-depleted marmosets.

Valsartan inhibited binding of AII to AT_1-receptors in aortic SMC, with a 30,000 fold higher affinity than for AII receptors in human myometrium, which are exclusively of the AT_2-receptor subtype. Valsartan is a competitive inhibitor at the receptor level. The high specificity for the AT_1-receptor subtype is substantiated by the fact that at a concentration of 10 μM valsartan lacks affinity for a series of other receptors, including: α_1-, α_2-, β_1-adrenoceptors, histamine$_1$, substance P, $GABA_A$, $GABA_B$, muscarinic, 5-HT_1 and 5-HT_2, and calcium channels (S. Bischoff, H. Bittiger, K. Hauser, H. Rogg, Research Dept. Ciba-Geigy, unpublished data). The specificity of the compound was confirmed in functional studies in rabbit aortic rings. In this preparation, valsartan inhibited AII-induced contractions whereas it did not affect contractions induced by 5-HT, potassium chloride, or noradrenaline, even at a concentration of 2 μM. At this very high concentration no contractions were observed in the aortic rings, indicating that the compound lacks agonistic properties and acts as a pure antagonist. As observed with other nonpeptide AT_1-receptor antagonists (Wong *et al.*, 1990c; 1991a; Chiu *et al.*, 1991a; Robertson *et al.*, 1992), the rightward displacement of the AII concentration-response curve induced by valsartan was accompanied by a reduction in the maximal response. In contrast, no reduction in the maximum contractions to AII has been observed in rabbit aorta treated with losartan (Chiu *et al.*, 1991b). This difference may be due to the alcoholic moiety in the structure of losartan, instead of the carboxylic acid moiety contained in compounds displaying the insurmountable type of antagonism. As demonstrated also for another AT_1-receptor antagonist, GR117289 (Robertson *et al.*, 1992), both effects were dependent on the incubation time. For valsartan, an incubation time of about 1 h was needed to reach equilibrium. The reduction in the maximum response to AII by these agents, described as insurmountable antagonism, has been ascribed to a slowly reversible antagonism at the receptor site (Timmermans *et al.*, 1991c; Robertson *et al.*, 1992). It is worth noting, however, that in bovine isolated glomerulosa cells, valsartan inhibited AII-induced aldosterone release, without affecting the maximum response to AII. This observation is compatible with a competitive antagonistic profile of valsartan. In addition, as depicted in Figure 2, valsartan displayed a competitive type of antagonism at the receptor level in SMC. This apparent discrepancy might be explained by the different protocols used, since in the binding and in the aldosterone release studies, valsartan and AII were given simultaneously, whereas in the aortic ring studies valsartan was given prior to the addition of AII. Thus, in the presence of an antagonist slowly dissociating in the aortic rings, the time for an agonist to reach an equilibrium with the receptor becomes longer. A similar profile to that of valsartan, was observed with the AT_1-receptor antagonist, L-158,809, which was a competitive inhibitor in binding and aldosterone release studies and non-competitive in rabbit aorta (Chang *et al.*, 1992).

The inhibition by valsartan of the AII-induced release of aldosterone *in vitro* is consistent with similar findings made with losartan (Smith *et al.*, 1992). Losartan and an analogue, DuP 532 are also reported to inhibit AII-induced aldosterone release *in vivo* in normotensive rats (Wong *et al.*, 1990b; 1991a). Similar results have been observed with val-

sartan at a dose of 10 mg kg^{-1} (de Gasparo *et al.*, 1992).

The specificity of the antagonistic effects of valsartan was also observed after oral administration *in vivo*. In pithed rats, valsartan displaced to the right the dose-pressor-curve of AII, without affecting responses to noradrenaline or electrical stimulation of the sympathetic outflow. These effects were observed 2, 4 h and to a lesser extent, 24 h after a single oral administration of valsartan. Thus, the long duration of the antihypertensive effect of valsartan, observed in renal hypertensive rats, appears to be due to a blockade of the AT_1-receptor. Long lasting inhibition of AII-induced pressor responses have also been reported with losartan in normal male volunteers (Christen *et al.*, 1991).

In conscious, renal hypertensive rats, valsartan induced dose-related decreases in blood pressure after both i.v. and oral administration. Transient tachycardia was observed after i.v. injection, but not after oral administration. As with systemic vasodilators, the initial increase in HR appeared to be due to a transient activation of the sympathetic nervous system, since it was prevented by pretreatment with propranolol. A similar increase in HR, has also been observed after i.v. administration of losartan in spontaneously hypertensive rats (Wong *et al.*, 1990d) and in conscious water-deprived and water-replete Brattleboro rats (Batin *et al.*, 1991). Particularly in water-deprived Brattleboro rats, which are characterized by a marked activation of the renin-angiotensin system, the hypotension induced by losartan was due to a marked increase in total peripheral conductance, indicating that this class of compounds possesses vasodilator properties (Batin *et al.*, 1991).

After i.v. or oral administration of valsartan, the antihypertensive effect of the compound in renal hypertensive rats persisted for up to 24 h, and the maximum effect was similar to that induced in the same model by an angiotensin converting-enzyme inhibitor. Compared to losartan, valsartan had a more rapid onset of action after oral administration, and its maximum effect was reached after about 1 h, whereas the maximum antihypertensive effect of losartan developed between 4 and 24 h after administration. This latency in the biological response to losartan in rats, which has also been observed by other authors (Wong *et al.*, 1991b), appears to be due to the formation of an active, hepatically-generated carboxylic acid metabolite, EXP3174. This active metabolite is approximately 20 times more potent than the parent compound (Wong *et al.*, 1990c; Timmermans *et al.*, 1991b) and after oral administration in hypertensive rats has an onset of action similar (Wong *et al.*, 1990c) to that reported here for valsartan.

The long duration of action of valsartan observed after single administration was confirmed in the repeated administration study, where valsartan was given once daily for 4 days. Despite its long action, no signs of accumulation of effect were observed. Although plasma concentrations of AII have been shown to increase after administration of AT_1-receptor antagonists (Bunkenburg *et al.*, 1991), no signs of tolerance to the antihypertensive effects were observed. Indeed, the data from this study indicate that in renal hypertensive rats the blood pressure can be persistently maintained at almost normotensive levels throughout the period of treatment.

The sodium-depleted marmoset is a primate model of a normal, but renin-angiotensin-dependent, blood pressure. It has been used to evaluate the hypotensive efficacy of renin inhibitors, since these agents are primate-specific and cannot be tested in rats (Wood *et al.*, 1989). In conscious, freely moving marmosets, with MAP measured by telemetry, valsartan decreased blood pressure after oral administration to an extent comparable to that observed after renin or converting-enzyme inhibitors (Wood *et al.*, 1989). Although the oral efficacy and duration of action of these two compounds was similar in the renal hypertensive rats, valsartan had a longer duration of action than losartan in the marmosets. As in the results described here, losartan also has a shorter duration of action in rhesus monkeys than in rats (Stearns *et al.*, 1992). This may be because nonhuman primates do not metabolize losartan to its more active metabolite. Liver slices from rhesus monkeys, in contrast to liver slices from rats and man, do not convert losartan to its more active carboxylic acid metabolite, but to less potent glucuronidation metabolites of the tetrazole moiety (Stearns *et al.*, 1992). As previously suggested the formation of an active metabolite in the liver may make it difficult to predict the dosage of losartan for patients with impaired liver function. Therefore, an orally effective AT_1-receptor antagonist that is not dependent for its action on an active metabolite could have advantages over losartan (Wong *et al.*, 1991a).

In conclusion, valsartan is a highly specific, orally active antagonist of the AT_1-receptor subtype. Its blood-pressure-lowering effect after oral administration is not accompanied by reflex tachycardia, and persists at least 24 h in renal hypertensive rats and 12–24 h in sodium-depleted marmosets.

We thank, R. Burdet, N. Flubacher, P. Forgiarini, E. Halmo, E. Hermes, M. Mele, D. Monna, H.P. Mueller, O. Peter, C. Rodriguez, C. Schnell, H. Thomann and S. Walther for their excellent technical support and Walter Fuchs for preparing the figures.

References

BATIN, P., GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1991). Cardiac haemodynamic effects of the non-peptide, angiotensin II-receptor antagonist, DuP 753, in conscious Long Evans and Brattleboro rats. *Br. J. Pharmacol.*, **103**, 1585–1591.

BLANKLEY, C.J., HODGES, J.C., KLUTCHKO, S.R., HIMMELSBACH, R.J., CHUCHOLOWSKI, A., CONNOLLY, C.J., NEERGAARD, S.J., VAN NIEUWENHZE, M.S., SEBASTIAN, A., QUIN, J., III, ESSENBURG, A.D. & COHEN, D.M. (1991). Synthesis and structure-activity relationships of a novel series of non-peptide angiotensin II receptor binding inhibitors specific for the AT_2 subtype. *J. Med. Chem.*, **34**, 3248–3260.

BOTTARI, S.P., TAYLOR, V., KING, I.N., BOGDAL, Y., WHITEBREAD, S. & DE GASPARO, M. (1991). Angiotensin II AT_2 receptors do not interact with guanine nucleotide binding properties. *Eur. J. Pharmacol., Mol. Pharmacol.*, **207**, 157–163.

BOTTARI, S.P., KING, I.N., REICHLIN, S., DAHLSTROEM, I., LYDON, N. & DE GASPARO, M. (1992). The angiotensin AT_2 receptor stimulates protein tyrosine phosphatase activity and mediates inhibition of particulate guanylate cyclase. *Biochem. Biophys. Res. Commun.*, **183**, 206–211.

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254.

BÜHLMAYER, P., CRISCIONE, L., FUHRER, W., FURET, P., DE GASPARO, M., STUTZ, S. & WHITEBREAD, S. (1991). Nonpeptidic angiotensin II antagonists: synthesis and *in vitro* activity of a series of novel naphthalene and tetrahydronaphthalene derivatives. *J. Med. Chem.*, **34**, 3105–3114.

BUMPUS, F.M., CATT, K.J., CHIU, A.T., DE GASPARO, M., GOODFRIEND, T., HUSAIN, A., PEACH, M.J., TAYLOR, D.G. JR & TIMMERMANS, P.B.M.W.M. (1991). Nomenclature for angiotensin receptors: a report of the nomenclature committee of the Council for High Blood Pressure Research. *Hypertension*, **17**, 720–721.

BUNKENBURG, B., SCHNELL, C., BAUM, H.-P., CUMIN, F. & WOOD, J.M. (1991). Prolonged angiotensin II antagonism in spontaneously hypertensive rats. Hemodynamic and biochemical consequences. *Hypertension*, **18**, 278–288.

CARINI, D.J., DUNCIA, J.V., JOHNSON, A.L., CHIU, A.T., PRICE, W.A., WONG, P.C. & TIMMERMANS, P.B. (1990). Nonpeptide angiotensin II receptor antagonists: n-((benzyloxy)benzyl) imidazoles and related compounds as potent antihypertensives. *J. Med. Chem.*, **33**, 1330–1336.

CHANG, R.S.L., SIEGEL, P.K.S., CLINESCHMIDT, B.V., MANTLO, N.B., CHAKRAVARTY, P.K., GREENLEE, W.J., PATCHETT, A.A. & LOTTI, V.J. (1992). In vitro pharmacology of L-158,809, a new highly potent and selective angiotensin II receptor antagonist. *J. Pharmacol. Exp. Ther.*, **262**, 133–138.

CHIU, A.T., HERBLIN, W.F., ARDECKY, R.J., MCCALL, D.E., CARINI, D.J., DUNCIA, J.V., PEASE, L.J., WONG, P.C., WEXLER, R.R., JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1989). Identification of angiotensin II receptor subtypes. *Biochem. Biophys. Res. Commun.*, **165**, 196–203.

CHIU, A.T., CARINI, D.J., DUNCIA, J.V., LEUNG, K.H., MCCALL, D.E., PRICE, W.A. JR, WONG, P.C., SMITH, R.D., WEXLER, R.R. & TIMMERMANS, P.B.M.W.M. (1991a). DuP 532. A second generation of nonpeptide angiotensin II receptor antagonists. *Biochem. Biophys. Res. Commun.*, **177**, 209–217.

CHIU, A.T., MCCALL, D.E., PRICE, W.A. Jr., WONG, P.C., CARINI, D.J., DUNCIA, J.V., WEXLER, R.R., YOO, S.E., JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1991b). *In vitro* pharmacology of DuP 753. *Am. J. Hypertens.*, **4** Suppl, 282S–287S.

CHIU, A.T., ROSCOE, W.A., MCCALL, D.E. & TIMMERMANS, P.B.M. W.M. (1991c). Angiotensin II-1 receptors mediate both vasoconstriction and hypertrophic responses in rat aortic smooth-muscle cells. *Receptor*, **1**, 133–140.

CHRISTEN, Y., WAEBER, B., NUSSBERGER, J., PORCHET, M., BORLAND, R.M., LEE, R.J., MAGGON, K., SHUM, L., TIMMERMANS, P.B.M.W.M. & BRUNNER, H.R. (1991). Oral administration of DuP 753, a specific angiotensin II receptor antagonist, to normal male volunteers: inhibition of pressor response to exogenous angiotensin I and II. *Circulation*, **83**, 1333–1342.

CONSENSUS TRIAL: THE CONSENSUS TRIAL STUDY GROUP (1987). Effects of enalapril on mortality in severe congestive heart failure. *N. Engl. J. Med.*, **316**, 1429–1435.

CRISCIONE, L., DE GASPARO, M., BUEHLMAYER, P., WHITEBREAD, S., RAMJOUE, H.P. & WOOD, J.M. (1992). Pharmacological profile of CGP 48933: a specific, orally active nonpeptide antagonist of AT_1 angiotensin II receptor subtype. *Hochdruck*, **12**, 43.

CRISCIONE, L., THOMANN, H., WHITEBREAD, S., DE GASPARO, M., BUEHLMAYER, P., HEROLD, P., OSTERMAYER, F.A. & KAMBER, B. (1990). Binding characteristics and vascular effects of various angiotensin II antagonists. *J. Cardiovasc. Pharmacol.*, **16**, S56–S59.

DE GASPARO, M., WHITEBREAD, S., LEVENS, N., RAMJOUE, H.P., CRISCIONE, L., ROGG, H., BAUM, H.P., BRECHLER, V., BUEHLMAYER, P., WOOD, J.M. & BOTTARI, S.P. (1992). Pharmacology of angiotensin II-receptor subtypes. In *Cellular and Molecular Biology of the Adrenal Cortex*. ed. Saez, J.M., Vol. 22, pp. 3–17. Colloque INSERM: John Libbey Eurotext Ltd.

DE LEAN, A., MUNSON, P.J. & RODBARD, D. (1978). Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curve. *Am. J. Physiol.*, **235**, E97–E111.

DUDLEY, D.T., HUBBELL, S.E. & SUMMERFELT, R.M. (1991). Characterization of angiotensin II (AT_2) binding sites in R3T3 cells. *Mol. Pharmacol.*, **40**, 360–367.

DUNCIA, J.V., CHIU, A.T., CARINI, D.J., GREGORY, G.B., JOHNSON, A.L., PRICE, W.A., WELLS, G.J., WONG, P.C., CALABRESE, J.C. & TIMMERMANS, P.B. (1990). The discovery of potent nonpeptide angiotensin II receptor antagonists: a new class of potent antihypertensives. *J. Med. Chem.*, **33**, 1312–1329.

FURUKAWA, Y., KISHIMOTO, S. & NISHIKAWA. (1982). *Hypotensive imidazole deriviates*. US Patent 4,340,598 issued to Takeda Chemical Industries, Ltd., Osaka, Japan. US patent.

GILLESPIE, J.S. & MUIR, T.C. (1967). A method of stimulating the complete sympathetic outflow from the spinal cord to the blood vessels in the pithed rat. *Br. J. Pharmacol.*, **30**, 78–87.

HERBLIN, W.F., CHIU, A.T., MCCALL, D.E., ARDECKY, R.J., CARINI, D.J., DUNCIA, J.V., PEASE, L.J., WONG, P.C., WEXLER, R.R., JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1991). Angiotensin II receptor heterogeneity. *Am. J. Hypertens.*, **4** Suppl, 299S–302S.

HOFBAUER, K.G. & WOOD, J.M. (1986). The renin-angiotensin system: inhibitors and antagonists. In *Handbook of Hypertension*, Vol 6: *Pathology of Hypertension, Regulatory Mechanisms* ed. Zanchetti, A. & Tarazi, R.C. pp. 466–488. Amsterdam: Elsevier Science Publisher.

HOFBAUER, K.G., ZSCHIEDRICH, H. & GROSS, F. (1976). Regulation of renin release and intrarenal formation of angiotensin: studies in the isolated rat kidney. *Clin. Exp. Pharmacol. Physiol.*, **3**, 73–93.

KENAKIN, T.P. (1984). Drugs and drug responses in isolated tissues. *Pharmacol. Rev.*, **36**, 165–222.

KRAMER, B.L., MASSIE, B.M. & TOPIC, N. (1983). Controlled trial of captopril in chronic heart failure: a rest and exercise hemodynamic study. *Circulation*, **67**, 807–816.

MANTLO, N.B., CHAKRAVARTY, P.K., ONDEYKA, D.L., SIEGL, P.K.S., CHANG, R.S., LOTTI, V.J., FAUST, K.A., CHEN, T.-B., SCHORN, T.W., SWEET, C.S., EMMERT, S.E., PATCHETT, A.A. & GREENLEE, W.J. (1991). Potent, orally active imidazo[4,5-*b*]pyridine-based angiotensin II receptor antagonists. *J. Med. Chem.*, **34**, 2919–2922.

MUNSON, P.J. & RODBARD, D. (1980). LIGAND: a versatile computerized approach for the characterisation of ligand binding systems. *Anal. Biochem.*, **107**, 220–239.

ONDETTI, M.A. (1991). Angiotensin converting enzyme inhibitors: an overview. *Hypertension*, **18** Suppl. 3, III134–III135.

ONDETTI, M.A., RUBIN, A. & CUSHMAN, D.W. (1977). Design of specific inhibitors of angiotensin converting enzyme: a new class of orally active antihypertensive agents. *Science*, **196**, 441–444.

PALS, D.T., MASUCCI, I., DENNING, G.S. JR, SIPES, F. & FESSLER, D.C. (1971). Role of the pressor action of angiotensin II in experimental hypertension. *Circ. Res.*, **29**, 673–681.

PALS, D.T., DENNING, Jr., G.S. & KEENAN, R.E. (1979). Historical development of saralasin. *Kidney Int.*, **15**, S7–S10.

ROBERTSON, M.J., BARNES, J.C., DREW, G.M., CLARK, K.L., MARSHALL, F.H., MICHEL, A., MIDDLEMISS, D., ROSS, B.C., SCOPES, D. & DOWLE, M.D. (1992). Pharmacological profile of GR117289 *in vitro*: a novel, potent and specific non-peptide angiotensin AT_1 receptor antagonists. *Br. J. Pharmacol.*, **107**, 1173–1180.

SCHNELL, R.C. & WOOD, J.M. (1993). Measurement of blood pressure and heart rate by telemetry in conscious unrestrained marmosets. *Am. J. Physiol.*, **264**, H1509–H1516.

SMITH, R.D., CHIU, A.T., WONG, P.C., HERBLIN, W.F. & TIMMERMANS, P.B.M.W.M. (1992). Pharmacology of nonpeptide angiotensin II receptor antagonists. *Annu. Rev. Pharmacol. Toxicol.*, **32**, 135–165.

STEARNS, R.A., MILLER, R.R., DOSS, G.A., CHAKRAVARTY, P.K., ROSEGAY, A., GATTO, G.J. & CHIU, S.-H.L. (1992). The metabolism of DuP 753, a nonpeptide angiotensin II receptor antagonist, by rat, monkey, and human liver slices. *Drug Metab. Dispos.*, **20**, 281–287.

TIMMERMANS, P.B.M.W.M., CARINI, D.J., CHIU, A.T., DUNCIA, J.V., PRICE, W.A. JR, WELLS, G.J., WONG, P.C., WEXLER, R.R. & JOHNSON, A.L. (1990). Nonpeptide angiotensin II receptor antagonists: a novel class of antihypertensive agents. *Blood Vessels*, **27**, 295– 300.

TIMMERMANS, P.B.M.W.M., CARINI, D.J., CHIU, A.T., DUNCIA, J.V., PRICE, W.A. JR, WELLS, G.J., WONG, P.C., JOHNSON, A.L. & WEXLER, R.R. (1991a). The discovery of a new class of highly specific nonpeptide angiotensin II receptor antagonists. *Am. J. Hypertens.*, **4** Suppl, 275S–281S.

TIMMERMANS, P.B.M.W.M., CARINI, D.J., CHIU, A.T., DUNCIA, J.V., PRICE, W.A. JR, WELLS, G.J., WONG, P.C., WEXLER, R.R. & JOHNSON, A.L. (1991b). Angiotensin II receptor antagonists: from discovery to antihypertensive drugs. *Hypertension*, **18** Suppl. 3, III136–III142.

TIMMERMANS, P.B.M.W.M., WONG, P.C., CHIU, A.T. & HERBLIN, W.F. (1991c). Nonpeptide angiotensin II receptor antagonists. *Trends Pharmacol. Sci.*, **12**, 55–62.

WEINSTOCK, J., KEENAN, R.M., SAMANEN, J., HEMPEL, J., FINKELSTEIN, J.A., FRANZ, R.G., GAITANOPOULOS, D.E., GIRARD, G.R., GLEASON, J.G., HILL, D.T., MORGAN, T.M., PEISHOFF, C.E., AIYAR, N., BROOKS, D.P., FREDRICKSON, T.A., OHLSTEIN, E.H., RUFFOLO, R.R. JR, STACK, E.J., SULPIZIO, A.C., WEIDLEY, E.F. & EDWARDS, R.M. (1991). 1-(Carboxybenzyl)-imidazole-5-acrylic acids: potent and selective angiotensin II receptor antagonists. *J. Med. Chem.*, **34**, 1514–1517.

WHITEBREAD, S., MELE, M., KAMBER, B. & DE GASPARO, M. (1989). Preliminary biochemical characterization of two angiotensin II receptor subtypes. *Biochem. Biophys. Res. Commun.*, **163**, 284–291.

WHITEBREAD, S.E., TAYLOR, V., BOTTARI, S.P., KAMBER, B. & DE GASPARO, M. (1991). Radioiodinated CGP 42112A: a novel high affinity and highly selective ligand for the characterization of angiotensin AT_2 receptors. *Biochem. Biophys. Res. Commun.*, **181,** 1365–1371.

WIEST, S.A., RAMPERSAUD, A., ZIMMERMAN, K. & STEINBERG, M.I. (1991). Characterization of distinct angiotensin II binding sites in rat adrenal gland and bovine cerebellum using selective nonpeptide antagonists. *J. Cardiovasc. Pharmacol.*, **17,** 177–184.

WONG, P.C., PRICE, W.A., CHIU, A.T., DUNCIA, J.V., CARINI, D.J., WEXLER, R.R., JOHNSON, A.L. & TIMMERMANS, P.B. (1990a). Nonpeptide angiotensin II receptor antagonists IX. Antihypertensive activity in rats of DuP 753, an orally active antihypertensive agent. *J. Pharmacol. Exp. Ther.*, **252,** 726–732.

WONG, P.C., PRICE, W.A. JR, CHIU, A.T., CARINI, D.J., DUNCIA, J.V., JOHNSON, A.L., WEXLER, R.R. & TIMMERMANS, P.B. (1990b). Nonpeptide angiotensin II receptor antagonists studies with EXP 9270 and DuP 753. *Hypertension*, **15,** 823–834.

WONG, P.C., PRICE, W.A. JR, CHIU, A.T., DUNCIA, J.V., CARINI, D.J., WEXLER, R.R., JOHNSON, A.L. & TIMMERMANS, P.B. (1990c). Nonpeptide angiotensin II receptor antagonists. XI. Pharmacology of EXP 3174: an active metabolite of DuP 753, an orally active antihypertensive agent. *J. Pharmacol. Exp. Ther.*, **255,** 211–217.

WONG, P.C., PRICE, W.A. JR, CHIU, A.T., DUNCIA, J.V., CARINI, D.J., WEXLER, R.R., JOHNSON, A.L. & TIMMERMANS, P.B. (1990d). Hypotensive action of DuP 753, an angiotensin II antagonist, in spontaneously hypertensive rats. Nonpeptide angiotensin II receptor antagonists. X. *Hypertension*, **15,** 459–468.

WONG, P.C., HART, S.D., CHIU, A.T., HERBLIN, W.F., CARINI, D.J., SMITH, R.D., WEXLER, R.R. & TIMMERMANS, P.B.M.W.M. (1991a). Pharmacology of DuP 532, a selective and noncompetitive AT_1 receptor antagonist. *J. Pharmacol. Exp. Ther.*, **259,** 861–870.

WONG, P.C., PRICE, W.A., Jr., CHIU, A.T., DUNCIA, J.V., CARINI, D.J., WEXLER, R.R., JOHNSON, A.L. & TIMMERMANS, P.B.M. W.M. (1991b). *In vivo* pharmacology of DuP 753. *Am. J. Hypertens.*, **4,** Suppl., 288S–298S.

WOOD, J.M., CRISCIONE, L., DE GASPARO, M., BUEHLMAYER, P., RUEEGER, H., STANTON, J.L., JUPP, R.A. & KAY, J. (1989). CGP 38 560 an orally active, low-molecular-weight renin inhibitor with high potency and specificity. *J. Cardiovasc. Pharmacol.*, **14,** 221–226.

(Received December 17, 1992
Revised May 12, 1993
Accepted May 18, 1993)