J. Pharm. Pharmacol. 1996, 48: 829–833
Received November 15, 1995
Accepted March 22, 1996

Comparative Cardiovascular Effects of the Angiotensin II Type 1 Receptor Antagonists ZD 7155 and Losartan in the Rat

INGA-LILL JUNGGREN, XIAOHE ZHAO, XIANGYING SUN AND THOMAS HEDNER

Division of Clinical Pharmacology, Department of Physiology and Pharmacology, University of Göteborg, Göteborg, Sweden

Abstract

Binding experiments show that ZD 7155 is a potent angiotensin II type 1 receptor antagonist. In this study this novel substance was studied in normotensive and hypertensive rats.

The relative potency and duration of the antihypertensive effects of ZD 7155 were compared with those of the reference substance, losartan. The inhibitory effects of both compounds on angiotensin II-induced pressor actions were studied in the conscious normotensive Sprague–Dawley (SD) rat and in the conscious, spontaneously hypertensive rat (SHR). Arterial blood pressure and heart rate (HR) were obtained by direct intraarterial recording. Angiotensin II infusion was administered intravenously in the dose range 53·3 ng–12·8 $\mu g\ kg^{-1}\ min^{-1}$ to the conscious rats. ZD 7155 was administered in a bolus dose of 1·082 $\mu mol\ kg^{-1}$ (0·51 $mg\ kg^{-1}$) and losartan in bolus doses of 2·165 and 6·495 $\mu mol\ kg^{-1}$ (1·0 and 3·0 $mg\ kg^{-1}$). In conscious SD rats, ZD 7155 and losartan behaved as competitive antagonists and the pressor response curve to angiotensin II was shifted to the right. Experiments in conscious SD rats also showed that ZD 7155 was approximately ten times as potent as losartan in suppressing the angiotensin II-induced pressor response (240 $ng\ kg^{-1}$; 10 min infusion). In addition, experiments with conscious rats demonstrated that ZD 7155 could suppress the angiotensin II-induced pressor response for approximately 24 h when ZD 7155 was administered intravenously in a 1·082 $\mu mol\ kg^{-1}$ bolus dose and angiotensin II was given at 240 $ng\ kg^{-1}$ (in a 10-min infusion). Experiments in conscious SHRs using ZD 7155 (1·082 $\mu mol\ kg^{-1}$) and losartan (6·495 $\mu mol\ kg^{-1}$) as intravenous boluses indicated that both ZD 7155 and the reference compound losartan exhibited a significant antihypertensive effect. These results demonstrate that ZD 7155 is a potent angiotensin II-type 1 antagonist which is approximately ten times as potent as losartan in suppressing the angiotensin II-induced pressor response. Furthermore, ZD 7155 may suppress the angiotensin II-induced pressor response for 24 h and in the SHR ZD 7155 induces a pronounced and persistent antihypertensive effect.

The cardiovascular effects of angiotensin-converting enzyme inhibitors are traditionally considered to be related to inhibition of angiotensin II generation. Because angiotensin-converting enzyme (kininase II) is not specific for angiotensin I but also converts a number of other bioactive peptides, a role for angiotensin II could not always be differentiated from that of other peptides, e.g. bradykinin (Zusman 1987; Ujhelyi et al 1989).

The receptor for the endogenous ligand angiotensin II was discovered some time ago by studies of the effects of peptide agonists and antagonists in isolated tissue (Regoli & Park 1972; Regoli 1979). The finding that the prototype biphenylimidazole angiotensin antagonist losartan did not share one of the binding epitopes with angiotensin II led to the suggestion that there were two distinct receptor subtypes, type 1 and type 2 angiotensin receptors (AT1 and AT2 receptors, respectively) (Bensoussan et al 1993; Chansel et al 1993). The recent evolution of selective and specific antagonists at both these receptor subtypes has provided the possibility of gaining further insight in the potential physiological and pathophysiological roles of angiotensin II.

The prototype AT1 receptor antagonist losartan was initially regarded as a classical competitive antagonist (MacFadyen & Reid 1994). The pharmacodynamic profile of this agent is, however, complicated, because it generates an active metabolite, EXP3174, which has non-competitive AT1 antagonistic properties (Rhaleb et al 1991; Timmermans et al 1991; Smith et al 1992; Widdop et al 1992). Several AT1 antagonists are now available which differ in their physicochemical and pharmacological characteristics (MacFadyen & Reid 1994). In this study the cardiovascular effects of the novel angiotensin II type 1 antagonist ZD 7155 (Oldham et al 1993; Hahn et al 1994) have been compared with the reference agent, losartan. The specific aims of the study were to compare the relative potency and duration of ZD 7155 with losartan on angiotensin II-induced pressor effects in conscious normotensive rats and to compare the antihypertensive effects of ZD 7155 and losartan in the conscious spontaneously hypertensive rat (SHR).

Correspondence: I.-L. Junggren, Department of Physiology and Pharmacology, University of Göteborg, Medicinargatan 7, S-413 90 Göteborg, Sweden.

Materials and Methods

Experimental animals

Experiments were conducted on male normotensive Sprague–Dawley rats and on SHRs, 220–300 g (Möllegaard Hansen Avelslaboratorie A/S, Denmark). Angiotensin II animals were housed in cages in groups of five, in an animal unit at 26°C with 60% humidity and with a 0500–0700 h light regimen; standard rat pellets and tap water were freely available. The experimental protocol for the conscious rat models was approved by the Animal Ethics Committee at the University of Göteborg, Sweden.

Surgical preparation of conscious rats

Animals for the conscious rat model were anaesthetized with metohexital (70 mg kg^{-1}, i.p.). The right jugular vein was cannulated with a PE-50 tube for intravenous administration of the various cardiovascular agents or saline. Immediately after surgery the jugular cannula was filled with saline. The left carotid artery was cannulated with a PE-50 tube for blood pressure (BP) recording.

Twenty to twenty-four hours after surgery the rats were placed individually in standard, opaque, plastic boxes (25·0 × 7·0 × 7·5 cm, i.d.). The boxes were large enough to enable the rats to move around freely. The catheter was connected to a Grass model 7D polygraph via a P23 DC transducer (Grass Instruments, St Louis, MO, USA). A cardiotachograph triggered by the arterial pulse was used to record heart rate (HR). The arterial catheter was connected by means of a side tube to a slow infusion pump which delivered saline continuously with a flow of 0·4–0·8 mL h^{-1} into the arterial catheter to prevent it becoming clogged.

The rats were left to become accustomed to the surroundings for 30 to 60 min before any experiments.

Experimental protocol

The cardiotachograph triggered by the arterial pulse recorded HR in beats min^{-1}. By means of these data the increase of mean basal blood pressure to maximum peak height was analysed and presented as the difference between the mean arterial blood pressure values (ΔMAP). The duration of the pressor response was also analysed.

Angiotensin II (Sigma, St Louis, MO, USA) was used in all experiments. angiotensin II was dissolved in saline containing 0·5% bovine serum albumin and administered as a 10-min infusion via the intravenous catheter.

In the dose-range experiments, angiotensin II was infused in incremental doses from 53·3 ng to 12·8 μg kg^{-1} min^{-1} with 30-min intervals. Five incremental doses were studied for each of the AT1 receptor antagonists in groups of 3–6 animals $dose^{-1}$.

The inhibitory effects of the AT1 receptor antagonists ZD 7155 and losartan on angiotensin II (240 ng kg^{-1})-induced increases in MAP and basal blood pressure were assessed. ZD 7155 was administered in an intravenous bolus dose of 1·082 μmol kg^{-1} (0·51 mg kg^{-1}) into the jugular vein of conscious Sprague–Dawley rats. For the reference compound losartan, intravenous bolus doses of 2·165 to 6·495 μmol kg^{-1} (1·0 to 3·0 mg kg^{-1}) were used. Saline served as a control for ZD 7155 and losartan. For each dose level of the AT1 antagonists, groups of seven animals were analysed. The AT1 antagonists were administered 5 min before the first dose of angiotensin II.

Drugs

Bovine serum albumin (Sigma), atropine (Sigma), methohexital (Brietal, Eli Lilly & Co, Indianapolis, USA), Hipertensin (angiotensin II, Ciba, Basle, Switzerland), ZD 7155 (5,7-diethyl-1-[2′-(1*H*-1,2,3,4-tetrazol-5-yl)biphenyl-4-ylmethyl]-1,2,3,4-tetrahydro-1,6-naphthyridin-2-one hydrochloride; AT1 receptor antagonist, Zeneca Pharmaceuticals, Manchester, UK), losartan (DuP 753, MK 954; AT1 receptor antagonist, DuPont Pharmaceuticals, Wilmington, Delaware, USA).

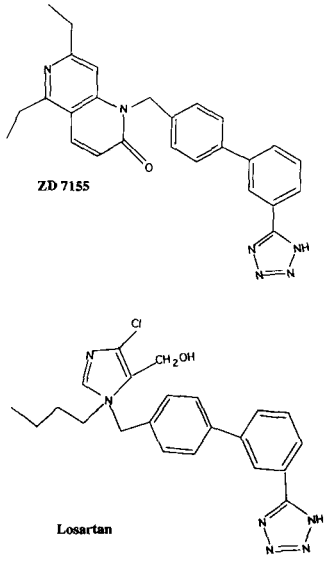

FIG. 1. Chemical structures of ZD 7155 (5,7-diethyl-1-[2′-(1*H*-1,2,3,4-tetrazol-5-yl)biphenyl-4-ylmethyl]-1,2,3,4-tetrahydro-1,6-naphthyridin 2-one hydrochloride) and losartan.

Statistical analysis

Angiotensin II values in the text, tables and figures are given as means ± s.e.m. Data were calculated using the Macintosh Statview II program on a Macintosh II computer. Group comparisons were performed using analysis of variance with either unpaired or paired Student's *t*-test. A probability (*P*) value < 0·05 was considered significant.

Results

The chemical structures of ZD 7155 and the reference agent, losartan, are presented in Fig. 1.

Dose-response effects of ZD 7155 and losartan

In the conscious rat model five increasing doses of ZD 7155 or losartan were given to enable examination of the inhibition of the angiotensin II-induced pressor response. The dose of angiotensin II given was 80 ng kg^{-1} min^{-1} infused during a 10-min period (Fig. 2). In these experiments ZD 7155 was shown to be approximately ten times more potent than losartan in suppressing the angiotensin II-induced pressor response.

Duration of inhibition of angiotensin II pressor effects

The duration of the inhibitory action of the two AT1 antagonists on the angiotensin II pressor responses was investigated in conscious SD rats. A single dose (1·082 μmol kg^{-1}) of ZD 7155 inhibited the angiotensin II (240 ng kg^{-1}, 10-min infusion at each interval)-induced pressor responses (Fig. 3). Almost complete blockade of the angiotensin II-induced pressor response was seen from 5 min to 2 h after ZD 7155 administration. The inhibitory effect of ZD 7155 was then slightly attenuated, but clearly significant 19 h after ZD 7155 administration. Twenty-four hours after the single ZD 7155 intravenous administration, the angiotensin II-induced pressor response was back to baseline. In contrast, the inhibitory effect of losartan (6·495 μmol kg^{-1}) on angiotensin II-induced pressor response lasted only 6 h after administration (data not shown).

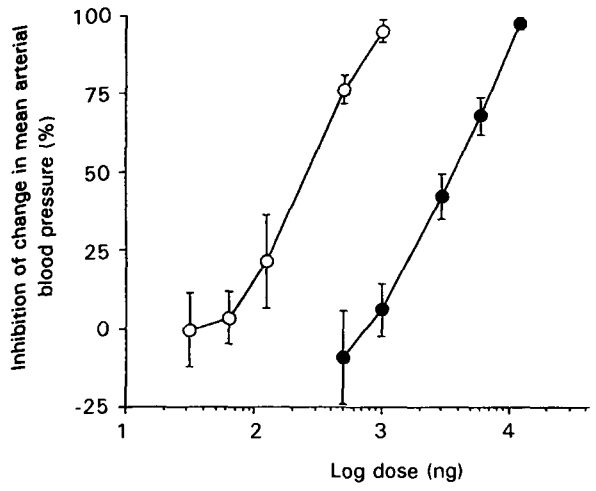

FIG. 2. Effects of increasing doses of ZD 7155 (○) and losartan (●) on the mean arterial pressure response to angiotensin II in conscious Sprague–Dawley rats. Angiotensin II was administered in a dose of 240 ng kg^{-1} (10-min infusion). Plot shows means ± s.e.m. of 3–6 animals in each group.

Inhibitory effects of ZD 7155 and losartan on angiotensin II pressor responses

The inhibitory effect of ZD 7155 on pressor and HR responses induced by incremental doses of angiotensin II was compared with that of the reference substance losartan in the conscious Sprague–Dawley rat model (Fig 4). ZD 7155 was given as a bolus intravenous dose of 1·082 μmol kg^{-1} and losartan in doses from 2·165 to 6·495 μmol kg^{-1}. The doses of angiotensin II used in these experiments were from 50 ng to 12·8 μg kg^{-1} min^{-1}. Both ZD 7155 and losartan significantly shifted the pressor response curve of angiotensin II to the right. The HR curve of angiotensin II was also shifted to the right. Thus, ZD 7155 and losartan both acted as competitive antagonists.

Antihypertensive effects

The antihypertensive effects of ZD 7155 (a bolus dose of 1·082 μmol kg^{-1}) and losartan (6·495 μmol kg^{-1}) were studied in conscious SHRs (Fig. 5). During the 6-h study both agents exhibited significant and comparable antihypertensive effects. The dose of losartan used was, however, six times that of ZD 7155. Progressive lowering of blood pressure was observed for both agents, and both curves significantly differed from the control recording 30 min after administration and up to 6 h. At the end of the experiment, the reduction of MAP was approximately 15–20%. None of the AT1 antagonists had any effect on HR.

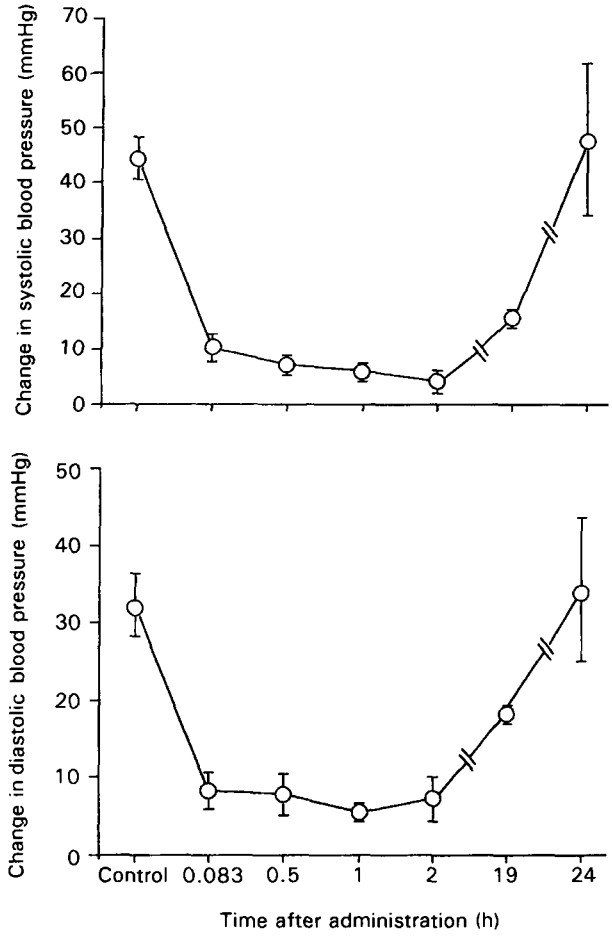

FIG. 3. Duration of inhibition of ZD 7155 on the pressor response (systolic blood pressure and diastolic blood pressure) to angiotensin II in conscious Sprague–Dawley rats. ZD 7155 was administered intravenously in a 1·082 μmol kg^{-1} dose and angiotensin II was given each time in a dose of 240 ng kg^{-1} (in a 10-min infusion). Plot shows means ± s.e.m. of seven animals in each group.

Discussion

The blood-pressure-lowering activity of selective AT1 antagonists such as losartan is dependent on the degree of activation of the renin-angiotensin system (Siegl 1993). The antihypertensive effect of losartan in standard models of experimental hypertension, such as in the SHR and in renal hypertensive rats and dogs is comparable with that of angiotensin-converting enzyme inhibitors. In low-renin models of hypertension, however, such as the DOCA-salt hypertensive rat, losartan does not reduce blood pressure (Wong et al 1990, 1992). In animals, in fact, losartan gives a blood-pressure-lowering profile identical with that given by the angiotensin-converting enzyme inhibitors, with the exception that the AT1 antagonist, unlike the angiotensin-converting enzyme inhibitors, does not potentiate the hypotensive response to bradykinin (Wong et al 1990, 1992).

This study has demonstrated that ZD 7155 is a potent competitive AT1 antagonist. Using losartan as a reference AT1 receptor antagonist we found that ZD 7155 was approximately ten times more potent than losartan in antagonizing angiotensin II-induced pressor effects. Binding experiments on guinea-pig adrenal gland membranes have demonstrated that ZD 7155 displaces (^{125}I)-angiotensin II from its binding sites in a concentration-dependent manner (Wong et al 1992). Further, in the isolated guinea-pig ileum, ZD 7155 potently shifted the angiotensin II dose-response curve to the right with a marked depression of the maximum response (Oldham et al 1993).

Experiments in conscious rats demonstrated that ZD 7155, in addition to its potent AT1 antagonistic effects, induced persistent suppression of the angiotensin II-induced pressor response. Whereas ZD 7155 significantly inhibited this

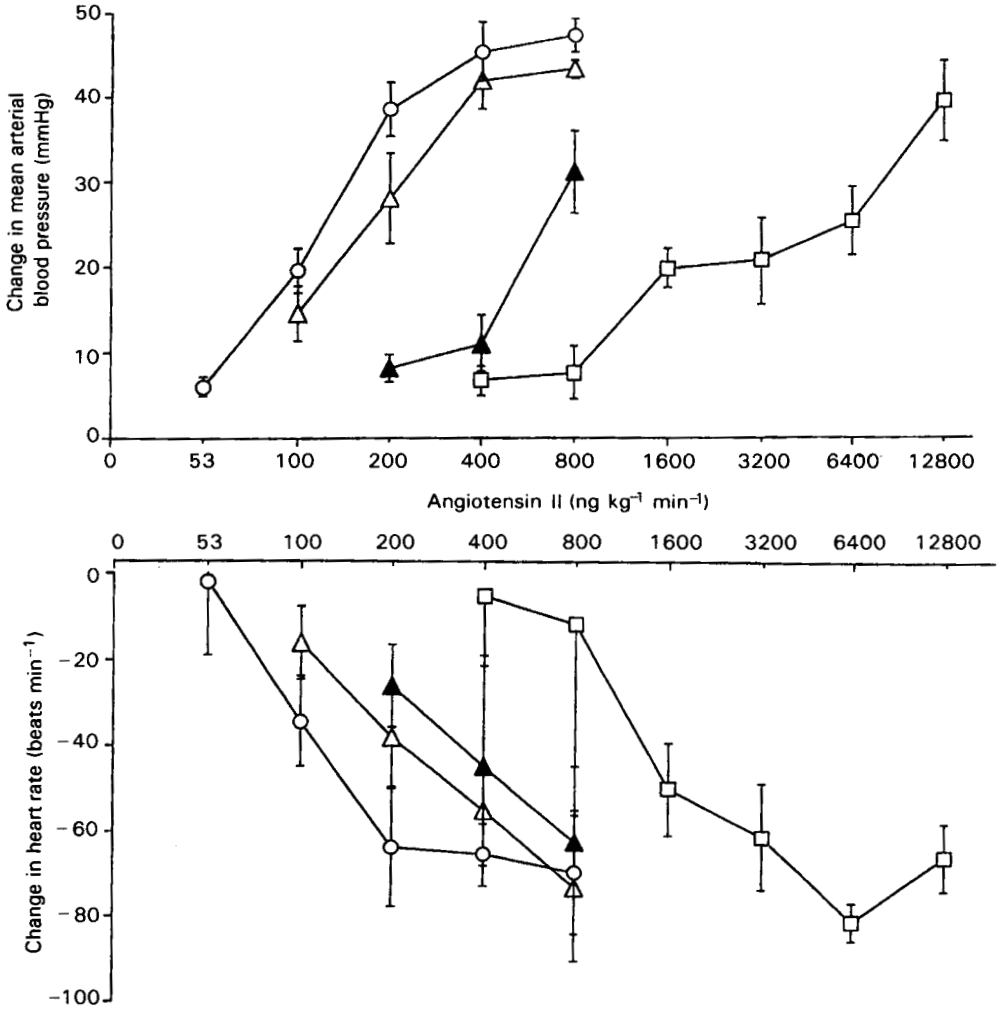

FIG. 4. Effects of ZD 7155 and losartan on the effects of incremental doses of angiotensin II in conscious Sprague–Dawley rats on mean arterial pressure and heart rate. Doses of angiotensin II used were 50 ng–12·8 μg kg^{-1} min^{-1}. Saline was used as control (○) for the angiotensin II type 1 receptor antagonists. ZD 7155 was given intravenously in a bolus dose of 1·082 μmol kg^{-1} (□), and losartan in the doses 2·165 (△) and 6·495 μmol kg^{-1} (▲). Plots show means ± s.e.m. of seven animals in each group.

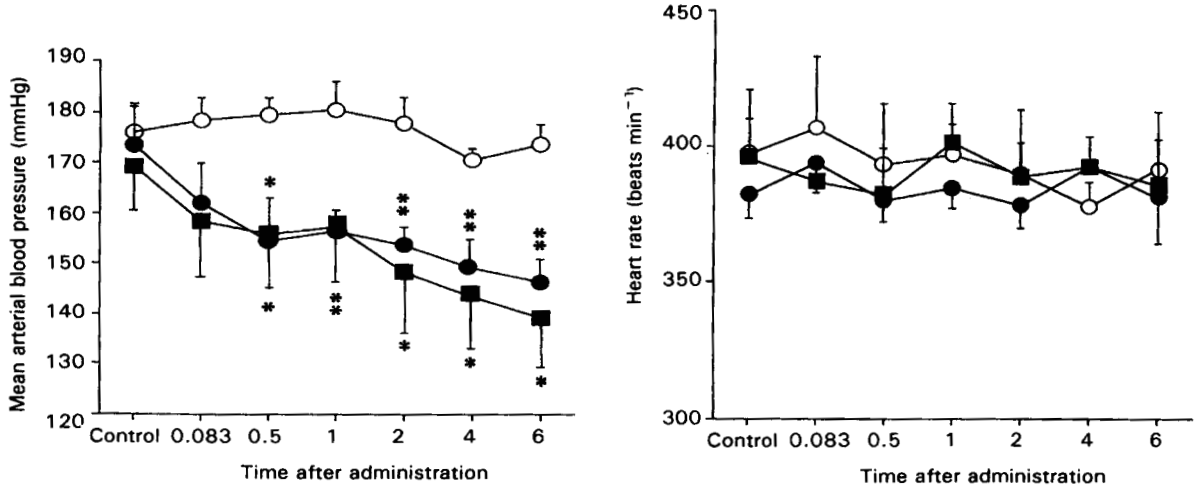

FIG. 5. Effects of ZD 7155 and losartan on mean arterial pressure and heart rate in conscious spontaneous hypertensive rats. Bolus intravenous doses of saline (○, control), ZD 7155 (●, 1·082 μmol kg^{-1}) and losartan (■, 6·495 μmol kg^{-1}) were given. Plots show means ± s.e.m. of six animals in each group. Significance compared with saline control: $^{*}P < 0.05$, $^{**}P < 0.01$.

response for approximately 24 h, the effects of losartan lasted for a considerably shorter time. ZD 7155 thus has the pharmacological profile of a potent and long-acting AT1 antagonist.

Several selective synthetic AT1 antagonists are now available (MacFadyen & Reid 1994). Both competitive and non-competitive agents have been developed and characterized. Unlike losartan, moreover, most of these compounds are not prodrugs.

Structure-activity studies of the biphenylimidazole AT1 antagonists have demonstrated the importance of a free carboxylic acid functionality located at the *ortho* position of the terminal aromatic ring. Replacement of this carboxylic acid functionality with a negatively charged tetrazole as in losartan and ZD 7155 (Fig. 1), will significantly enhance the affinity of the compounds for the AT1 receptor site (Chiu et al 1990a, b). Thus, as demonstrated by evidence from currently available selective AT1 antagonists, a hydrogen-donating or -accepting function seems to be critical for functional interaction with the AT1 receptor site.

In the Sprague–Dawley rat model, ZD 7155 induced a pronounced and persistent antihypertensive effect. After single intravenous administrations ZD 7155 (1·082 μmol kg^{-1}) and losartan (6·495 μmol kg^{-1}) both significantly reduced blood pressure in the SHR. The antihypertensive actions of the AT1 antagonists were seen as soon as 30 min after intravenous administration and significant and similar 10–20% reductions of MAP remained after 6 h.

Losartan and ZD 7155 are effective after peroral dosage (Oldham et al 1993; Timmermans et al 1993) and there is no development of tolerance to the antihypertensive action of losartan after repeated daily peroral dosing (Timmermans et al 1993).

In addition to reducing blood pressure, an AT1 antagonist such as losartan may inhibit the 'growth response' to angiotensin II in isolated smooth muscle cell preparations, because this response seems to be dependent on the AT1 receptor subtype (Timmermans et al 1993). By analogy with the angiotensin-converting enzyme inhibitors, blockade of the AT1 receptor site has also proven to be effective in reversing cardiac hypertrophy in animal models of hypertension (Timmermans et al 1993).

In conclusion, the data presented demonstrate that ZD 7155 acts as an AT1 receptor antagonist which is more potent in suppressing the angiotensin II-induced pressor response and produces an antihypertensive effect in the SHR which is more persistent than that of the reference compound losartan.

Acknowledgement

This work was supported by the Swedish Medical Research Foundation (proj grant 8642), The Medical Faculty, University of Göteborg and a project grant from Zeneca Pharmaceuticals. We gratefully acknowledge losartan as a gift from MSD/DuPont.

References

Bensoussan, M., Mitchell, T., Reilly, T., Timmermanns, P. B. M. W. M., Verroust, P. J., Ronco, P. M. (1993) Immunological reactivity of angiotensin II receptor antagonists: possible implications for receptor binding sites. Eur. J. Pharmacol. 247: 169–175

Chansel, D., Vandermeersch, S., Pham, P., Ardaillou, R. (1993) Characterization of (^{3}H)losartan receptors in isolated rat glomeruli. Eur. J. Pharmacol. 247: 193–198

Chiu, A. T., McCall, D. E., Aldrich, P. E., Timmermanns, P. B. M. W. M. (1990a) (^{3}H) DuP 753, a highly potent and specific radioligand for the angiotensin II-I receptor subtype. Biochem. Biophys. Res. Comm. 172: 1195–1202

Chiu, A. T., McCall, D. E., Price, W. A., Wong, P. C., Carini, D. J., Duncia, J. V., Wexler, R. R., Sung, E. Y., Johnson, A. L., Timmermans, P. B. M. W. M. (1990b) Nonpeptide angiotensin II receptor antagonists, VII. Cellular and biochemical pharmacology of DuP 753, an orally active antihypertensive agent. J. Pharmacol. Exp. Ther. 252: 711–718

Hahn, A. W., Jonas, U., Buhler, F. R., Resink, T. J. (1994) Activation of human peripheral monocytes by angiotensin II. FEBS Lett. 347: 178–180

MacFadyen, R. J., Reid, J. L. (1994) Angiotensin receptor antagonists as a treatment for hypertension. J. Hypertension 12: 1333–1338

Oldham, A. A., Allott, C. F., Major, J. S., Pearce, R. J., Roberts, D. A., Russell, S. T. (1993) Zeneca ZD 7155. A novel, potent and orally-effective angiotensin II receptor antagonist. Br. J. Pharmacol. 109(S): 136P

Regoli, D. (1979) Receptors for angiotensin: a critical analysis. Can. J. Physiol. Pharmacol. 57: 129–139

Regoli. D., Park, W. K. (1972) The pressor and myotropic effects and the antagonistic properties of several analogs of angiotensin II. Can. J. Physiol. Pharmacol. 50: 99–112

Rhaleb, N.-E., Rouissi, N., Nantel, F., D'Orleans-Juste, P., Regoli, D. (1991) DuP 753 is a specific antagonist for the angiotensin receptor. Hypertension 17: 480–484

Siegl, P. K. S. (1993) Discovery of losartan, the first specific nonpeptide angiotensin II receptor antagonist. J. Hypertension 11 (Suppl 3): S19–22

Smith, R. D., Chiu, A. T., Wong, P. C., Herblin, W. F., Timmermanns, P. B. M. W. M. (1992) Pharmacology of nonpeptide angiotensin II receptor antagonists. Ann. Rev. Pharmacol. Toxicol. 32: 135–165

Timmermans, P. B. M. W. M., Wong, P. C., Chiu, A. T., Herblin, W. F. (1991) Nonpeptide angiotensin II receptor antagonists. Trends Pharmacol. Sci. 12: 55–62

Timmermans, P. B. M. W. M., Wong, P. C., Chiu, A. T., Herblin, W. F., Benfield, P., Carini, D. J., Lee, R. J., Wexler, R. R., Saye, J. A. M., Smith, R. D. (1993) Angiotensin II receptors and angiotensin II receptor antagonists. Pharmacol. Rev. 45: 205–251

Ujhelyi, M. R., Fergusson, R. K., Vlasses, P. H. (1989) Angiotensin-converting enzyme inhibitors; mechanistic controversies. Pharmacotherapy 9: 351–362

Widdop, R. E., Gardiner, K. M., Kemp, P. A., Bennett, T. (1992) Inhibition of the haemodynamic effects of angiotensin II in conscious rats by AT-receptor antagonists given after the AT1-receptor antagonist EXP 3174. Br. J. Pharmacol. 107: 873–880

Wong, P. C., Price, W. A., Chiu, A. T. (1990) Nonpeptide angiotensin II receptor antagonists. Hypertension 15: 823–834

Wong, P. C., Chiu, A. T., Duncia, J. V., Herblin, W. F., Smith, R. D., Timmermanns, P. B. M. W. M. (1992) Angiotensin II receptor antagonists and receptor subtypes. Trends Endocrinol. Metab. 3: 211–217

Zusman, R. M. (1987) Effects of converting enzyme inhibitors on the renin angiotensin aldosterone, bradykinin, and arachidonic acid-prostaglandin systems; correlation of chemical structure and biological activity. Am. J. Kidney Dis. 10 (suppl): 13–23