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Pharmacological Characterization of KR-30988, a Novel Non-peptide AT_1 Receptor Antagonist, in Rat, Rabbit and Dog

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Abstract

The pharmacological profile of KR-30988, a non-peptide AT_1-selective angiotensin receptor antagonist, has been investigated by use of a variety of experimental models in-vitro and in-vivo.

KR-30988 inhibited the specific binding of [^{125}I][Sar^1, Ile^8]-angiotensin II to the recombinant AT_1 receptor from man with a potency similar to that of losartan (IC50 values, the concentrations of drugs displacing 50% of specific binding, 13·6 and 12·3 nM, respectively), but did not inhibit the binding of [^{125}I]CGP 42112A to recombinant AT_2 receptor from man (IC50 $> 10\,\mu$M for both drugs). Scatchard analysis showed that KR-30988 interacted competitively with recombinant AT_1 receptor from man in the same manner as losartan. In functional studies with rat and rabbit aorta, KR-30988 non-competitively inhibited the contractile response to angiotensin II ($pD_2, = -\log$ EC50 (where EC50 is the dose resulting in 50% of a reference contraction), 8·64 and 7·73, respectively) with a 20–85% decrease in the maximum contractile responses, unlike losartan. In pithed rats intravenous KR-30988 resulted in a non-parallel shift to the right of the dose–pressor response curve to angiotensin II (ID50 value, the dose inhibiting the pressor response to angiotensin II by 50%, 0·09 mg kg^{-1}) with a dose-dependent reduction in the maximum responses; in this antagonistic effect KR-30988 was 20 times (approx.) more potent than losartan (ID50 1·74 mg kg^{-1}). In conscious renal hypertensive rats oral administration of KR-30988 produced a dose-dependent and long-lasting (> 24 h) anti-hypertensive effect; the potency was six times that of losartan (ED30 values, the dose reducing mean arterial blood pressure by 30 mmHg, 0·48 and 2·97 mg kg^{-1}, respectively). In conscious furosemide-treated dogs oral administration of KR-30988 produced a dose-dependent and long-lasting (> 8 h) hypotensive effect with a rapid onset of action (time to E_{max}, the maximum effect, 1–2 h); KR-30988 was eight times more potent than losartan (ED20, the dose reducing mean arterial blood pressure by 20 mmHg, 1·04 and 7·96 mg kg^{-1}, respectively).

These results suggest that KR-30988 is a potent, orally active selective AT_1 receptor antagonist with a mode of insurmountable antagonism.

The renin–angiotensin system is physiologically important in the control of blood pressure and overactivity of the system is now implicated in the pathogenesis of hypertension (Vallotton 1987). Angiotensin II, the primary mediator of the renin–angiotensin system, has several physiological actions such as vasoconstriction, aldosterone release and potentiation of catecholamine release; it thus plays a major role in the determination of long-term blood-pressure control (Ganten et al 1991; Timmermans et al 1993; Griendling et al 1994). Angiotensin II has also been shown to be important in ventricular and vascular remodelling (Gobbons & Dzau 1994), which are highly prevalent in hypertension. These deleterious effects of angiotensin II are mainly mediated through stimulation of the AT_1 receptor subtype (Timmermans et al 1993; Griendling et al 1994). Recently, the AT_1 receptor

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antagonists have been developed as a new class of antihypertensive drug. Losartan, the first orally active non-peptidic AT_1 receptor antagonist, was shown to be effective in the treatment of essential hypertension (Wong et al 1990a, 1991a; Dahlof et al 1995; Smith et al 1996) and beneficial effects in the treatment of congestive heart failure have also been demonstrated (Dickstein et al 1994; Crozier et al 1995). AT_1 receptor antagonists are classified as surmountable and insurmountable. For losartan, AT_1 antagonism is surmountable whereas that of its major metabolite EXP3174 is insurmountable (Wong et al 1990b).

Several AT_1 receptor antagonists, which differ in their physiochemical and pharmacological characteristics, are now available (Wienen et al 1993; Ziai et al 1996; Ojima et al 1997). KR-30988 (2-butyl-5-dimethoxymethyl-6-phenyl-3-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-3*H*-imidazo [4,5-*b*] pyridine, Figure 1) was synthesized by the Korea Research Institute of Chemical Technology (KRICT, Taejon, Korea) and belongs to a novel class of non-peptide angiotensin II receptor antagonist with high affinity for the AT_1 receptor. KR-30988 is structurally different from losartan and its series of imidazole analogues in having an imidazopyridine ring skeleton with an acetal group. It is known that intravenously administered KR-30988 has potent antihypertensive effects in two high-renin animal models—conscious renal-ligated hypertensive rats and normotensive rats under anaesthesia with a combination of urethane and α-chloralose (Lee & Shin 1994).

The specific aims of the study were to characterize and compare the pharmacological properties of KR-30988 with those of losartan by examining its antagonistic effects on the binding of [^{125}I][Sar^1, Ile^8]-angiotensin II and [^{125}I]CGP 42112A to recombinant AT_1 and AT_2 receptor subtypes from man, on the angiotensin II-induced contraction of rabbit and rat aortic segments, on the angiotensin II-induced pressor response in pithed rats, and on antihypertensive effects in renal hypertensive rats and furosemide-treated beagle dogs.

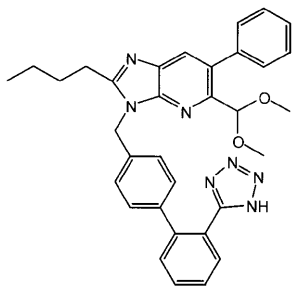

Figure 1. The chemical structure of KR-30988.

Materials and Methods

Chemicals

KR-30988, L-158809, PD-123177 and losartan were synthesized at the Bio-Organic Science Division, KRICT. Sodium pentobarbital was purchased from Hanlim (Seoul, Korea) and ketamine hydrochloride from Yuhan (Seoul, Korea). [Sar^1, Ile^8]-angiotensin II, angiotensin II acetate, arterenol bitartrate, vasopressin acetate and isoproterenol hydrochloride were purchased from Sigma (St Louis, MO). [^{125}I][Sar^1, Ile^8]-angiotensin II and [^{125}I]CGP 42112A (2200 Ci mmol^{-1}) were obtained from NEN Life Science Products (Boston, MA). KR-30988 and losartan were dissolved in dimethylsulphoxide. They were further diluted in buffer for angiotensin II binding assay and isolated tissue experiments, and dissolved in 0·05 M KOH in saline and suspended in Tween 80 for intravenous and oral administration, respectively. All chemicals were prepared immediately before use.

Radioligand binding assay

Binding assays were performed in 96-well plates by incubating samples of recombinant AT_1 and AT_2 receptors from man (BioSignal, Montreal, Canada) with 0·21 nM [^{125}I][Sar^1, Ile^8]-angiotensin II and 0·5 nM [^{125}I]CGP 42112A, respectively. The assay buffer (pH 7·4) contained Tris (50 mM), $MgCl_2$ (5 mM), EDTA (1 mM) and bovine serum albumin (0·1%). Specific binding of [^{125}I][Sar^1, Ile^8]-angiotensin II and [^{125}I]CGP 42112A was determined experimentally from the difference between counts in the absence and presence of unlabelled angiotensin II and [Sar^1, Ile^8]-angiotensin II (10 μM). After incubation at 37°C for 60 min (or 180 min for AT_2 receptor), the incubation mixtures were filtered through GF/C glass-fibre filters (Wallac, Turku, Finland) which had been presoaked in polyethylenimine (0·3%) and washed with ice-cold Tris buffer (50 mM, pH 7·4) by use of the Inotech (Dottikon, Switzerland) harvester. Captured radioactivity was counted by means of MicroBeta (Wallac). The inhibition by antagonists of specific [^{125}I][Sar^1, Ile^8]-angiotensin II and [^{125}I]CGP 42112A binding was estimated in terms of IC50 values, the molar concentrations of unlabelled drugs necessary to displace 50% of specific binding. The K_i value was calculated from equation 1:

$$K_i = IC50/(1 + L/K_d) \quad (1)$$

where L is the concentration of [^{125}I][Sar^1, Ile^8]-angiotensin II or [^{125}I]CGP 42112A (Cheng & Prusoff 1973). Data from binding experiments were

analysed by non-linear regression, by use of the PRISM computer programme (GraphPad Software, San Diego, CA).

In-vitro potency in rat and rabbit aorta

Aortic rings from male Sprague–Dawley rats, 350–450 g (KRICT, Taejon, Korea), and male New Zealand White rabbits, 2–3 kg (Samyook, Suwon, Korea), were prepared without endothelium and mounted in tissue baths containing oxygenated (95% O_2–5% CO_2) Krebs bicarbonate buffer at 37°C as described elsewhere (Shin et al 1998). In experiments with rat aorta (resting tension 2 g), a cumulative concentration–contractile response curve for angiotensin II was determined for each tissue after 30 min incubation with KR-30988 and losartan. The tissues were rinsed several times and left to return to baseline tension. The tissues were then contracted with 122·7 mM K^+ solution to obtain a reference contractile response, as a percentage of which all the responses from rat aorta were expressed. In experiments with rabbit aorta (resting tension 2 g), the first control cumulative concentration–contractile response curve for angiotensin II was determined to ensure stable reactivity to subsequently added angiotensin II. The tissues were left, with repeated washing, to recover baseline tension and the second cumulative concentration–contractile response curve for angiotensin II was established for each tissue after 30-min incubation with KR-30988 or losartan. Responses from rabbit aorta were expressed as a percentage of the maximum angiotensin II response obtained from the first cumulative concentration–response curve. The isometric contraction was recorded with force-displacement transducers (Grass FT03; Grass Instruments, Quincy, MA) and displayed on a chart recorder (Multicorder MC 6625, Hugo Sachs Electronic, Freiburg, Germany). The calculated pD_2 value ($= -\log EC50$, where EC50 is the dose resulting in half the reference contractile response) for KR-30988 was obtained from linear regression of log dose–response data, and the pA_2 value for losartan was determined according to the Schild equation (Schild 1947).

In separate experiments to study possible interaction between KR-30988 and losartan, isolated rabbit aorta was first incubated with KR-30988 or vehicle for 30 min and subsequently with losartan (1 μM) or vehicle for another 10 min. The concentration–response curve for angiotensin II was then obtained in the presence of KR-30988 and losartan. In the second series of experiments a similar protocol was repeated except that the tissues were incubated first with losartan or vehicle for 10 min and then with KR-30988 or vehicle for 30 min.

In-vivo potency and specificity as angiotensin II antagonist in pithed rats

Male Sprague–Dawley rats (KRICT), 350–450 g, were anaesthetized, cannulated and pithed as described elsewhere (Gillespie & Muir 1967). Rats were ventilated with room air by means of a rodent ventilator (model 7025; Ugo Basile, Varese, Italy; frequency 60 cycles min^{-1}; stroke volume 1 mL/100 g body weight). The left femoral artery and vein were cannulated for arterial blood-pressure measurement and intravenous administration of drugs, respectively. The arterial catheter was connected to an Isotec pressure transducer (Healthdyne, Marietta, GA) coupled to a Graphtec Linearcorder (model 3310; Graphtec, Tokyo, Japan). Heart rate was derived from arterial pulse pressure via a cardiotachometer (Type 576; Graphtec). The animals were pretreated with a single intravenous (i.v.) dose of KR-30988, losartan or vehicle (0·05 M KOH, 1 mL kg^{-1}) 15 min before injection of angiotensin II. Only one full sequential dose–response curve for angiotensin II was obtained from each rat. Dose–pressor response curves for norepinephrine, vasopressin and isoproterenol were also determined in the presence or absence of KR-30988 at 0·3 mg kg^{-1}, to determine the specificity of this antagonist. The results were expressed as mmHg of diastolic arterial blood pressure. The dose (ID50) of compounds that inhibited the pressor response to angiotensin II (10 μg kg^{-1}, i.v.) by 50% was calculated by linear regression as an indirect measure of antagonism.

Antihypertensive effects in conscious renal hypertensive rats

The renal hypertensive rats were prepared by a complete ligation of the left renal artery as described elsewhere (Cangiano et al 1979; Lee & Shin 1994; Lee et al 1998). Six days after ligation the animals' femoral arteries were surgically prepared for recording arterial blood pressure (Lee et al 1998). The animals were left for one day to recover and stabilize in individual cages. On the day of the experiment, rats were kept moving free in individual cages in a quiet room and the arterial catheter was connected to a pressure transducer (CDX-III; Modular Instruments, Malvern, PA) coupled to a physiograph (Modular 8000 Signal processor; Modular Instruments), and heart rate was derived from the blood pressure pulse, both parameters being analysed and stored by Biowindow software (Modular Instruments). Arterial blood pressure and heart rate were monitored for 6 h and then again

24 h after single oral administration of KR-30988 and losartan. The results were expressed as mmHg of mean arterial blood pressure. The dose (ED30) of compounds that reduced mean arterial blood pressure by 30 mmHg was calculated by linear regression.

Hypotensive effects in furosemide-treated dogs

Male beagle dogs (Samyook, Suwon, Korea), 8–12 kg, were anaesthetized and cannulated as described elsewhere (Lee et al 1998). Two days after catheter implantation, animals were trained to stand in a sling (Daejong, Seoul, Korea) for continuous measurement of arterial blood pressure via a Grass P23XL pressure transducer, then continuous recording on a Gould 2000 physiograph (Gould, Cleveland, OH). Heart rate was derived from the arterial pressure pulse by means of the ECG/Biotacho amplifier module of the Gould 2000 physiograph. To activate the renin–angiotensin system, animals were treated with furosemide ($10\,mg\,kg^{-1}$) twice, 18 h (intramuscularly) and 2 h (intravenously) before the experiment, as described by Wong et al (1991b). Food and water were withdrawn from these dogs after the first dose of furosemide. Arterial blood pressure and heart rate were monitored for 8 h after single oral administration of KR-30988 and losartan. The results were expressed as mmHg of mean arterial blood pressure. The dose (ED20) of compounds that reduced mean arterial blood pressure by 20 mmHg was calculated by linear regression.

Statistical analysis

All values are expressed as means ± s.e.m. Data were analysed by means of Student's *t*-test or one-way analysis of variance then by the Dunnett test for multiple comparisons (Sigma Stat; Jandel, San Rafael, CA). In all comparisons the difference was considered to be statistically significant at $P < 0{\cdot}05$.

Results

Radioligand binding assay

Against the recombinant AT_1 receptor from man, [^{125}I][Sar^1, Ile^8]-angiotensin II interacted with a single population of binding sites with a dissociation constant (K_d) of $0{\cdot}24 \pm 0{\cdot}01$ nM. The corresponding number of binding sites labelled by the radioligand was $46{\cdot}3 \pm 0{\cdot}7$ fmol $(mg\,protein)^{-1}$. KR-30988, losartan, L-158809 and PD-123177 competed dose-dependently with 0·21 nM [^{125}I][Sar^1, Ile^8]-angiotensin II for the binding sites of recombinant AT_1 from man; their inhibition curves seemed to be monophasic (Figure 2A, B). KR-30988 had specific affinity for the recombinant AT_1 receptor from man (IC50 $13{\cdot}6 \pm 7{\cdot}3$ nM, K_i $5{\cdot}6 \pm 3{\cdot}0$ nM) without any binding affinity for the recombinant AT_2 subtype, against which PD-123177 had moderate activity (IC50 $4{\cdot}3 \pm 1{\cdot}4\,\mu$M, K_i $1{\cdot}8 \pm 0{\cdot}6\,\mu$M). In the displacement of labelled angiotensin II from the recombinant AT_1 receptor from man the potency of KR-30988 was equal to that of losartan (IC50 $12{\cdot}3 \pm 1{\cdot}4$ nM, K_i $5{\cdot}1 \pm 0{\cdot}6$ nM) and less than that of L-158809 (IC50 $1{\cdot}44 \pm 0{\cdot}34$ nM). The Hill coefficients for inhibition by KR-30988, losartan and L-158809 were 1·18, 0·86 and 0·99, respectively, not significantly different from unity. The results from the saturation binding assay using [^{125}I][Sar^1, Ile^8]-angiotensin II conducted in the presence of KR-30988 (10 nM) and losartan (10 nM) are depicted in Figure 2C. Scatchard transformations of [^{125}I][Sar^1, Ile^8]-angiotensin II saturation curves revealed that these two antagonists did not affect the total number of binding sites labelled by [^{125}I][Sar^1, Ile^8]-angiotensin II, but increased the dissociation constant of the radioligand by factors of 1·62 (K_d 0·39 nM) with KR-30988 and 1·37 (K_d 0·33 nM) with losartan, compared with control data. The saturation binding assay also showed their competitive interaction with the receptor.

In-vitro potency in rat and rabbit aorta

KR-30988 and losartan inhibited angiotensin II-induced contractions of the rat aorta in a concentration-dependent manner (Figure 3), but with different types of antagonism. KR-30988 (10^{-9}, 3×10^{-9} and 10^{-8} M) caused a non-parallel rightward shift in the concentration–contractile response curve to angiotensin II with a significant reduction in the maximum contractile response (calculated pD_2 8·64; Figure 3A). In contrast, losartan (10^{-7}, 3×10^{-7} and 10^{-6} M) caused a parallel rightward shift in the concentration–response curve without any changes in the maximum contractile response (pA_2 8·02; slope of the Schild plot 1·03; Figure 3C). Similar results were observed for rabbits (pD_2 of KR-30988 7·73; pA_2 and slope of the Schild plot of losartan 7·59 and 1·34, respectively; Figures 3B, D).

Preincubation of rabbit tissue with KR-30988 (3×10^{-8} M) for 40 min (KR-30988 + vehicle) and 30 min (vehicle + KR-30988) reduced the maximum contractile response to angiotensin II by approximately 50% (Figure 4). However, for tissue incubated for 30 min with KR-30988 (3×10^{-8} M)

and then for 10 min with losartan (10^{-6} M) the subsequent angiotensin II concentration–response curves were similar to those for losartan only in their position and shape without the reduction of the maximum contractile response seen with KR-30988 only (Figure 4A). Reverse incubation of tissue with losartan (10^{-6} M) before treatment with KR-30988 (3×10^{-8} M) resulted in similar findings (Figure 4B). These results indicate that the insurmountable antagonism of KR-30988 can be reversed by addition of a high concentration of losartan, an angiotensin AT_1-selective surmountable receptor antagonist.

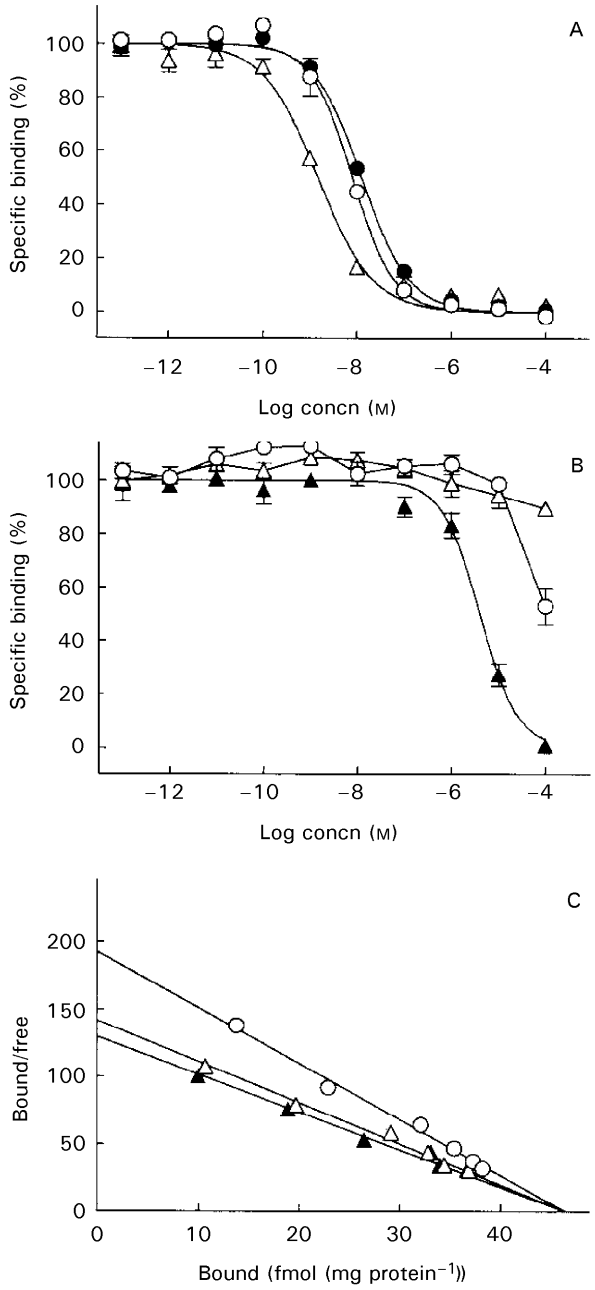

Figure 2. Inhibition by KR-30988 (○), losartan (●), L-158809 (△) and PD-123177 (▲) of specific [^{125}I][Sar^1, Ile^8]-angiotensin II binding to the recombinant AT_1 receptor from man (A) and specific [^{125}I]CGP 42112A binding to the recombinant AT_2 receptor from man (B). C. Scatchard transformations of saturation binding data for specific [^{125}I][Sar^1, Ile^8]-angiotensin II binding to the recombinant AT_1 receptor from man in the absence (○) or presence of KR-30988 (10 nM, ▲) or losartan (10 nM, △). The data points represent the means from three separate experiments run in quadruplicate.

In-vivo potency and specificity of angiotensin II antagonist in pithed rats

In pithed rats treated with vehicle (control group) under the experimental conditions used, the mean diastolic arterial pressure and heart rate were $36{\cdot}7 \pm 1{\cdot}7$ mmHg and $314 \pm 9{\cdot}4$ beats min^{-1}, respectively. The baseline values for diastolic arterial pressure and heart rate were similar for all groups of pithed rats. Cumulatively administered angiotensin II induced a gradual increase in diastolic arterial pressure with dose (E_{max}, the maximum effect, $112{\cdot}0 \pm 7{\cdot}5$ mmHg; ED50 (the dose reducing the mean arterial blood pressure by 50 mmHg) $0{\cdot}68 \pm 0{\cdot}05$ μg kg^{-1}; Figure 5). Pretreatment with KR-30988 and losartan did not significantly affect diastolic arterial pressure. KR-30988 (0·03–0·3 mg kg^{-1}, i.v.) not only caused a dose-dependent rightward shift in the dose–pressor response curve to angiotensin II with an ID50 value of 0·09 mg kg^{-1}, but also significantly reduced the maximum pressor response to angiotensin II (Figure 5A). Losartan (1·0–10·0 mg kg^{-1}, i.v.) dose-dependently shifted the dose–pressor response curve to angiotensin II parallel to the right, with an ID50 value of 1·74 mg kg^{-1}, but without any change in the maximum response to angiotensin II, unlike KR-30988 (Figure 5B). At an intravenous dose of 0·3 mg kg^{-1} KR-30988 did not alter the dose–response curves to norepinephrine, vasopressin and isoproterenol (Figure 6).

Antihypertensive effects in conscious renal hypertensive rats

The effects of the orally administered KR-30988 (0·3, 1·0 and 3·0 mg kg^{-1}) on mean arterial blood pressure and heart rate in conscious renal hypertensive rats are shown in Figure 7A. The mean predose values of mean arterial blood pressure and heart rate were 161 ± 8 mmHg and 380 ± 18 beats min^{-1} in renal hypertensive rats. KR-30988 elicited a dose-dependent decrease in mean arterial blood pressure with a gradual onset of the effect (10 min), the maximum being reached 1–3 h after the dose. For all doses the antihypertensive effects of KR-30988 persisted at a significant level for 24 h

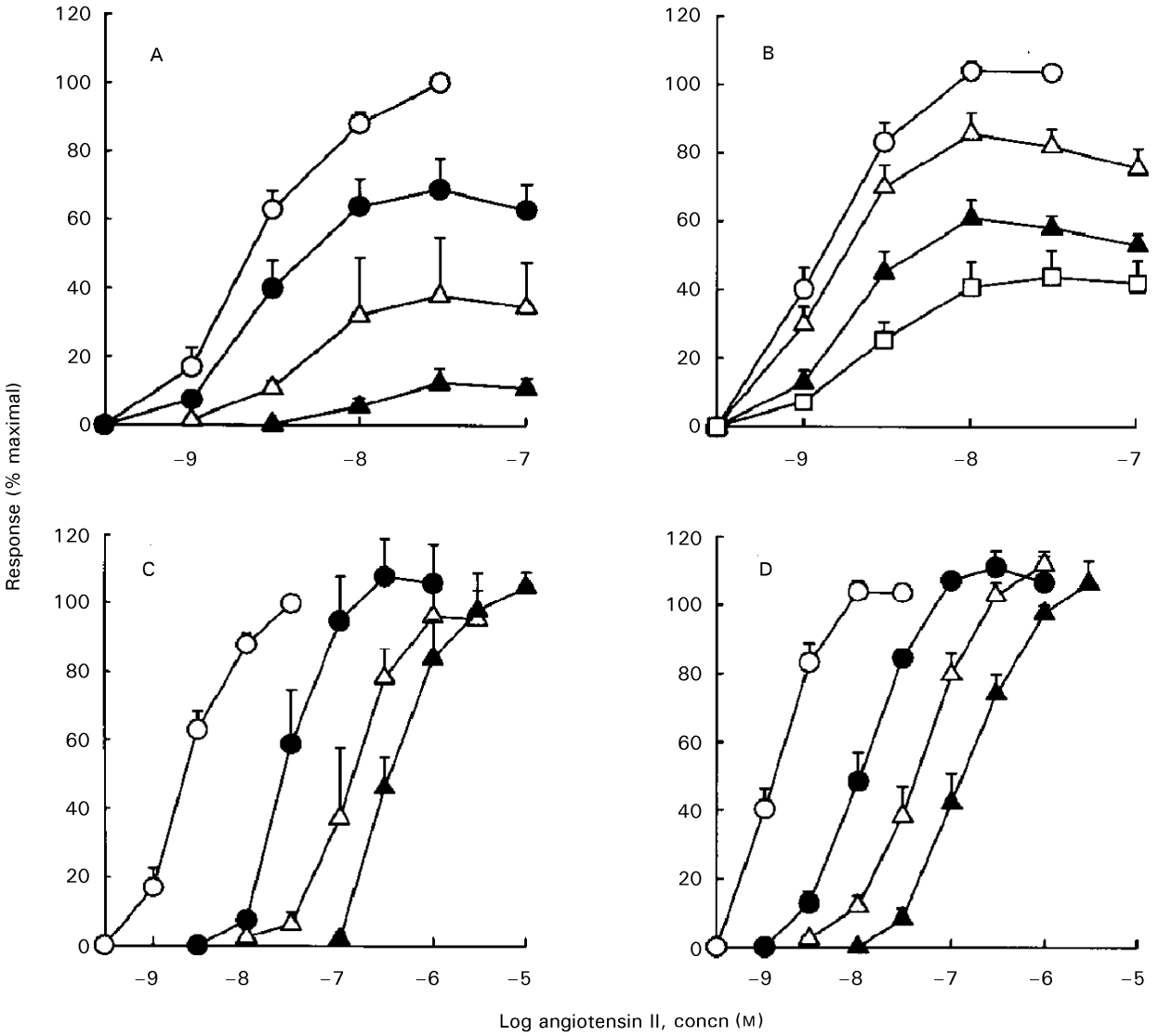

Figure 3. Effects of KR-30988 (A, B; ●, 10^{-9} M; △, 3×10^{-9} M; ▲, 10^{-8} M ; □, 3×10^{-8} M; ○, vehicle) and losartan (C, D; ●, 10^{-7} M; △ 3×10^{-7} M; ▲, 10^{-6} M; ○, vehicle) on the concentration–contractile response curve to angiotensin II in isolated rat aorta (A, C) and rabbit aorta (B, D). The data points represent the mean percentages of the maximum response ± s.e.m. (n = 4–8).

after the dose (ED30 $0{\cdot}48 \pm 0{\cdot}21$ mg kg^{-1}), but without any significant change in heart rate (data not shown). Orally administered losartan (3 and 10 mg kg^{-1}) elicited a dose-dependent decrease in mean arterial blood pressure (ED30 $2{\cdot}97 \pm 1{\cdot}02$ mg kg^{-1}) with slower action than KR-30988 (time to maximum 4–6 h; Figure 7B).

Hypotensive effects in furosemide-treated dogs

The effects of the orally administered KR-30988 (1·0, 3·0 and 10·0 mg kg^{-1}) and losartan (3, 10 and 30 mg kg^{-1}) on mean arterial blood pressure and heart rate in furosemide-treated dogs are shown in Figure 8. The mean predose values of mean arterial blood pressure and heart rate were 102 ± 4 mmHg and 116 ± 10 beats min^{-1} in furosemide-treated dogs. KR-30988 and losartan caused a dose-dependent decrease in mean arterial blood pressure with a gradual onset of the effect (10 min), the maximum effect being reached 1–3 h after the dose. The hypotensive effects of KR-30988 and losartan (ED20 $1{\cdot}04 \pm 0{\cdot}67$ and $7{\cdot}96 \pm 0{\cdot}94$ mg kg^{-1}, respectively) lasted over 8 h after administration of the dose ($P < 0{\cdot}05$), and these effects were not accompanied by any significant changes in heart rate at all doses used (heart rate data not shown).

Discussion

To characterize the pharmacological specificity of the newly synthesized KR-30988 for angiotensin II

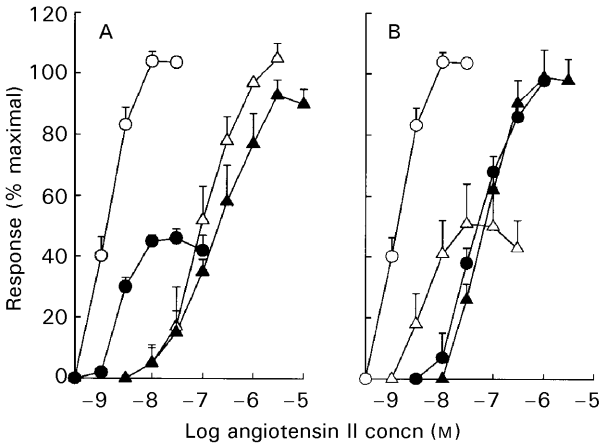

Figure 4. Effects of KR-30988 (3×10^{-8} M) and losartan (10^{-6} M) alone or in combination on the concentration–contractile response curve to angiotensin II in isolated rabbit aorta. A. The aorta was treated with KR-30988 or its vehicle for 30 min and then with losartan or its vehicle for another 10 min: ○, vehicle + vehicle; ●, KR-30988 + vehicle; △, vehicle + losartan; ▲, KR-30988 + losartan. B. The aorta was treated with losartan or its vehicle for 10 min and then with KR-30988 or its vehicle for another 30 min: ○, vehicle + vehicle; ●, losartan + vehicle; △, vehicle + KR-30988; ▲, losartan + KR-30988. The data points represent means ± s.e.m. (n = 4–6).

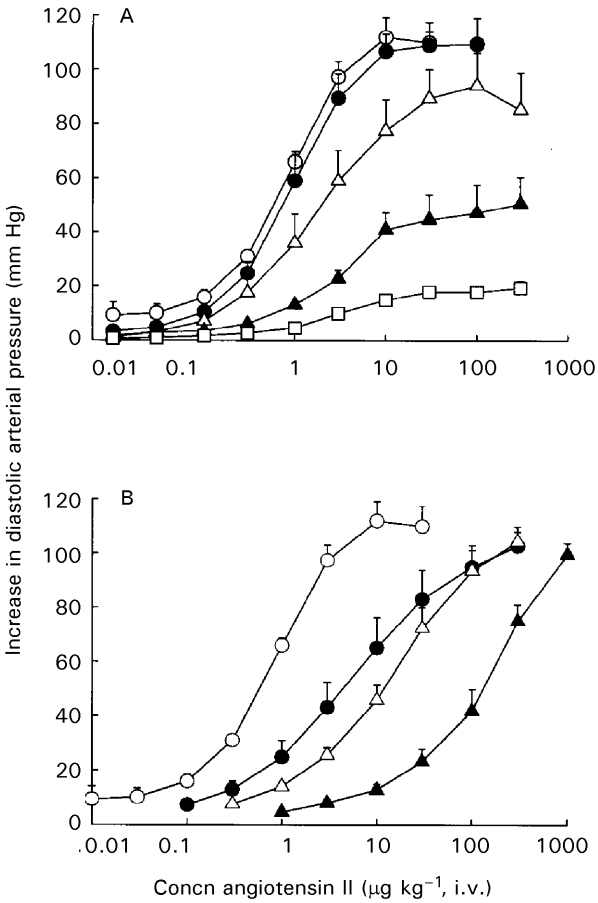

Figure 5. Effects of intravenously administered KR-30988 (A) (○, vehicle; and ●, 0·03; △, 0·06; ▲, 0·1; and □, 0·3 mg kg^{-1}) and losartan (B) (○, vehicle; and ●, 1·0; △, 3·0; ▲, 10·0 mg kg^{-1}) on the log dose–pressor response curve to angiotensin II in anaesthetized pithed rat. The data points represent means ± s.e.m. (n = 6–9).

receptors, the affinity of KR-30988 and losartan for these receptors have been assessed and compared in radioligand binding studies using recombinant angiotensin receptors from man. KR-30988 and losartan totally displaced specifically bound [^{125}I][Sar^1, Ile^8]-angiotensin II from recombinant angiotensin AT_1 receptor, with equal potency (IC50 13·55 and 12·30 nM, respectively). In contrast, KR-30988 at $\leq 100\,\mu$M had no interaction with recombinant angiotensin AT_2 receptor from man, from which PD 123177, an AT_2-selective antagonist, displaced specifically bound [^{125}I]CGP 42112A. In this study the affinity of PD-123177 for the AT_2 receptor was much lower (micromolar affinity) than that measured in previous studies with receptor from a different source (Wienen et al 1993), probably because of differences between the characteristics of receptor sources for the AT_2 receptor. These results from the binding studies suggest that KR-30988 is highly selective for the AT_1 receptor site. Analysis of the competition curve showing characteristics of monophasic inhibition indicated binding of KR-30988 to a single class of AT_1 receptor with a Hill coefficient of 0·96. In further radioligand saturation experiments KR-30988 caused an increase in the dissociation constant of [^{125}I][Sar^1, Ile^8]-angiotensin II without reduction in the maximum binding capacity (B_{max}) of recombinant AT_1 receptor from man. These data are strong evidence that KR-30988 competitively interacts with AT_1 receptors in the same way as losartan.

Several functional in-vitro and in-vivo studies were performed to characterize the mode of interaction of KR-30988 with the AT_1 receptor. In rat and rabbit aorta KR-30988 caused a non-parallel rightward shift in the concentration–response curve to angiotensin II with a reduction of the maximum contractile response by 31% to 87%, suggesting insurmountable antagonism of angiotensin II-induced contraction (pD_2 8·64 and 7·73, respectively). This unusual pharmacological behaviour exerted by KR-30988 has previously been reported for other non-peptide AT_1 antagonists such as candesartan, BIBR 277 or EXP 3174 (Wienen et al 1993; Panek et al 1995; Ojima et al 1997), whereas losartan caused a parallel rightward shift in the concentration–response curve without any changes in maximum contractile response (pA_2 7·59 and 8·02 in rabbit and rat aorta, respectively), indicating a competitive antagonism. This phenomenon of

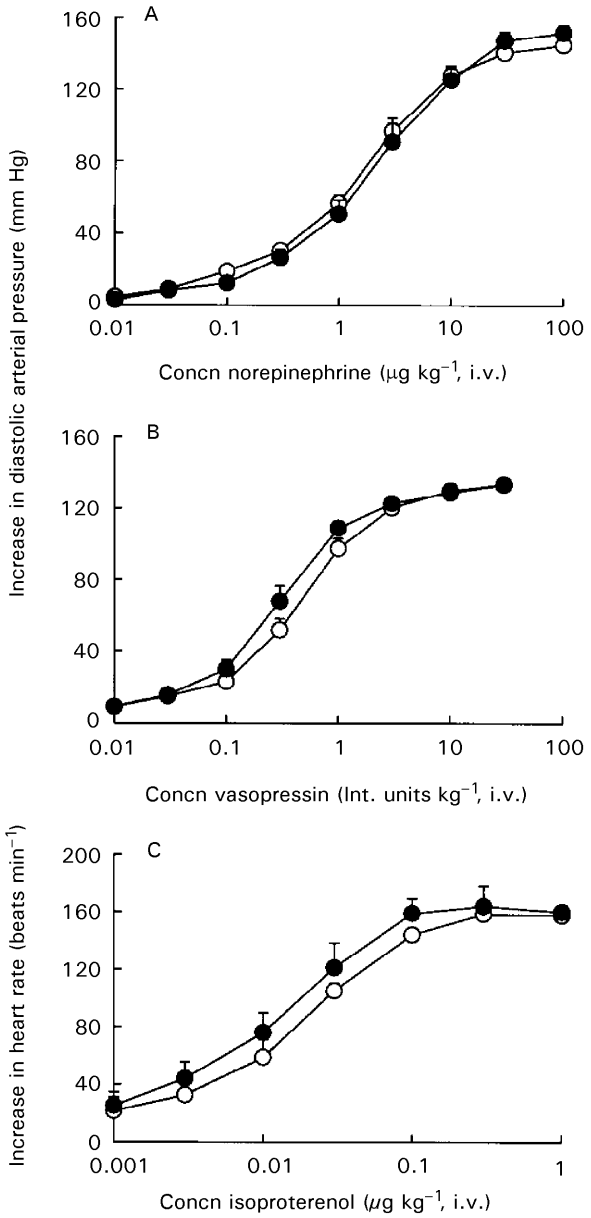

Figure 6. Effects of intravenously administered vehicle (○) and KR-30988 (0·3 mg kg^{-1}, ●) on the log dose–pressor response curve to norepinephrine (A) and vasopressin (B) and on the log dose–tachycardiac response curve to isoproterenol (C) in the anaesthetized pithed rat. The data points represent means ± s.e.m. (n = 5–6).

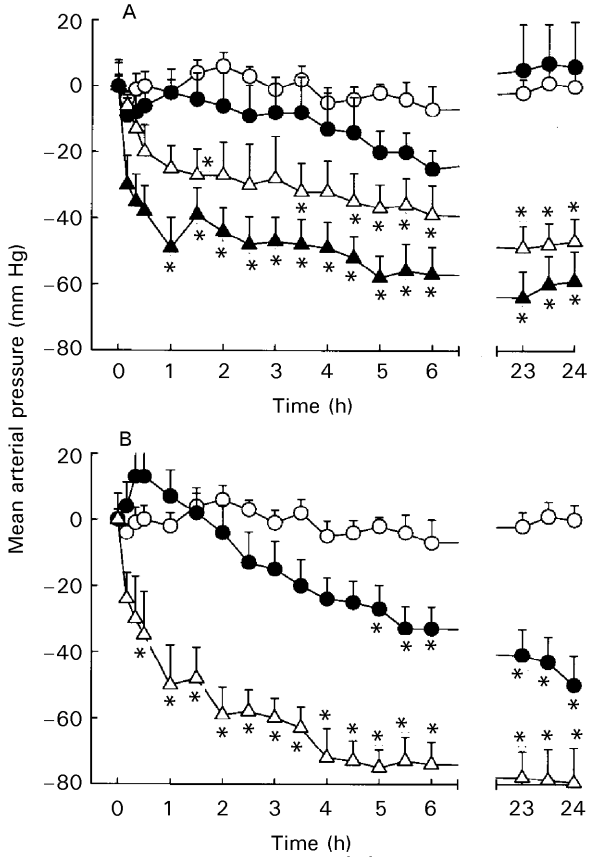

Figure 7. Effects of orally administered KR-30988 (A) (●, 0·3; △, 1·0; ▲, 3·0 mg kg^{-1} and ○, vehicle) and losartan (B) (●, 3·0; △, 10·0 mg kg^{-1} and ○, vehicle) on mean arterial blood pressure in conscious renal hypertensive rats. The data points represent the mean percentage change from the control, ± s.e.m. (n = 4–6). *$P < 0.05$ compared with control.

insurmountable antagonism by KR-30988 was also reproduced in an anaesthetized pithed rat, i.e. KR-30988 caused a rightward shift in the dose–pressor response curve to angiotensin II with a dose-dependent reduction in the maximum pressor response to angiotensin II. In contrast, in the pithed rat losartan caused a rightward parallel shift in the dose–pressor response curve to angiotensin II without reducing the maximum response. Several hypothetical mechanisms proposed to explain insurmountable antagonism of AT_1 receptor antagonists include action on multiple receptors, slow dissociation of the receptor-antagonist complex, and allosteric modification of receptors (Wienen et al 1992). It remains unclear, however, how insurmountable antagonism can be displayed in isolated vessels and in-vivo by non-peptide AT_1 receptor antagonists, including KR-30988, for which antagonism was competitive in the binding study.

To characterize the nature of the insurmountable antagonism of KR-30988 further we performed interaction studies by incubating isolated tissue with this compound and losartan, a surmountable angiotensin AT_1 receptor antagonist (Wong et al 1990b). The KR-30988 (30 nM)-induced reduction in the maximum response to angiotensin II was abolished almost completely by addition of a high concentration (1 μM) of losartan. This finding was similar to those reported previously for other insurmountable angiotensin II antagonists (Wong & Timmermans 1991; Ojima et al 1997). These results suggest that most of the KR-30988 mole-

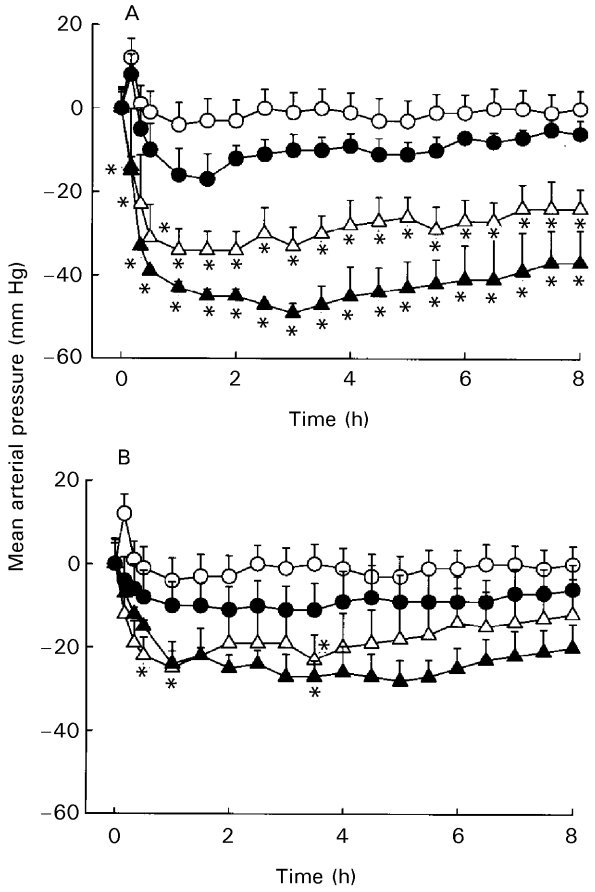

Figure 8. Effects of orally administered KR-30988 (A) (●, 1·0; △, 3·0; ▲, 10·0 mg kg^{-1} and ○, vehicle) and losartan (B) (●, 3·0; △, 10·0; ▲, 3·0 mg kg^{-1} and ○, vehicle) on mean arterial blood pressure in conscious furosemide-treated dog. The data points represent the mean percentage change from the control, ± s.e.m. (n = 4–7). *$P < 0.05$ compared with control.

cules bound to the receptors are replaced by losartan so that subsequently added angiotensin II can compete for the receptors occupied by losartan. Thus, KR-30988 and losartan might interact with the same binding sites, and it is unlikely that KR-30988 binds irreversibly to angiotensin II receptors.

KR-30988 was shown to be 20 times (approx.) more potent than losartan in blocking the pressor effect of angiotensin II in the pithed rat (ID50 0·09 and 1·74 mg kg^{-1} for KR-30988 and losartan, respectively). The selective interaction of KR-30988 with angiotensin II receptors was further substantiated by results from functional experiments demonstrating no effects of KR-30988 on the pressor response to norepinephrine and vasopressin, or on the heart rate response to isoproterenol in pithed rat.

In this study, the haemodynamic profile and the in-vivo potencies of KR-30988 and losartan were compared in animal models of hypertension in which the activated renin–angiotensin system was known to play an important role in the development and maintenance of blood pressure. In renal hypertensive rats, orally administered KR-30988 produced a dose-dependent antihypertensive effect with six times more potency than losartan (ED20 0·48 and 2·97 mg kg^{-1}, respectively). KR-30988 produced the maximum antihypertensive effect more rapidly than losartan, which exerted its maximum antihypertensive effect between 4 and 6 h after the dose with slow onset of action, mainly because of the in-vivo generation of the active metabolite EXP3174 after oral administration in this species (Wong et al 1990b). The antihypertensive effect of KR-30988 became much stronger 24 h after oral administration at higher doses. The findings from the renal hypertensive rats indicate that KR-30988 exerts long-lasting antihypertensive effects with a haemodynamic profile somewhat different from that of losartan, probably because of different absorption after oral administration, or different metabolism, or both.

The hypotensive effects of KR-30988 were further studied in furosemide-treated conscious dogs, another animal with a high plasma renin level (Wong et al 1991b), to compare the results with those from the rat model of hypertension. In this animal model the oral hypotensive effect of KR-30988 was approximately eight times stronger than that of losartan (ED20 1·04 and 7·96 mg kg^{-1}, respectively). Comparison of the results from renal hypertensive rats and furosemide-treated dogs showed that oral antihypertensive effects and haemodynamic profiles were similar (rapid arrival of E_{max} and long duration) for both species.

Despite the potent antihypertensive activity of KR-30988 in rats and dogs, reflex tachycardia was not observed (data not shown), whereas it is with other types of antihypertensive drug, including calcium-channel blockers and potassium-channel activators (Sorkin & Clissold 1987; Longman et al 1988). Although the lack of reflex tachycardia in the presence of reduced blood pressure is also observed with blockers of the renin–angiotensin system such as angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists (Cody 1984; Wong et al 1991a), the underlying mechanism is still unclear, although venous dilation, enhanced vagal tone or reduction of the sympathetic baroreceptor response may be involved (Cody 1984).

In summary, the results from this study show that KR-30988 is highly selective for AT_1 receptors and exerts insurmountable antagonism in functional studies and competitive antagonism in binding assays. In renal hypertensive rats, orally administered KR-30988 had significant long-lasting (> 24 h) antihypertensive effects; potency was six

times that of losartan and onset of action was more rapid. In furosemide-treated conscious dogs, orally administered KR-30988 was eight times (approx.) more potent than losartan at reducing blood pressure; duration of action was long—at least 8 h after the dose. These pharmacological profiles indicate that KR-30988 is a highly potent, orally active AT_1-selective receptor antagonist. Further studies are needed to evaluate the clinical therapeutic potential of this compound.

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