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In vivo Pharmacological Evaluation of Newly Synthesized Nonpeptidic AT_1 Receptor Antagonists in Rats

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This study was conducted to characterize the *in vivo* pharmacology of KR-30988, KR-30992 and losartan, new AT_1 antagonists, given as i.v. cumulative doses, in two animal models of high renin, conscious renal artery-ligated hypertensinve rats (RHRs) and normotensive rats anesthetized with urethane (900 mg/kg, i.p.) and α-chloralose (90 mg/kg, i.p.), with a special emphasis on the pharmacological characterization of the latter model. In conscious RHRs, KR-30988, KR-30992, losartan and captopril caused a dose-dependent decrease in blood pressure, their relative potencies (ED_{20}) being 0.057, 0.208, 0.164 and 0.018 mg/kg i.v., repectively. In anesthetized rats, 2 hours after anesthesia, plasma renin activity was increased from 7.31 to 34.07 ng/ml/h, the level approximately 1.5 times greater than the highest level in RHRs. In anesthtized rats, the ED_{20}s for all four compounds were 0.011, 0.046, 0.035 and 0.004 mg/kg i.v., repectively. By comparison, ED_{20}s from anesthetized rats were 4 to 5 times smaller than those from conscious RHRs, with a good correlation ($r=0.999$) noted between ED_{20}s from two groups of rats. The data on ED_{20} may indicate the higher sensitivity of anesthetized rats to the hypotensive activity of the compounds and the same order of potencies in two models. These results suggest that, in addition to RHRs, the normotensive rats anesthetized as above can serve as a suitable model for the rapid pharmacological evaluation of AT_1 receptor antagonists.

Key words: AT_1 receptor, Losartan, Renal hypertensive rat, Urethane, α-Chloralose

INTRODUCTION

Inhibitors of the renin-angiotensin system (RAS) have proven to be powerful tools in defining the physiology of this biochemical cascade as well as therapeutically important agents for the treatment of hypertension and heart failure. Different types of inhibitors of the RAS that have been developed thus far, include ACE inhibitors, renin inhibitors and peptidic angiotensin II (A II) receptor antagonists. However, these classes of compounds are known to possess limitations with respect to oral bioavailability, selectivity for the RAS system, incomplete prevention of A II formation, partial agonistic effects, and short duration of action. The recent discovery of nonpeptidic A II receptor antagonists such as losartan (DuP 753) devoid of most of these limitations in both animals and humans (Timmermans *et al.*, 1993; Chiu *et al.*, 1990; Wong *et al.*, 1990a, 1990b), paved the way for the design of other more potent, and orally active compounds.

These compounds are excellent tools for *in vivo* pharmacological studies to demonstrate the importance of the RAS in controlling blood pressure and fluid balance under physiologic and pathophysiologic conditons because they are highly selective for A II receptors, p.o. active and do not have A II agonistic activity (Shibouta *et al.*, 1993; Chiu *et al.*, 1990; Wong *et al.*, 1990a, 1990b). With respect to subtypes of A II receptors, most of the compounds of this class under development as potential antihypertensives are selective for AT_1 receptors, mainly because most of what we know about A II is associated with AT_1 receptor subtypes, and much of the physiological function of AT_2 receptor subtypes remains obscure (Timmermans *et al.*, 1993). As for the *in vivo* pharmacological studies of these nonpeptide AT_1 receptor antagonists, all the animal models used were those where the RAS was known to be activated. Consequently, losartan, the most well-characterized of this group, is an effective antihypertensive agent in renal hypertensive rats (one-

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ligated two-kidney type), a high renin model of experimental hypertension, but not in deoxycorticosterone acetate-salt hypertensive rats, a low renin model (Wong *et al.*, 1990b). However, losartan or captopril after acute i.v. administration did not lower blood pressure in conscious normotensive rats with normal renin levels, where the RAS system plays a minimal role in the control of blood pressure.

In this study, we characterized the pharmacological profiles of two novel nonpeptide AT_1 receptor antagonists, KR-30988 and KR-30992, given i.v. in renal artery-ligated rats and normotensive rats anesthetized with urethane and α-chloralose, with a special emphasis on the usefulness of the anesthetized rats as an animal model for pharmacological evalution of A II receptor antagonists. Losartan, AT_1 selective compound, and captopril, an ACE inhibitor, were also used for comparison as pharmacological tools in these experiments.

MATERIALS AND METHODS

Materials

Urethane and α-chloralose were purchased from Sigma Chemical Company (USA), and captopril was a gift from Boryung pharmaceutical Co. (Korea). Losartan, KR-30988 and KR-30992 were synthesized at Korea Research Institute of Chemical Technology (KRICT, Korea). [^{125}I]A II and an A I radioimmunoassay kit were purchased from DuPont-NEN (USA). Urethane was dissolved in isotonic saline and α-chloralose in propylene glycol with heating. All the drugs were prepared just before use.

Animals

Male Sprague-Dawley rats weighing 300-400 g were used in this study. They were purchased from the Department of Experimental Animal, KRICT and kept in a storage room under the conditions of constant temperature, relative humidity and illumination (12-h light, 12-h dark cycle) until the day of experiment, with free access to food and tap water.

Renal Hypertensive Rats (RHRs)

RHRs of 2-kidney, 1-ligation types were prepared as follows. Rats were anesthetized with ketamine·HCl (125 mg/kg, i.p.) and a small incision was made on the left side of abdomen. The left renal artery was separated from the vein near the junction with the aorta, taking care not to traumatized the vein, and then a complete ligature of 4-0 sterile silk was placed on the renal artery. After ligation, the incision was closed by carefully suturing the muscle layer with 4-0 silk and then the skin with metallic clips (Cangiano *et al.*, 1979). To delineate and confirm the development of high blood pressure and plasma renin activity (PRA) over days of ligation, animals that underwent the operations were divided into groups of days 0 (before operation), 6, 7, 8 and 28 days postoperativity for the measurement of systolic blood pressure and plasma renin activity. Indirect systolic blood pressure was measured using the tail cuff method with Multichannel 8000 (TSE, Germany) from the conscious rat.

Conscious Renal Hypertensive Rats (con-RHR)

As it was shown that animals from groups of day 6, 7 and 8 revealed good correlation between systolic blood pressure and plasma renin activity, rats from these groups were considered a model for acute renal hypertension and used as hypertensive rats in this study when systolic blood pressure was more than 180 mmHg.

After the animals were anesthetized with ketamine·HCl (125 mg/kg, i.v.), the left carotid artery and right jugular vein were exposed and cannulated with the catheters filled with heparinized saline (20 IU/ml) for the continuous monitoring of blood pressure and drug injections, respectively. The distal ends of the catheters were passed through a subcutaneous tunnel to exit dorsally, posterior to the head and between the scapulas. The animals were allowed to recover from anesthesia for at least 3 hours in individual cages. Then the catheter inserted in the carotid artery was connected to a pressure transducer (Isotec, Health dyne, USA) coupled to a phsiograph (Linearcorder WR3310, Hugo Sachs, Germany) to monitor the systemic arterial pressure. Heart rate was derived from the systemic arterial pressure pulses measured by the ECG/Rate coupler of the physiograph.

Losartan and KR-30992 were dissolved in 0.05 N KOH solution, and KR-30988 in 5% ethanol/5% cremophor. Captopril was dissolved in 0.9 w/v% NaCl. AT_1 antagonists were intravenously administered at cumulative doses of 0.001-3 mg/kg (1 ml/kg) at 15 minute intervals via a catheter inserted into the right jugular vein. Captopril given at the same doses i.v. cumulatively was also included for comparison. Data were expressed as percentage change of blood pressure and heart rate from baseline values.

Anesthetized Normotensive Rats (ane-NR)

Normotensive rats were anesthetized with a combination of urethane (900 mg/kg, i.p.) and α-chloralose (90 mg/kg, i.p.). The rats breathed room air via a tracheotomy tube connected to a rodent ventilator (Harvard apparatus, UK; stroke volume, 1 ml/100 g, 60 cycles/min). Systemic arterial pressure was measured and continuously monitored via a catheter (heparinized, 20 IU/ml) inserted in the left carotid artery, which

was connected to Grass P23XL pressure transducer and a Gould 2000 physiograph. Heart rate was derived from the systemic arterial pressure pulse by the ECG/Biotacho amplifier module of the Gould 2000 physiograph. Rectal temperature was maintained at 36.5±0.5℃ by thermistor-controlled radiant heat. Forty minutes after surgery, when consistent control values for all the parameters were possible to obtain, the experiment was conducted. AT_1 antagonists and captopril were intravenously administered according to the same protocol as above.

Plasma Renin Activity (PRA) Assay

To compare plasma renin activity in con-RHRs and ane-NRs, PRA was measured in the animals from 4 groups of renal arterial ligation and 1 group of combined anesthesia (2 hours after anesthesia) as follows. 1.5 Milliliter of blood was withdrawn by heart puncture from each animal under light ether anesthesia into a prechilled syringe containing sufficient EDTA solution (final concentration: 1 mg/ml). Collected samples were maintained in an ice bath and the plasma separated by centrifugation at 4℃ for 15 minutes (1200×g). The clear plasma was stored frozen at −80℃ until the day of assay. After thawing of the frozen plasma samples in an ice bath, plasma renin activity was determined by radio immunoassay (Haber *et al.*, 1969) using angiotesin I [^{125}I] assay kit (Du Pont-New England Nuclear), and the activity was expressed as ng/ml/hr of angiotensin I generated.

Statistical Analysis

Data were expressed as mean± S.E.M. The difference between groups was evaluated by Student's t-test for unpaired data as appropriate, with $p<0.05$ being considered statistically significant.

RESULTS

Antihypertensive Effect in Conscious Renal Hypertensive Rats

Fig. 1 shows the effects of cumulative i.v. doses of AT_1 antagonists and captopril (0.001-3 mg/kg) on mean arterial pressure (MAP) and heart rate (HR) in conscious renal hypertensive rats. Basal values of MAP and HR in groups of RHRs used for this study were similar and pooled as 178.2± 3.98 mmHg and 431.1± 9.67 beats/min, respectively.

KR-30988 at doses over 0.03 mg/kg i.v. dose-dependently decreased MAP and the maximal effect at each dose was reached approximately 5-10 min after i.v. dosing. The reduction in MAP at the highest dose tested (3 mg/kg, i.v.) was 50.4% and the hypotensive effect at this dose was long-lasting and thus substantial

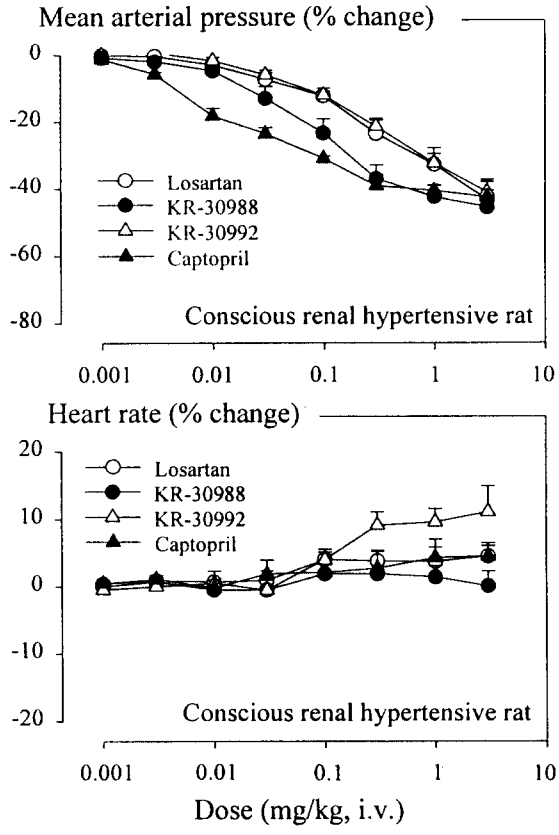

Fig. 1. Effects of cumulative i.v. doses of several AT_1 receptor antagonists and captopril (0.001-3 mg/kg) on mean arterial pressure and heart rate in conscious renal hypertensive rat. Data represent the mean± S.E.M. (n=5-6).

even at over 2 hours after dosing. Losartan also started reducing MAP significantly at the dose over 0.03 mg/kg i.v. and the reduction in MAP was 58% at 3 mg/kg i.v.. The hypotensive effects and dose-response curve for KR-30992 were similar to those for losartan. Depressor activity of captopril in RHRs was greater than those for all three AT_1 antagonists tested, as shown in dose-response curves.

The ED_{20} values (the dose which decreased the basal MAP by 20%) for KR-30988, KR-30992, losartan and captopril were 0.057, 0.206, 0.164 and 0.018 mg/kg i.v., respectively (Table I). As judged by ED_{20} values, the antihypertensive potency of KR-30988 was shown to be three times stronger than that of losartan in conscious renal hypertensive rat. Heart rate was not significantly changed by AT_1 antagonists tested and captopril at almost all i.v. doses used (Fig. 1).

Antihypertensive Effects in Anesthetized Normotensive Rats

The effects of cumulative i.v. doses of AT_1 receptor antagonists and captopril (0.001-3 mg/kg) were examined in normotensive rats anesthetized with urethane

Table I. ED_{20} values of AT_1 antagonists and captopril for depressor effects on mean arterial pressure (MAP) in conscious renal hypertensive rats (con-RHR) and anesthetized normotensive rats (ane-NR).

	ED_{20}[a] SAPm (mg/kg, i.v.)		ED_{20} in con-RHR
	con-RHR	ane-NR	/ED_{20} in ane-NR
Losartan	0.164	0.033	4.95
KR-30988	0.057	0.011	5.05
KR-30992	0.206	0.046	4.52
Captopril	0.018	0.003	5.35

[a] ED_{20}, the effective dose to lower the initial mean arterial pressure by 20%.

and α-chloralose, a model of high renin state (Fig. 2). Basal values of MAP and HR were 123.1± 3.10 mmHg and 446.7± 10.77 beats/min, respectively. In anesthetized rats, KR-30988 at 0.001-1 mg/kg i.v. markedly decreased MAP dose dependently, but no further reduction in MAP was noted at 3 mg/kg i.v. KR-30988 had a fast onset in its hypotensive activity, with the maximal effect being reached at 5-10 min after dosing of each dose. The maximal reduction in MAP was 57.8% from the basal values at 1 mg/kg i.v. and the pressor effect was still marked at over 2 hours after dosing at 3 mg/kg i.v. Losartan at over 0.01 mg/kg also decreased MAP dose-dependently with the maximal effect of 58.0% at 3 mg/kg i.v. Similar hypotensive effects were also observed with KR-30992 at doses tested with a similar maximal reduction in MAP despite greater ED_{20}. The profile of dose-dependency of KR-30988 and captopril was dissilmilar to that for losartan and KR-30992 in that the former reached the plateau although losartan and KR-30992 did not.

The ED_{20} values for KR-30988, KR-30992, losartan and captopril were 0.011, 0.046, 0.033 and 0.003 mg/kg i.v., respectively (Tabel I). As judged by ED_{20} values, KR-30988 was about three times as potent as losartan in hypotensive activity in anesthetized normotensive rat model. Three AT_1 antagonists tested and captopril caused a dose-dependent decrease in heart rate in this model (Fig. 2).

The ratios of ED_{20} in conscious renal hypertensive rats to ED_{20} in anesthetized normotensive rats were 5.05, 4.52, 4.95 and 5.35 for KR-30988, KR-30992, losartan and captopril (Table I).

Plasma Renin Activity (PRA)

In most of the rats subjected to the ligation of the renal artery, systolic blood pressure started increasing on day 3 and 4, reached its maximum on days 6-8 ($p<0.01$) and decreased thereafter on day 28, but still significantly greater than day 0 ($p<0.01$) (Table II). On day 6, 7 and 8 after ligation, there was a significant change ($p<0.01$) in PRA from a control level of 7.31± 0.63 ng/ml/hr Ang I to 19-22 ng/ml/hr Ang I, but on day 28, it returned to control level, despite the maintenance of high blood pressure, indicating good correlation between development of hypertension and PRA, only in the acute phase of renal hypertension in this model.

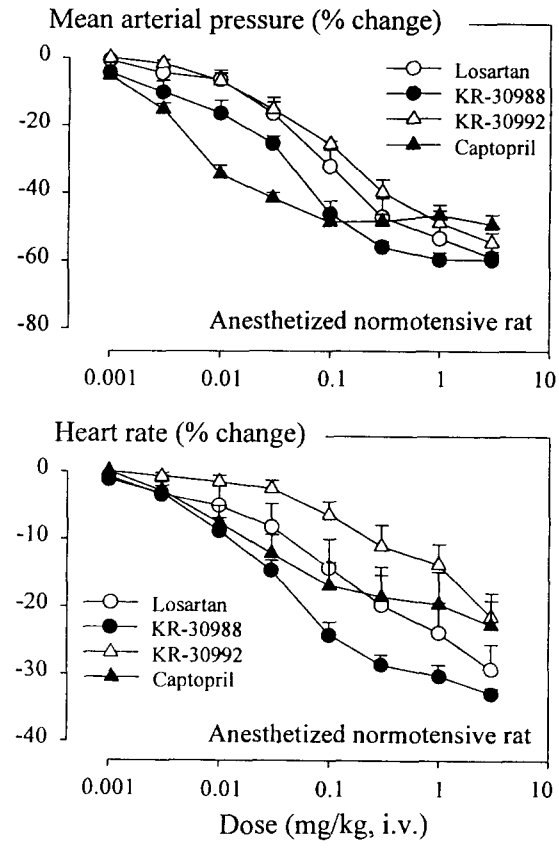

Fig. 2. Effects of cumulative i.v. doses of several AT_1 receptor antagonists and captopril (0.001-3 mg/kg) on mean arterial pressure and heart rate in normotensive rats anesthetized with urethane and α-chloralose. Data represent the mean± S.E.M. (n=4-6).

In about 10-20% of rats that underwent the surgery for renal arterial ligation, neither development of hypertension nor change in PRA was noted with an apparent morphological evidence of complete renal infarction in the kidney on the side subjected to surgery.

On the other hand, in rats anesthetized with a combination of urethane and α-chloralose, about a five fold increase in PRA was noted as compared with control normotensive rats in conscious state and PRA measured from anesthetized rats were also significantly larger than those from RHRs ($p<0.01$).

DISCUSSION

In rats of 2-kidney, 1-ligation type, it has been shown that PRA is elevated in the acute phase of hypertension (6-8 days after ligation), but returns to the

Table II. Plasma renin activity levels (PRA), systolic blood pressure (SBP) and heart rate (HR) before and 6, 7, 8 and 28 days after ligation of left renal artery (con-RHR) and 2 hours after anesthesia (ane-NR). Data are expressed as mean± S.E.M.

	Basal value	con-RHR (days after renal artery ligation)				ane-NR
		6	7	8	28	
PRA (ng/ml/hr)	7.31± 0.63	22.00± 2.82*	20.16± 3.42*	19.74± 2.29*	6.41± 0.88	34.07± 1.58*
SBP (mmHg)	154.2± 1.8	191.5± 4.3*	206.9± 9.4*	214.8± 13.7*	173.7± 3.1*	145.7± 4.9
HR (beats/min)	355.8± 24.0	364.3± 16.8	407.7± 34.1	427.2± 22.1	345.2± 13.1	447.2± 13.7
No. of animals	34	8	7	9	9	9

*$p<0.01$ compared with conscious normotensive rats (basal value).

normal level despite the consistence of high blood pressure in the chronic phase of hypertension (28 days after ligation). This finding, observed by other researchers (Cangiano *et al.*, 1979; Fernandez *et al.*, 1977), appears to indicate the possible role of the circulating renin angiotensin system in the development of hypertension in the acute phase, but not in the chronic phase of renal hypertension in this rat model.

In this study, we demonstrated that KR-30988 and KR-30992, newly synthesized nonpeptidic AT_1 receptor antagonists, have potent antihypertensive activity in the acute phase of conscious renal hypertensive rats, their i.v. potency being approximately 3 times greater than and comparable to losartan, respectively. Previous studies from this laboratory have shown that KR-30988 and KR-30992 are potent and selective AT_1 receptor antagonists for *in vitro* preparations including radioligand binding experiments with rat liver and functional experiments with isolated helical strips of rabbit aorta, with the relative potencies being similar to those observed for *in vivo* assays (unpublished data). The hypotension induced by all the drugs tested was not accompanied by any significant changes in HR at 0. 001-3 mg/kg i.v. The lack of tachycardia or insignificant effects on HR displayed by the drugs tested in this study appear to be common to blockers of the renin-angiotensin system, although the mechanism is still unclear and may be due to venous dilatation, enhanced vagal tone or reduction of the sympathetic baroreceptor responses to a decrease in blood pressure associated with the inhibition of the renin-angiotensin system (Cody, 1984).

In the second part of the study, we measured the effects of compounds on MAP and HR in anesthetized rats. We adopted a combination of urethane and α-chloralose for anesthesia because of the following reasons: Firstly, the combination of these two anesthesthetics has minimal effects on vascular smooth muscle as compared with other commonly used anesthetics such as pentobarbital and ketamine (Smith *et al.*, 1980; Hof, 1983; Mauck *et al.*, 1961). Secondly, the combined use of urethane and α-chloralose has been reported to induced a significant increase in PRA in rats (up to six fold; Faber, 1989), which was confirmed in this study, thus providing the pharmacological basis for the possible increase in sensitivity to blood pressure lowering effects of drugs acting via the inhibition of the renin angiotensin system. All the AT_1 receptor antagonists tested and captopril lowered MAP in the anesthetized normotensive rats. This finding appears to indicate that, in rats anesthetized with a combination of urethane and α-chloralose, the elevated renin is involved in the maintenance of blood pressure in rats under anesthesia via the cascade of the renin-angiotensin system and that the hypotensive effects of AT_1 antagonists tested are mainly mediated via the blockade of the vasoconstrictor influence A II, the main active metabolite of the RAS. The mediation of hypotensive effects of KR-30988 and KR-30992 via the blockade of AT_1 receptors may be further supported by the *in vitro* findings that they did not have any selective antagonism against contractile responses induced by KCl, norepinephrine, serotonin and PGF2 in isolated helical strips of rabbit aorta, while they antagonized the contraction induced by A II in this preparation (unpublished data). However we could not rule out the possibility of the involvement of other mechanisms as it is known that many anesthetics including urethane and α-chloralose may cause release of vasopressin (Dyball, 1975), either through direct action on nerves or kidney or indirectly through reduction in vascular pressures and activation of baroreflexes.

In the present study, the same order of potency was observed for the four compounds tested in conscious RHRs and anesthetized normotensive rats, with their ED_{20} ratios in two groups of rats within the range of 4-5. These findings appear to reveal the fact that the anesthetized rat is more sensitive (approximately 4-5 fold) to the depressor activity of AT_1 receptor antagonists and ACE inhibitors than the conscious renal hypertensive rat.

In summary, our results indicate that the normotensive rat anesthetized with a combination of urethane and α-chloralose could serve as a valuable high renin animal model that offers a method for rapid pharmacological evaluation of the candidates for novel antihypertensives acting via the inhibition of the renin-angiotensin system, especially AT_1 receptor antagonists.

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REFERENCES CITED

Chiu, A. T., McCall, D. E., Price, W. A., Wong, P. C., Carini, D. J., Duncia, J. V., Wexler, R. R., Yoo, S. E., Johnson, A. L. and Timmermans, P. B. W. M., Nonpeptide angiotensin II receptor antagonists. VII. Cellular and biochemical pharmacology of DuP 753, an orally active anhihypertensive agent. *J. Pharmacol. Exp. Ther.,* 252(2), 711-718 (1990).

Cody, R. J., Haemodynamic responses to specific renin-angiotensin inhibitors in hypertensive and congestive heart failure. *Drugs,* 28, 144-169 (1984)

Dyball, R. E. J., Potentiation of neurohypophyseal hormone release by urethane. *J. Physiol. Lond.,* 245, 119 (1975).

Faber, J. E., Effects of althesin and urethan-chloralose on neurohumoral cardiovasscular regulation. *Am. J. Physiol.,* 256, R757-R765 (1989).

Hof, R. P., Calcium antagonist and the peripheral circulation: differences and similarities between PY 108-068, nicardipine, verapamil and diltiazem. *Br. J. Pharmacol.,* 78, 375-394 (1983).

Haber, E., Koemer, T., Page, L. B., Kliman B. and Purnode, A., Application of a radio immunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. *J. Clin. Endocr.,* 29, 1349-1355 (1969).

Mauck, H. P., Freund, J. and Porter, R. R., Variability of cardiac output in dogs under chloralose and urethane anesthesia. *Toxicol. Appl. Pharmacol.,* 3, 380-385 (1961).

Shibouta, Y., Inada, Y., Ojima, M., Wada, T., Noda, M., Sanada, T., Kubo, K., Kogara, Y., Naka, T. and Nishikawa, Pharmacological profile of a highly potent and long-acting angiotensin II receptor antagonist, 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)biphyenyl-4yl]methyl] 1H-benzimidazole-7-carboxylic acid (CV-11974), and its prodrug, (±)-1-(cyclohexyloxycarbonyloxy)-ethyl 2-ethoxy-1-[[2'-(1H-tetrazole-5-yl)biphenyl-4-yl%methyl]-1H-benzimidazole-7-carboxylate (TCV-116). *J. Pharmacol. Exp. Ther.,* **266**(1), 114-120 (1993).

Smith, T. L. and Hutching, P. M., Anesthetic effects on hemodynamics of spontanuously hypertensive and Wistar-Kyoto rats. *Am. J. Physiol.,* 238, H539-H 544 (1980)

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. and Smith, R. D., Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol. Rev.,* 45(2), 205-251 (1993).

Wong, P. C., Price, W. A., Chiu, A. T., Duncia, J. V., Carini, D. J., Wexler, R. R., Johnson, A.L. and Timmermans, P. B. M. W. M., Nonpeptide angiotensin II receptor antagonists. VIII. Characterization of functional antagonism displayed by DuP 753, an orally active antihypertensive agent. *J. Pharmcol. Exp. Ther.,* 252(2), 719-725 (1990a).

Wong, P. C., Price, W. A., Chiu, A. T., Duncia, J. V., Carini, D. J., Wexler, R. R., Johnson, A. L. and Timmermans, P. B. M. W. M., Nonpeptide angiotensin II receptor antagonists. IX. Antihypertensive activity in rats of DuP 753, an orally active antihypertensive agent. *J. Pharmcol. Exp. Ther.,* 252(2), 726-732 (1990b).