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In vivo pharmacological characterization of UP 269-6, a novel nonpeptide angiotensin II receptor antagonist

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Abstract

UP 269-6, 5-methyl-7-propyl-8-[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]-1,2,4-triazolo[1,5-*c*]pyrimidin-2(3*H*)-one is a novel nonpeptide angiotensin II receptor antagonist. In vivo studies were performed to evaluate UP 269-6 for its angiotensin II antagonistic action. In pithed rats, i.v. administration of UP 269-6 (0.03–1 mg/kg) shifted dose dependently to the right the dose-pressor response curve for angiotensin II and decreased the maximum response. The angiotensin II antagonistic effect of UP 269-6 was as potent as that of L-158,809 (5,7-dimethyl-2-ethyl-3-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-imidazo[4,5-*b*]pyridine) and 10 times more potent than that of losartan. UP 269-6 antagonized the angiotensin II sympathetic-mediated tachycardiac response. UP 269-6 inhibited dose dependently the pressor response to angiotensin II with an ID_{50} of 4.5 μg/kg, i.v. in conscious normotensive dogs. Oral administration of UP 269-6 (0.1 to 30 mg/kg) resulted in a dose-dependent and long-lasting inhibition of the angiotensin II-induced pressor response in conscious normotensive rats and dogs. Compared to losartan, UP 269-6 presented a more rapid onset of action. UP 269-6 caused similar angiotensin II antagonistic effects in rats and dogs but the duration of the effect was greater in dogs than in rats. UP 269-6 did not alter the tachycardiac response to isoproterenol and the pressor response to vasopressin. UP 269-6 was demonstrated to be devoid of agonistic properties in rats and dogs. Furthermore, UP 269-6 did not induce hypotension and did not cause alteration in heart rate and ECG waveforms in dogs even at a dose 1000 times higher than the angiotensin II antagonistic effective dose. These results demonstrate that UP 269-6 is a potent and specific angiotensin II receptor antagonist and does not possess agonistic properties.

Keywords: UP 269-6; Losartan; Angiotensin II; Angiotensin II receptor antagonist; Blood pressure; Heart rate

1. Introduction

The renin-angiotensin system consists of a cascade of enzymatic reactions leading to angiotensin II, a powerful vasoconstrictor. Angiotensin II is an octapeptide produced by the action of angiotensin-converting enzyme on angiotensin I. The renin-angiotensin system has been demonstrated to play an important role in the regulation of blood pressure and fluid volume homeostasis, and to be involved in the pathogenesis of many cardiovascular diseases (for reviews see Ganten et al., 1991; Hofbauer and Wood, 1986; Vallotton, 1987; Peach and Dostal, 1990). Blockade of the renin-angiotensin system with angiotensin-converting enzyme inhibitors has been shown to be clinically effective in the treatment of hypertension and congestive heart failure (Cody, 1986; Stumpe, 1987; Williams, 1988; Lavie et al., 1991). However, there is evidence to suggest that unwanted side effects of angiotensin-converting enzyme inhibitors such as cough or angioedema (Just, 1989; Coulter and Edwards, 1987; Parish and Miller, 1992; Roberts and Wuertz, 1991) result from the lack of specificity of angiotensin-converting enzyme for angiotensin I. Angiotensin-converting enzyme not only cleaves angiotensin I, but also hydrolyses bradykinin and substance P (Benjamin and Webb, 1990; Regoli and Barabé, 1980), and angiotensin-converting enzyme inhibitors affect the metabolism of these endogenous peptides in addition to that of angiotensin I. Blockade of the renin-angiotensin system by a selective receptor antagonist of angiotensin II would be expected to display a similar therapeutic profile to an angiotensin-

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converting enzyme inhibitor but might lack the undesirable side effects thought to be related to potentiation of bradykinin or substance P (McEwan and Fuller, 1989; Morice et al., 1989; Berkin, 1989; Thysell et al., 1988; Chin and Buchan, 1990). Furthermore, there is evidence that angiotensin II can be formed in vivo by the action of proteases other than angiotensin-converting enzyme (Dzau, 1989; Okamura et al., 1990). Thus, inhibition of angiotensin-converting enzyme may not be the most effective way to block the action of angiotensin II. Blockade at the angiotensin II receptors may be a direct and more specific approach to interrupt the renin-angiotensin system. Angiotensin II has been shown to interact with more than one receptor subtype (Chiu et al., 1989), and these subtypes have been named angiotensin AT_1 and AT_2 receptors (Bumpus et al., 1991). The angiotensin AT_1 receptor subtype has been identified as that which mediates the well-known effects of angiotensin II, such as contraction of vascular smooth muscle, aldosterone and adrenaline release, water intake and cellular proliferation (Criscione et al., 1990; Timmermans et al., 1991a; Chiu et al., 1991; Smith et al., 1992). The discovery by the DuPont group of the first orally active, nonpeptide angiotensin AT_1 receptor antagonist, losartan (DuP 753), has provided an important new tool to explore the physiological and pathophysiological role of angiotensin II. Losartan is the most clinically advanced nonpeptide angiotensin II receptor antagonist to date (Smith et al., 1992; Timmermans et al., 1993) and has been demonstrated to be an effective antihypertensive agent in human.

The purpose of this report was to evaluate the properties of a novel nonpeptide angiotensin AT_1 receptor antagonist, UP 269-6 (5-methyl-7-propyl-8-[(2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]-1,2,4-triazolo-[1,5-*c*]pyrimidin-2-(3*H*)-one). The structure of UP 269-6 is depicted in Fig. 1 (Nicolaï et al., 1994). The effects of UP 269-6 on blood pressure, heart rate, and the responses to angiotensin II, angiotensin III, and other agents were evaluated in the pithed rat, conscious rat, and conscious dog.

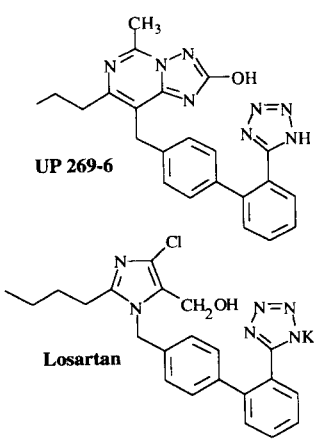

Fig. 1. Chemical structures of UP 269-6, 5-methyl-7-propyl-8-[(2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]-1,2,4-triazolo[1,5-*c*]pyrimidin-2-(3*H*)-one and losartan.

2. Materials and methods

2.1. Effects in anesthetized pithed rats

Male OFA Sprague-Dawley rats (290–320 g) (Iffa Credo, L'Arbresle, France) were anesthetized with sodium pentobarbitone (60 mg/kg, i.p.) following atropine (1 mg/kg, i.p.). A tracheotomy was performed and artificial ventilation with oxygen-enriched room air was started with an Edco volume-controlled rodent respirator (model 804, Phymep, Paris, France) at a frequency of 60 cycles/min with a volume of 1.4 ml/100 g body weight. A catheter was inserted into the left carotid artery and the left jugular vein for recording of arterial blood pressure and for intravenous drug administration, respectively. The arterial catheter was connected to a pressure transducer (Statham P23XL, Spectramed, Montigny-le-Bretonneux, France) coupled to a Gould 8400S polygraph (Gould Electronique, Ballainvilliers, France). Heart rate was derived from the arterial pulse pressure using a cardiotachometer (Biotach amplifier, Gould Electronique). Subsequently, the animals were pithed by inserting a steel rod (2 mm in diameter) via the orbit and the foramen magnum, and the pithing rod was driven down into the whole length of the spinal canal (Gillepsie and Muir, 1967). The vagus nerves were cut and the right carotid was tied. The animals were kept warm at 37°C by means of a thermostat-controlled heating board. Arterial blood pressure and heart rate were continuously recorded through the whole experiment.

In a first series of experiments, the i.v. potency of the angiotensin II receptor antagonistic effect of UP 269-6 was evaluated. After the surgery was completed, experiments were started after a 15–20-min period of equilibration. To construct the control dose-response curves, angiotensin II (0.003–3000 μg/kg) was administered intravenously. Each dose of angiotensin II was injected in a volume of 0.05 ml/300 g body weight followed by 0.15 ml of 0.9% saline. For the lower doses of angiotensin II (0.003–0.03 μg/kg), which induced an increase in diastolic blood pressure of less than 20 mmHg, full recovery of the response was permitted. For the higher doses (0.1–3000 μg/kg), angiotensin II was injected cumulatively with each successive injection being given immediately after the maximal effect of the preceding dose. Thereafter, sufficient time was allowed to ensure that blood pressure and heart rate

had completely returned to the control basal values. Drugs were intravenously administered 15 min before the initiation of the second angiotensin II dose-response curve. Groups of 6 rats received a single i.v. administration of vehicle, UP 269-6 (0.03, 0.1, 0.3 and 1 mg/kg), losartan (1, 3 and 10 mg/kg), L-158,809 (0.1 mg/kg) or propranolol (1 mg/kg). Each rat served as its own control.

Increases in diastolic pressure (Δ mmHg) and heart rate (Δ beats/min) were used to construct the angiotensin II dose-pressor response curve and the angiotensin II dose-heart rate response curve, respectively. 5 min after i.v. injection of the angiotensin II receptor antagonists, their effects on blood pressure and heart rate were measured.

In a second series of experiments, isoproterenol (0.001–10 μg/kg) was given intravenously in a cumulative manner to construct a dose-heart rate response curve for isoproterenol before and 15 min after i.v. administration of 1 mg/kg of UP 269-6 to ascertain the specificity of UP 269-6 effect.

2.2. Effects in conscious normotensive rats

One week before the experiment was conducted, male OFA Sprague-Dawley rats weighing 280–300 g were anesthetized with ketamine (Imalgène 500, Rhône-Mérieux, Lyon, France) (100 mg/kg, i.p.). Using standard aseptic methods, the right femoral artery and the left femoral vein were cannulated with polyethylene tube (Clay Adams, Rua, Marne-La-Vallée, France). The catheters were tunnelled under the dorsal skin and were exteriorized through a tether (Instech X 625 SS, Phymep) sutured to the neck muscles. When not in use, the vascular catheters were filled with an aqueous solution of polyvinylpyrrolidone (500 mg/ml) (Prolabo, Paris, France) and heparin (500 IU/ml), and closed with a metal plug. After recovery from surgery, the animals were housed individually in cages with free access to water and food for at least 1 week.

On the day of the experiment, rats were placed moving free into individual boxes in a quiet room. The free end of the arterial catheter was attached to a swivel-tether system which was connected to a Statham P23XL pressure transducer placed at the level of the animal's heart when in normal position for monitoring arterial pressure. Continuous perfusion (flow rate: 0.5 ml/h) via the arterial catheter with heparinized isotonic glucose solution (25 IU/ml in 5% glucose) prevented any clots. A floating polyethylene tubing was connected to the femoral venous catheter for i.v. injections. Arterial blood pressure was continuously monitored on a Beckman R411 polygraph (Sensormedic, The Netherlands) and the analogical signal was simultaneously converted into a digital signal by means of an on-line data acquisition system (Buxco Electronics, Sharon, CT, USA) and collected on a PC AT computer. From the instantaneous arterial pressure signal, systolic, diastolic and mean blood pressures and heart rate were calculated for each cardiac cycle. Blood pressure and heart rate values were then averaged for each 6-s period. At the end of a 60-min equilibration period, angiotensin II (150–200 ng/kg, 0.15–0.2 ml/rat) was rapidly injected i.v. to increase systolic blood pressure by at least 50 mmHg. Angiotensin II was administered twice at 10-min intervals to establish a reproducible control angiotensin II pressor response (Δ mmHg). After each injection, the catheter was rinsed twice with 0.2 ml of saline. Each rat was then treated with a single oral dose (1 ml/100 g via a feeding needle) of UP 269-6 (0.1, 0.3, 1, 3, 10 or 30 mg/kg), losartan (10 mg/kg) or vehicle ($n = 6$ per group). For the oral route, agents were given as an aqueous suspension (Tween 80 (5%), arabic gum, sodium chloride: 0.2 ml, 10 g, 1 g qs 1 l). Angiotensin II was subsequently injected at 15-min intervals for 1 h after oral administration of the angiotensin II receptor antagonist, then at 30-min intervals for the next 4 h. Angiotensin II challenges were also repeated 24 h after oral administration in rats treated with the highest dosages or the vehicle. In each daily experiment eight rats were tested simultaneously and the animals were randomly assigned to receive either vehicle, UP 269-6 or losartan. The angiotensin II pressor response after the administration of angiotensin II receptor antagonist was compared at each time to that obtained for the pretreatment control.

2.3. Effects in conscious normotensive dogs

Male Beagle dogs weighing 12–15 kg (Elevage Harlan, Gannat, France) were anesthetized with Propofol (8 mg/kg, i.v.) (Diprivan, I.C.I-Pharma, Cergy, France). After endotracheal intubation, animals were artificially ventilated using a volumetric respirator (Ohmeda, Coignières-Maurepas, France) and anesthesia was maintained by a mixture of 1.5–3% isoflurane (Forene, Abbott, Rungis, France), 60% nitrous oxide (CFPO, Bonneuil-Sur-Marne, France) and 40% oxygen (Air Liquide, Nanterre, France). Under aseptic conditions, the left carotid artery was dissected free from the surrounding tissues and a small piece of filamentous dacron tissue (USCI sauvage, Bard, Gentilly, France) was set around the artery over a length of 5–6 cm, so as to fully cover the dissected part of the artery. The skin was finally sewn over the dacron material, surrounding the vessel to provide a permanent carotid artery loop to allow direct recording of arterial blood pressure. Antibiotic, analgesic and anti-inflammatory therapies were given for 1 week after surgery. After recovery from the surgery, a minimum of 3 weeks was allowed to train dogs to stand quietly in a Pavlov sling

(Charles River, Saint Aubin-Lès-Elbeuf, France) for 6-h periods. Each dog was allowed to recover for at least 1 week after each test before being included in any other experiment. Dogs were fasted for 18 h prior to the experiment. The day of the experiment, a winged needle infusion set (Butterfly-R, Abbott) was placed into the cephalic vein for i.v. administrations. A catheter (Leader cath 115.09, Vygon, Ecouen, France) was percutaneously inserted into the carotid artery loop under local anesthesia and coupled to a Statham P23XL pressure transducer for direct recording of systolic and diastolic blood pressures. Heart rate was recorded using a cardiotachometer triggered by pulsatile arterial pressure waves. Hemodynamic parameters were continuously recorded on a Gould 8400S or Beckman R611 polygraph. The electrocardiogram (ECG) was derived from limb lead II and was recorded and analyzed using a special canine ECG analyzer (Fukuda M.E. Cardisuny 501 AX-D, O.C.E.M, Rambouillet, France). PR and QT intervals and QRS widths were automatically measured. The corrected QT (QTc) was calculated using Bazett's formula (Bazett, 1920): QTc = measured QT (ms)/$\sqrt{RR}$ (s).

In the first series of experiments, the i.v. potency and duration of angiotensin II antagonistic effects of UP 269-6 were studied. After an appropriate equilibration period, angiotensin II (0.1 μg/kg, 0.1 ml/kg) was injected i.v. twice at 10-min intervals. Changes (Δ mmHg) in systolic blood pressor response to angiotensin II challenges were used to establish a control angiotensin II pressor response. Angiotensin II was subsequently injected 10 min after each successive increasing i.v. dose of UP 269-6 or vehicle. Each dog received either UP 269-6 at 0.1, 0.3, 1, 3, 10, 30, 100, 300, 1000, 3000 and 10000 μg/kg as a bolus i.v. injection or vehicle administered in the same conditions ($n = 6$ per group). Each successive dose was injected at 30-min intervals in a volume of 0.1 ml/kg and 0.2 ml/kg for the highest dose. UP 269-6 was dissolved in 1 M phosphate buffer solution at an initial concentration of 50 mg/ml and UP 269-6 solutions were prepared just before use with further dilutions in distilled water. All solutions were used after filtration through a 0.22-μm filter (Single use filter units, Millex-GV millipore, Poly Labo, Strasbourg, France). Effects on hemodynamic and ECG parameters were evaluated 2, 9, 20 and 30 min after injection of each dose of UP 269-6 or vehicle and were always noted before angiotensin II injection and compared with the control pretreatment period.

In the second series of experiments, the p.o. potency and duration of the angiotensin II and angiotensin III antagonistic effects of UP 269-6 were examined and effects on the vasopressin pressor response were studied to assess the selectivity and specificity of UP 269-6 for the angiotensin II receptor. Angiotensin II (0.1 μg/kg), angiotensin III (0.2 μg/kg) and vasopressin (0.025 IU/kg) were sequentially injected i.v. before administration of a single oral dose of UP 269-6 (0.1, 0.3, 1, 3, 10, 30 mg/kg) or vehicle ($n = 6$ per group). UP 269-6 was given as a suspension as described above. Changes (Δ mmHg) in systolic blood pressure were used to establish control pressor responses to agonists. Angiotensin II was subsequently injected 30 min after drug administration and angiotensin II, angiotensin III and vasopressin were sequentially injected at various

Table 1
Basal diastolic pressure and heart rate values in pithed normotensive rats

Group		Basal diastolic pressure (mmHg)			Basal heart rate (beats/min)		
		Before control angiotensin II dose-response curve	Before 2nd angiotensin II dose-response curve		Before control angiotensin II dose-response curve	Before 2nd angiotensin II dose-response curve	
			Before drug i.v. administration	After drug i.v. administration		Before drug i.v. administration	After drug i.v. administration
Vehicle	0.1 ml/kg	45 ± 2	49 ± 3	51 ± 3	344 ± 9	342 ± 6	340 ± 7
UP 269-6	0.03 mg/kg	47 ± 2	48 ± 4	43 ± 3	338 ± 9	335 ± 12	334 ± 14
	0.1 mg/kg	43 ± 2	43 ± 4	35 ± 3 [a]	344 ± 17	343 ± 12	341 ± 12
	0.3 mg/kg	41 ± 2	43 ± 3	30 ± 2 [a]	350 ± 4	340 ± 5	342 ± 6
	1 mg/kg	45 ± 3	48 ± 8	31 ± 2 [a]	350 ± 9	350 ± 9	348 ± 9
Losartan	1 mg/kg	47 ± 3	51 ± 4	35 ± 3 [a]	351 ± 3	348 ± 9	343 ± 8
	3 mg/kg	44 ± 2	48 ± 3	32 ± 2 [a]	352 ± 8	362 ± 20	358 ± 22
	10 mg/kg	50 ± 2	52 ± 4	36 ± 4 [a]	339 ± 10	330 ± 11	328 ± 11
L-158,809	0.1 mg/kg	42 ± 4	45 ± 3	30 ± 1 [a]	374 ± 8	385 ± 6	365 ± 6

Hemodynamic parameters were measured before control and second angiotensin II dose-response curves were made. For the latter, values were measured before and 15 minutes after i.v. administration of angiotensin II receptor antagonists. Values are expressed as the means ± S.E.M. for six rats per group.

[a] Significant difference ($P < 0.05$) versus before drug i.v. administration.

times during the following 6 h (1, 2, 4 and 6 h), and in most of the groups, effects of vasopressor agonists were also tested 24 h after administration. Effects on hemodynamic and ECG parameters were evaluated 0.5, 1, 2, 3, 4, 5 and 6 h after oral administration and compared with the control pretreatment period. The ECG was recorded only for the vehicle-treated group and the 10 and 30 mg/kg UP 269-6-treated groups.

2.4. Statistics

Data are reported as means ± S.E.M. The statistical analysis was performed using Student's *t*-test for unpaired or paired observations, one-way analysis of variance and subsequently a Student's *t*-test with adjustment for multiple comparisons. ED_{50} values for angiotensin II (dose of angiotensin II required to produce a 50% maximum response) and 95% confidence limits were computed by a linear least-squares regression analysis. The dose-ratio of the ED_{50} values for angiotensin II obtained after and before test compound injection was calculated. Linear regression slope after treatment was compared to its own control with Student's *t*-test. The difference was considered to be significant when the *P* value was less than 0.05.

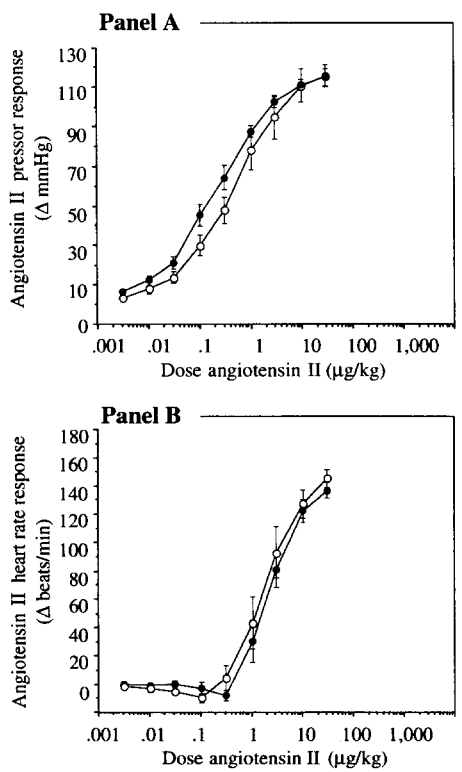

Fig. 2. Dose-pressor curves for angiotensin II (panel A) and dose-heart rate response curves for angiotensin II (panel B) in anesthetized pithed rats. Data are expressed as the means ± S.E.M. ($n = 6$). • First control dose-response curve for angiotensin II, ○ second dose-response curve for angiotensin II, after intravenous administration of vehicle.

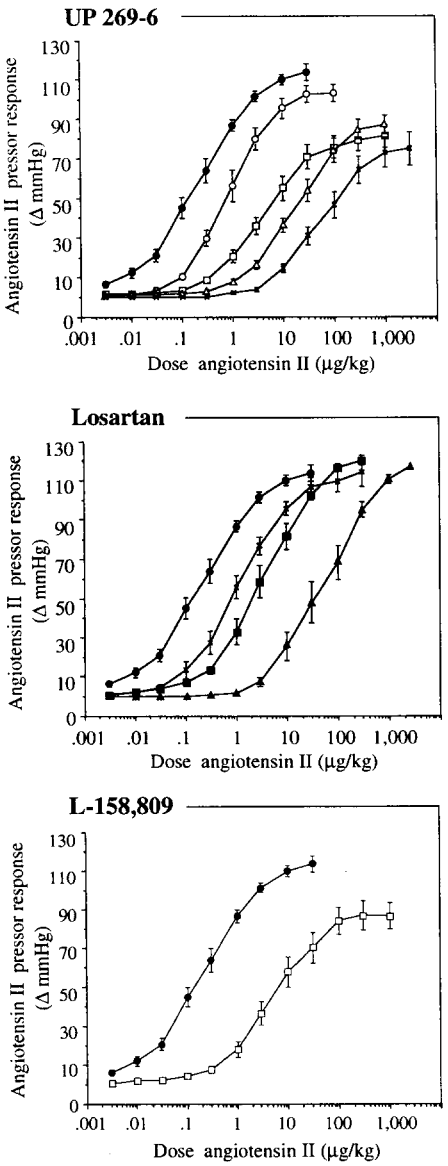

Fig. 3. Effects of UP 269-6, losartan and L-158,809, administered i.v., on the dose-pressor response curve for angiotensin II in anesthetized pithed rats. Data are expressed as the means ± S.E.M. ($n = 6$). • Vehicle, ○ UP 269-6 0.03 mg/kg, □ UP 269-6 or L-158,809 0.1 mg/kg, △ UP 269-6 0.3 mg/kg, × UP 269-6 or losartan 1 mg/kg, ■ losartan 3 mg/kg, ▲ losartan 10 mg/kg.

2.5. Drugs

UP 269-6, L-158,809 and losartan (DuP 753) were synthesized for pharmacological use as the free acid (UP 269-6 and L-158,809) and potassium salt (losartan) in the Research Chemistry Department of Carpibem (Rueil-Malmaison, France). Propranolol hydrochloride, isoproterenol hydrochloride, angiotensin III acetate salt and vasopressin ([Arg^8]vasopressin) aqueous solution were purchased from Sigma Chemical Co (distributed by Coger, Paris, France). Angiotensin II-amide (Val^5-angiotensin II-asp-β-amide: Hypertensin Ciba) was purchased from Ciba-Geigy (Basle, Switzerland).

Table 2
Effects of UP 269-6, losartan and L-158,809 on the pressor response to angiotensin II in anaesthetized pithed rat

Compound	Dose mg/kg	ED_{50} for angiotensin II μg/kg	95% confidence limits μg/kg	Maximum pressor response to angiotensin II Δ mmHg	ED_{50} ratio [b]	Parallel shift [c]
Vehicle	–	0.22	0.17– 0.28	114 ± 4	0.6	Yes
UP 269-6	0.03	0.91	0.70– 1.19	103 ± 4	3.1	Yes
	0.1	2.69	1.90– 3.81	79 ± 6 [a]	8.9	No
	0.3	15.21	11.81–19.58	87 ± 5 [a]	52.4	No
	1	47.54	32.94–68.62	75 ± 9 [a]	153.3	No
Losartan	1	1.11	0.87– 1.42	110 ± 5	3.4	Yes
	3	3.17	2.42– 4.13	110 ± 2	15.1	Yes
	10	52.82	39.12–71.84	118 ± 1	155.3	Yes
L-158,809	0.1	4.14	2.84– 6.03	81 ± 5 [a]	14.8	No

Values represent the dose (ED_{50}) of angiotensin II that induced 50% of the maximum pressor response obtained after treatment with vehicle or angiotensin II receptor antagonist. Maximum pressor responses to angiotensin II are expressed as the means ± S.E.M. ($n = 6$).
[a] Significant difference ($P < 0.05$) versus vehicle-treated group.
[b] ED_{50} ratio represents ED_{50} for angiotensin II calculated from the second angiotensin II dose-pressor response curve in the presence of the antagonist divided by ED_{50} for angiotensin II calculated from the first (control) angiotensin II dose-response curve in the absence of the antagonist.
[c] Parallel shift was determined by comparison of the linear regression slope with its own control (yes = parallel; no = nonparallel).

3. Results

3.1. Effects in pithed rats

Pithed, normotensive rats had an initial diastolic blood pressure and heart rate of 45.1 ± 0.9 mmHg and 346 ± 3 beats/min ($n = 48$), respectively. There were no significant differences in the baseline values between any of the groups. Table 1 indicates basal diastolic pressure and heart rate before control and second dose-response curves for angiotensin II. Before the second angiotensin II dose-response curve was made, hemodynamic parameters had completely returned to basal control values. The intravenous administration of vehicle or 0.03 mg/kg of UP 269-6 did not significantly affect diastolic blood pressure. Treatment with higher

Table 3
Hemodynamic effects of vehicle, UP 269-6 (0.1, 0.3, 1, 3, 10, 30 mg/kg) and losartan (10 mg/kg) p.o. in conscious normotensive rats

Group		Time after treatment (h)						
		0	0.5	1	2	3	5	24
		Mean blood pressure (mmHg)						
Vehicle	0.1 ml/kg	109 ± 2	103 ± 3	101 ± 4	98 ± 4	94 ± 3	91 ± 4	102 ± 3
UP 269-6	0.1 mg/kg	107 ± 2	103 ± 2	104 ± 3	100 ± 3	95 ± 2	91 ± 2	
	0.3 mg/kg	104 ± 2	97 ± 3	94 ± 5	94 ± 3	92 ± 3	87 ± 5	
	1 mg/kg	103 ± 2	100 ± 3	93 ± 2	90 ± 3	91 ± 3	88 ± 2	95 ± 4
	3 mg/kg	102 ± 2	95 ± 3	88 ± 2 [a]	87 ± 2	89 ± 1	85 ± 2	94 ± 3
	10 mg/kg	104 ± 1	97 ± 2	91 ± 3	87 ± 3	88 ± 4	83 ± 2	94 ± 3
	30 mg/kg	108 ± 2	100 ± 2	94 ± 5	93 ± 3	91 ± 4	87 ± 3	93 ± 3
Losartan	10 mg/kg	107 ± 2	103 ± 3	100 ± 4	97 ± 3	96 ± 3	87 ± 3	97 ± 5
		Heart rate (beats/min)						
Vehicle	0.1 ml/kg	381 ± 7	406 ± 6	419 ± 11	420 ± 11	419 ± 14	413 ± 10	383 ± 18
UP 269-6	0.1 mg/kg	391 ± 16	413 ± 12	436 ± 22	413 ± 21	417 ± 13	397 ± 17	
	0.3 mg/kg	384 ± 14	396 ± 22	408 ± 23	426 ± 20	404 ± 15	390 ± 13	
	1 mg/kg	365 ± 10	408 ± 15	397 ± 12	387 ± 13	390 ± 14	376 ± 15	355 ± 9
	3 mg/kg	370 ± 11	405 ± 14	393 ± 13	414 ± 17	420 ± 5	393 ± 13	383 ± 12
	10 mg/kg	394 ± 15	453 ± 16	442 ± 23	431 ± 18	432 ± 20	400 ± 20	408 ± 19
	30 mg/kg	405 ± 10	464 ± 19 [a]	454 ± 15 [a]	449 ± 18	435 ± 17	437 ± 7	395 ± 15
Losartan	10 mg/kg	380 ± 8	418 ± 10	398 ± 18	420 ± 7	426 ± 8	405 ± 4	410 ± 10

Values are expressed as the means ± S.E.M. for six to seven rats per group.
[a] Significant difference ($P < 0.05$) versus vehicle-treated group.

doses of UP 269-6, L-158,809 and losartan significantly lowered diastolic blood pressure. UP 269-6 decreased diastolic blood pressure by -8.5 ± 3.8, -13.0 ± 2.4 and -17.2 ± 2.7 mmHg at 0.1, 0.3 and 1 mg/kg, respectively. The reduction in diastolic blood pressure evoked by losartan was -16.5 ± 2.2, -15.8 ± 2.1 and -15.5 ± 4.0 mmHg at 1, 3 and 10 mg/kg, respectively. L-158,809 (0.1 mg/kg) caused a decrease in diastolic blood pressure by -15.2 ± 2.8 mmHg. Heart rate was not affected by any compounds at any doses used.

Cumulative administration of angiotensin II induced a dose-dependent increase in diastolic blood pressure and heart rate. As shown in Fig. 2, no tachyphylaxis to angiotensin II was observed when a second angiotensin II dose-response curve was made in our experimental conditions. The maximum increase in diastolic blood pressure and heart rate induced by angiotensin II was 114 ± 6 mmHg and 143 ± 6 beats/min, respectively, in the control period and 114 ± 4 mmHg and 135 ± 5 beats/min after vehicle administration. UP 269-6 shifted dose dependently the dose-pressor response curve for angiotensin II to the right and reduced the maximal pressor response to angiotensin II (Fig. 3, Table 2). UP 269-6 at 0.03 mg/kg caused a rightward significant shift of the dose-pressor response curve for angiotensin II in a parallel manner and induced a nonsignificant decrease of the angiotensin II maximal pressor response. The ratio of the ED_{50} values for angiotensin II obtained before and after 0.03 mg/kg i.v. of UP 269-6 was 3.1. Administered at 0.1, 0.3 and 1 mg/kg, UP 269-6 shifted the curve to the right in a nonparallel manner and significantly depressed the maximal pressor responses to angiotensin II by 24–34% compared to the angiotensin II maximal effect obtained after vehicle administration. The ratio of the ED_{50} values for angiotensin II was 8.9, 52.4, and 153.3, respectively. Losartan at 1, 3 and 10 mg/kg i.v. shifted dose dependently and significantly the dose-pressor response curve for angiotensin II to the right in a parallel manner without altering the maximum pressor response to angiotensin II (Fig. 3). The ratio of the ED_{50} values was 3.4, 15.1 and 155.3, respectively (Table 2). L-158,809 (0.1 mg/kg) elicited an equivalent antagonistic effect at the same dosage of UP 269-6 (ratio of ED_{50} values for angiotensin II: 14.8 versus 8.9, respectively) and similarly caused a nonparallel rightward shift of the dose-pressor response for angiotensin II. The maximal response to angiotensin II was significantly reduced by 29% (Fig. 3).

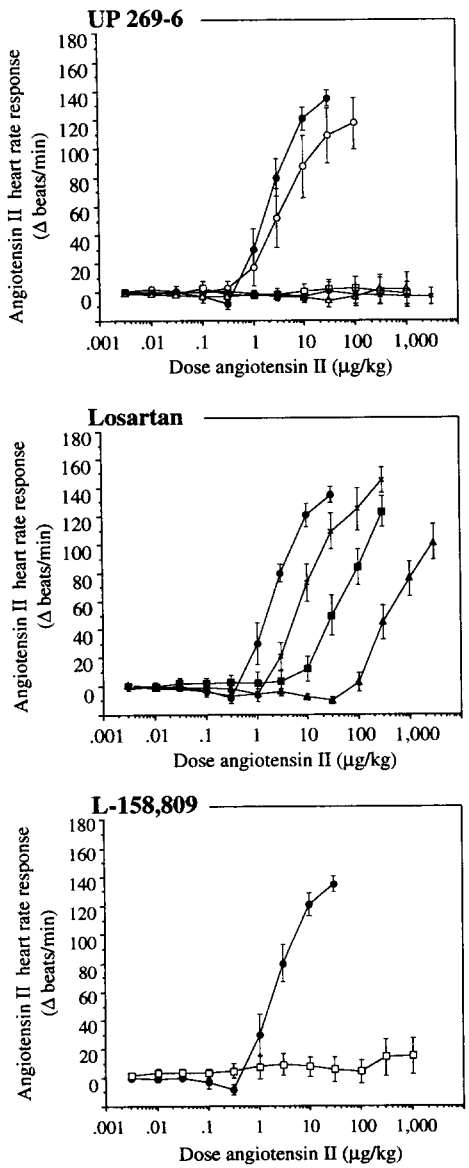

Fig. 4. Effects of UP 269-6, losartan and L-158,809, administered i.v., on the dose-heart rate response curve for angiotensin II in anesthetized pithed rats. Data are expressed as the means ± S.E.M. ($n = 6$). ● Vehicle, ○ UP 269-6 0.03 mg/kg, □ UP 269-6 or L-158,809 0.1 mg/kg, △ UP 269-6 0.3 mg/kg, × UP 269-6 or losartan 1 mg/kg, ■ losartan 3 mg/kg, ▲ losartan 10 mg/kg.

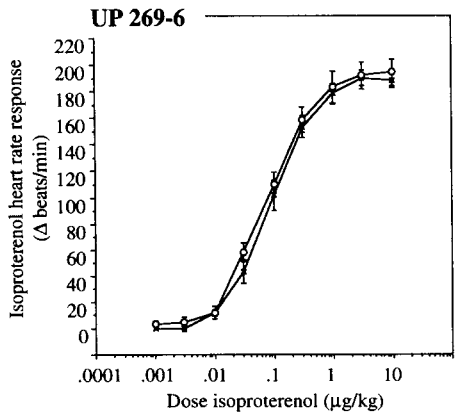

Fig. 5. Effects of UP 269-6, administered i.v., on the dose-heart rate response curve for isoproterenol in anesthetized pithed rats. Data are expressed as the means ± S.E.M. ($n = 6$). ○ Vehicle, × UP 269-6 1 mg/kg.

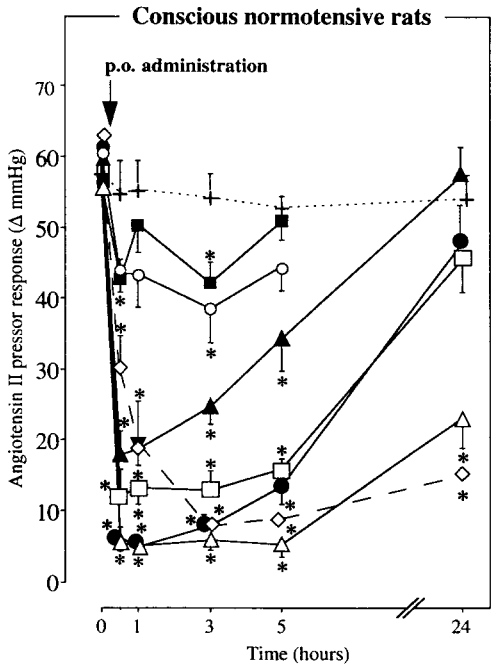

Fig. 6. Effects of vehicle, UP 269-6 (0.1, 0.3, 1, 3, 10 and 30 mg/kg, p.o.) and losartan (10 mg/kg, p.o.) on the pressor response to angiotensin II (150–200 ng/kg, i.v.) in conscious normotensive rats. Values are expressed as the means ± S.E.M. ($n = 6$). * Significant difference ($P < 0.05$) versus pretreatment values. + Control, ◇ losartan 10 mg/kg, ■ UP 269-6 0.1 mg/kg, ○ UP 269-6 0.3 mg/kg, ▲ UP 269-6 1 mg/kg, □ UP 269-6 3 mg/kg, ● UP 269-6 10 mg/kg, △ UP 269-6 30 mg/kg.

UP 269-6 at 0.03 mg/kg i.v. induced a small shift to the right of the angiotensin II dose-heart rate response curve, while the higher doses (0.1, 0.3 and 1 mg/kg) completely abolished the heart rate response to angiotensin II (Fig. 4). In contrast, losartan (1, 3 and 10 mg/kg) shifted dose dependently to the right in a parallel manner the dose-heart rate response curve for angiotensin II without altering the maximum heart rate response (Fig. 4). L-158,809, as observed with UP 269-6, elicited full inhibition of the heart rate response to angiotensin II (Fig. 4). The positive chronotropic response induced by angiotensin II was fully inhibited after blockade of cardiac β_1-adrenoceptors with 1 mg/kg, i.v. of propranolol. To exclude a direct β_1-adrenoceptor antagonistic effect of UP 269-6, a dose-heart rate response curve for isoproterenol was made before and after administration of 1 mg/kg of UP 269-6. UP 269-6, at a dose which fully inhibited angiotensin II pressor and heart rate responses, did not modify the positive chronotropic response induced by isoproterenol, indicating its lack of direct β_1-adrenoceptor blocking activity (Fig. 5).

3.2. Effects in conscious normotensive rats

Table 3 shows the effects of oral administration of UP 269-6 and losartan on blood pressure and heart rate in conscious normotensive rats. The oral administration of UP 269-6 induced a slight decrease in arterial blood pressure in a nondose-dependent manner. As compared to that of the vehicle-treated group, the hypotensive effect reached a significant level only with the dose of 3 mg/kg, 1 h after treatment. No significant change in heart rate was noted, except a transient rise observed from 0.5 to 1 h after administration of 30 mg/kg of UP 269-6. Losartan (10 mg/kg) did not significantly modify the basal hemodynamic parameters.

During the control period, the angiotensin II systolic blood pressure response was in a range of 55 ± 2 to 62 ± 3 mmHg in the different groups of rats. In vehicle-treated animals, this response was stable over the whole observation period (Fig. 6). UP 269-6 inhibited in a dose-related manner the pressor responses to angiotensin II (Fig. 6). The maximum percentages of inhibition were 26, 45, 70, 84, 94 and 94% after oral administration of 0.1, 0.3, 1, 3, 10 and 30 mg/kg, respectively. UP 269-6 at 0.1 and 0.3 mg/kg produced a significant inhibition of the angiotensin II pressor responses from 2 to 3 h and from 1.5 to 4.5 h after administration, respectively. UP 269-6 (1–30 mg/kg) inhibited significantly the pressor response to angiotensin II from 15 min up to 5 h after oral treatment. 24 h after oral administration, the angiotensin II pressor response was still inhibited by 23, 22 and 58% at 3, 10 and 30 mg/kg, respectively, and this inhibition was statistically significant for the highest dose. The an-

Table 4
Hemodynamic effects of vehicle and increasing intravenous doses of UP 269-6 in conscious normotensive dogs

Parameter	Group	Dose (mg/kg)											
		0	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
MAP (mmHg)	Vehicle	96 ± 4	94 ± 4	98 ± 5	98 ± 6	98 ± 4	100 ± 4	101 ± 5	102 ± 4	104 ± 5	106 ± 4	103 ± 6	109 ± 6
	UP 269-6	89 ± 5	91 ± 5	90 ± 5	89 ± 5	88 ± 7	90 ± 4	87 ± 5	88 ± 6	90 ± 5	89 ± 6	91 ± 6	90 ± 7
HR (beats/min)	Vehicle	80 ± 3	71 ± 4	78 ± 6	70 ± 6	68 ± 4	67 ± 4	71 ± 5	71 ± 5	76 ± 3	77 ± 9	76 ± 6	86 ± 11
	UP 269-6	77 ± 2	72 ± 5	65 ± 3	65 ± 4	66 ± 4	64 ± 3	67 ± 4	69 ± 4	63 ± 3	66 ± 3	69 ± 3	72 ± 7

Values are expressed as the means ± S.E.M. for six dogs. Measurements of mean arterial pressure (MAP) and heart rate (HR) were performed 20 min after each i.v. administration.

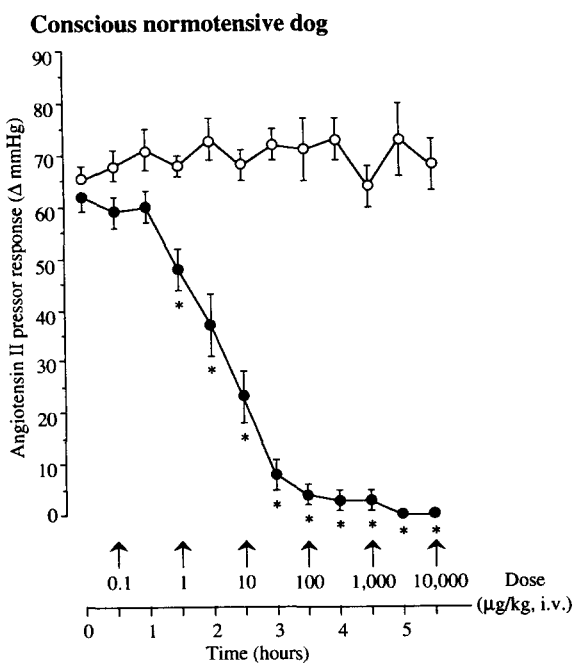

Fig. 7. Effects of UP 269-6 and vehicle on the pressor response to angiotensin II (100 ng/kg, i.v.) in conscious normotensive dogs. Arrows indicate intravenous administration of UP 269-6 (μg/kg) or vehicle (0.1 ml/kg or 0.2 ml/kg). Values are expressed as the means ± S.E.M. ($n = 6$). * Significant difference ($P < 0.05$) versus pretreatment values. ○ Vehicle, ● UP 269-6.

giotensin II inhibitory effect of losartan (10 mg/kg p.o.) had a clearly slower onset than that of UP 269-6 with a maximal blockade of 89%, 2 h after dosing (Fig. 6). A significant inhibition was still observed 24 h after drug administration (76% of angiotensin II control response).

3.3. Effects in conscious normotensive dogs

Consecutive intravenous administration of increasing doses of UP 269-6 (0.1–10000 μg/kg) had no effect on the baseline blood pressure in conscious dog (Table 4). Heart rate tended to decrease in the vehicle-treated group and in the UP 269-6-treated group during the observation period. These decreases in heart rate were slight and not dose-related. UP 269-6, at all doses tested, did not alter electrocardiographic parameters except the R–R-interval and the QT-interval, which were influenced by the heart rate decreases. There was no significant difference in the control pressor response to angiotensin II between vehicle- and UP 269-6-treated-groups. As shown in Fig. 7, pressor responses to angiotensin II were unaffected by successive administration of the vehicle. UP 269-6 inhibited dose dependently the angiotensin II pressor response. A significant inhibition was reached after intravenous administration of 1 μg/kg and the maximal inhibition was obtained between 30 and 10000 μg/kg (Fig. 7). The calculated i.v. ID_{50} was 4.5 μg/kg (3.1–6.7).

Administered at 0.1, 0.3, 1, 3, 10 and 30 mg/kg, p.o. in conscious normotensive dogs, UP 269-6 exerted no statistically significant effect on the hemodynamic (Table 5) and electrocardiographic parameters measured. The control systolic pressor responses induced by intravenous administration of angiotensin II, angiotensin III and vasopressin are presented in Table 6. Pressor responses to agonists were modified neither over the 6-h period nor 24 h after oral administration of vehicle. UP 269-6 administered orally caused a significant dose-related inhibitory effect on the angiotensin II

Table 5
Hemodynamic effects of vehicle and oral administration of UP 269-6 (0.1, 0.3, 1, 3, 10, 30 mg/kg) in conscious normotensive dogs

Group		Time after treatment (h)					
		0	0.5	1	3	6	24
		Mean blood pressure (mmHg)					
Vehicle	0.1 ml/kg	96 ± 4	91 ± 4	94 ± 4	92 ± 3	100 ± 4	98 ± 4
UP 269-6	0.1 mg/kg	98 ± 6	92 ± 6	97 ± 4	94 ± 5	96 ± 7	
	0.3 mg/kg	98 ± 4	95 ± 4	93 ± 6	93 ± 6	101 ± 6	
	1 mg/kg	91 ± 5	83 ± 4	81 ± 4	89 ± 5	91 ± 5	88 ± 6
	3 mg/kg	85 ± 4	79 ± 3	81 ± 4	82 ± 6	87 ± 3	81 ± 5
	10 mg/kg	96 ± 6	92 ± 6	88 ± 5	90 ± 5	93 ± 5	89 ± 5
	30 mg/kg	98 ± 3	92 ± 6	93 ± 4	88 ± 4	90 ± 4	98 ± 3
		Heart rate (beats/min)					
Vehicle	0.1 ml/kg	65 ± 3	66 ± 5	70 ± 5	64 ± 4	74 ± 6	74 ± 6
UP 269-6	0.1 mg/kg	75 ± 4	75 ± 5	74 ± 4	79 ± 8	72 ± 6	
	0.3 mg/kg	75 ± 3	77 ± 4	76 ± 7	73 ± 5	82 ± 6	
	1 mg/kg	69 ± 3	66 ± 2	66 ± 3	67 ± 3	75 ± 5	69 ± 3
	3 mg/kg	67 ± 1	66 ± 3	73 ± 5	69 ± 3	77 ± 2	74 ± 2
	10 mg/kg	69 ± 7	74 ± 7	71 ± 5	71 ± 4	70 ± 8	74 ± 5
	30 mg/kg	62 ± 3	69 ± 4	74 ± 5	66 ± 4	75 ± 6	81 ± 5

Values are expressed as the means ± S.E.M. for six dogs.

pressor response (Fig. 8). UP 269-6 at 0.1 and 0.3 mg/kg decreased significantly the pressor response to angiotensin II, and a 22 and 48% maximal inhibition of control angiotensin II responses was observed, respectively, 1 h after oral treatment. After oral administration of 1 mg/kg, the angiotensin II inhibition was sustained for at least 6 h and the angiotensin II pressor response was maximally decreased by 74%. UP 269-6 administered at 3, 10 and 30 mg/kg greatly inhibited the pressor response to angiotensin II by 86, 98 and 100%, respectively, and the angiotensin II receptor antagonistic effect lasted for 24 h (36, 59 and 52% of control angiotensin II pressor response, respectively).

In these experimental conditions, angiotensin III (0.2 μg/kg) was a vasoconstrictor agent as potent as angiotensin II (0.1 μg/kg). Pressor responses to angiotensin III were reproducible over the 6-h period and 24 h after dosing in the vehicle-treated group. UP 269-6 was at least equally potent in blocking the pressor responses to angiotensin II and angiotensin III (Fig. 8).

Pressor actions of vasopressin were also evaluated in this experiment in order to assess the specificity for the angiotensin II receptor of the tested compounds. Oral administration of UP 269-6 (0.1–30 mg/kg) did not modify vasopressin pressor responses.

4. Discussion

The results of these in vivo studies provide evidence that UP 269-6 is a potent, highly specific, and orally active angiotensin II receptor antagonist. Moreover, these data clearly demonstrate that UP 269-6 is devoid of partial agonistic properties.

In the anesthetized pithed rat, UP 269-6 at 0.1, 0.3 and 1 mg/kg i.v. caused nonparallel shifts to the right of the dose-pressor response curve for angiotensin II and reduced the maximum pressor response to angiotensin II, suggesting insurmountable antagonism (Wong et al., 1990a; Wong and Timmermans, 1991). L-158,809 (0.1 mg/kg) also exhibited a noncompetitive angiotensin II antagonism. In contrast, a rightward parallel shift of the angiotensin II dose-pressor response curve without reduction in the maximum response was observed in pithed rat treated with losartan. This pattern is consistent with a competitive and reversible interaction of losartan with the angiotensin AT_1 receptor as previously described (Wong and Timmermans, 1991). Compared to losartan, UP 269-6 was about 10 times more potent in blocking the pressor effect of angiotensin II in the pithed rat. UP 269-6 has also been demonstrated to be a noncompetitive antagonist of angiotensin II-induced contractions of rabbit aorta (Caussade et al., 1995).

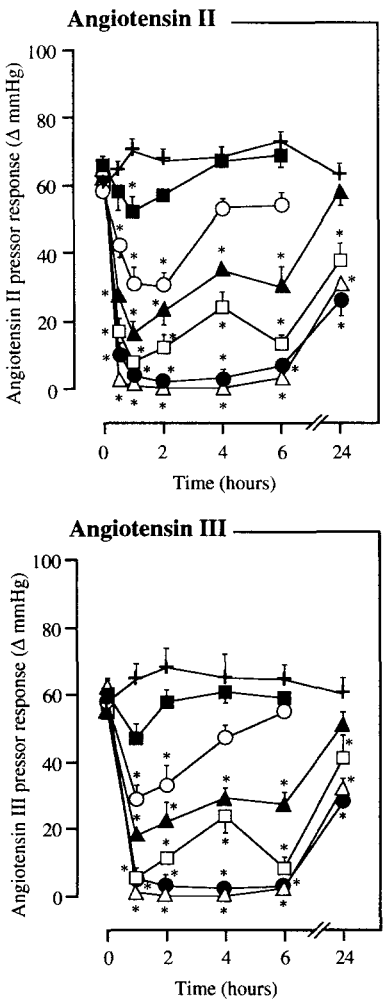

Fig. 8. Effects of vehicle and UP 269-6 (0.1, 0.3, 1, 3, 10 and 30 mg/kg, p.o.) on the pressor response to angiotensin II (100 ng/kg, i.v.) and to angiotensin III (200 ng/kg, i.v.) in conscious normotensive dogs. Values are expressed as the means ± S.E.M. ($n = 6$). * Significant difference ($P < 0.05$) versus pretreatment values. + Vehicle, ■ UP 269-6 0.1 mg/kg, ○ UP 269-6 0.3 mg/kg, ▲ UP 269-6 1 mg/kg, □ UP 269-6 3 mg/kg, ● UP 269-6 10 mg/kg, △ UP 269-6 30 mg/kg.

Table 6
Basal values of the pressor responses to angiotensin II, angiotensin III and vasopressin in conscious normotensive dogs

	Dose mg/kg	Angiotensin II 0.1 μg/kg Δ mmHg	Angiotensin III 0.2 μg/kg Δ mmHg	Vasopressin 0.025 IU/kg Δ mmHg
Vehicle	–	60 ± 3	58 ± 4	32 ± 2
UP 269-6	0.1	66 ± 3	60 ± 2	29 ± 7
	0.3	58 ± 1	58 ± 4	36 ± 5
	1	62 ± 3	55 ± 2	33 ± 4
	3	60 ± 3	55 ± 2	31 ± 2
	10	63 ± 3	58 ± 6	33 ± 4
	30	64 ± 3	62 ± 3	38 ± 3

Values are expressed as the means ± S.E.M. ($n = 6$). There was no significant difference in the pressor response induced by angiotensin II, angiotensin III or vasopressin among the different treated groups prior to the drug administration.

The angiotensin II chronotropic response may be attributed to angiotensin II-induced presynaptic facilitation of norepinephrine release, which subsequently activates cardiac β_1-adrenoceptors, since propranolol exerted a minimal inhibitory effect on the pressor response to angiotensin II and nearly completely inhibited the heart rate responses to angiotensin II in pithed rats, as previously reported (Zimmerman, 1981). Ganglionectomy greatly reduced the tachycardiac response to angiotensin II, demonstrating that angiotensin II has a presynaptic effect (Knappe and Van Zwieten, 1988; Zhang et al., 1993). Furthermore, attenuation of norepinephrine prejunctional reuptake (Starke, 1971), sensitization of postjunctional receptors to catecholamines (Clough et al., 1983) and release of catecholamines from the adrenal medulla (Wong et al., 1990c) may partly explain the angiotensin II chronotropic response. In our experimental conditions, UP 269-6 at 0.03 mg/kg shifted to the right the dose-tachycardiac response curve for angiotensin II. In contrast, UP 269-6 at higher doses (0.1–1 mg/kg) and L-158,809 (0.1 mg/kg) entirely suppressed the heart rate response to angiotensin II. Losartan (1–10 mg/kg, i.v.) failed to suppress completely the tachycardiac response to angiotensin II but shifted dose dependently to the right the angiotensin II dose-chronotropic response curve in a parallel manner, as previously reported (Wong et al., 1990c). The reason for the different qualitative inhibition of the angiotensin II-mediated sympathetic action between UP 269-6 or L-158,809 and losartan is not known and remains to be elucidated. These distinct sympathoinhibitory properties are probably not related to differences between angiotensin II antagonistic potency or hemodynamic responses exhibited since losartan (10 mg/kg) and UP 269-6 (1 mg/kg) were equipotent in inhibiting angiotensin II pressor responses and induced similar hemodynamic effects in the pithed rat. Furthermore, oral or intravenous administration of UP 269-6 in conscious rats and dogs did not modify heart rate as discussed below, indicating no interaction of UP 269-6 with the sympathetic nervous system of these intact preparations. Angiotensin II is known to increase heart rate through peripheral prejunctional facilitation of norepinephrine release but also probably through vagal tone withdrawal (Lumbers et al., 1979). Different effects of UP 269-6, L-158,809 and losartan on the possible angiotensin II-induced withdrawal of vagal tone can be ruled out since the present experiment was performed in atropine-pretreated pithed rats. A possible explanation of the discrepancy in the inhibition of the angiotensin II-induced tachycardiac response is that UP 269-6 possesses direct cardiac β_1-adrenoceptor antagonistic activity, but this is very unlikely because UP 269-6 (1 mg/kg, i.v.) did not modify the dose-heart rate response curve for isoproterenol. Another possibility is that the sympathoinhibitory effects of angiotensin II receptor antagonists may be not homogeneously distributed. Moreau et al. (1993) have shown that losartan exerts sympathoinhibitory effects against the vascular but not the chronotropic cardiac response to spinal cord stimulation in pithed spontaneously hypertensive rats, thereby demonstrating a difference in the sensitivity of prejunctional angiotensin II receptors at the cardiac and vascular level. Whether different functional prejunctional angiotensin II receptors exist at the cardiac level remains to be demonstrated. Whether noncompetitive (UP 269-6 and L-158,809) and competitive angiotensin II receptor antagonism (losartan) can explain the discrepancy in the inhibition of the angiotensin II-mediated tachycardia remains to be elucidated.

UP 269-6, L-158,809 and losartan lowered arterial blood pressure in the pithed rat, which can be probably attributed to the blockade of the vasoconstrictor effect of endogenous angiotensin II as plasma renin activity is high in the pithed rat (De Jonge et al., 1982; Wong et al., 1989, 1990b). Furthermore, these results indicate that UP 269-6 is devoid of angiotensin II agonistic properties in this model. UP 269-6 at all i.v. doses did not modify the baseline heart rate in pithed rats. Since the heart rate of the pithed and atropine-treated rat represents the intrinsic heart rate due to the absence of a functional autonomic nervous system in this model, our results suggest that UP 269-6 is devoid of direct myocardial effects.

In the conscious normotensive dog, increasing i.v. doses of UP 269-6 inhibited dose dependently the pressor response to angiotensin II with an ID_{50} of 0.0045 mg/kg. In similar experimental conditions, losartan, administered at 1, 3 and 10 mg/kg i.v, induced a dose-dependent inhibition of the angiotensin II pressor response with an ID_{50} of about 1 mg/kg (Wong et al., 1991). Compared to losartan, UP 269-6 was about a 200 times more potent angiotensin II receptor antagonist. Losartan generates a small amount of the active carboxylic acid metabolite EXP3174 in dogs, which may explain its weaker action in this species (Wong et al., 1991). Even at doses up to 10 mg/kg i.v., a dose 2000 times higher than the ID_{50} for the angiotensin II pressor response, UP 269-6 did not increase arterial blood pressure in the conscious dog, confirming that UP 269-6 is devoid of angiotensin II receptor agonistic properties.

In addition, UP 269-6 did not induce hypotension and did not interfere with cardiac electrical conductance since, even up to 10 mg/kg i.v., UP 269-6 caused no alterations in ECG waveforms and heart rate in the conscious normotensive dog. A slight decrease in heart rate was observed in all animals during the 6-h observation period and this decrease may be related to the experimental conditions since no difference between vehicle- and UP 269-6-treated groups was found.

The potency and duration of the angiotensin II antagonistic effects of UP 269-6 were investigated after oral administration in conscious rats and dogs. In both species, UP 269-6 antagonized dose dependently the angiotensin II-induced increase in blood pressure and the angiotensin II antagonistic effect of UP 269-6 was very fast and long lasting.

In the conscious normotensive rat, UP 269-6 (0.1–30 mg/kg, p.o.) induced a significant dose-related inhibition of the pressor response to angiotensin II. Administered at 10 mg/kg, UP 269-6 and losartan showed similar angiotensin II antagonistic potency. Compared to losartan, UP 269-6 presented a more rapid onset of action and its maximum inhibitory effect was reached 15–30 min after oral administration whereas the maximum angiotensin II antagonistic effect of losartan developed between 2 and 3 h after administration. This latency in the biological response to losartan in rats may be attributed to the in vivo generation of the active metabolite EXP3174 after oral administration of losartan in this species (Wong et al., 1990a). The high potency and long duration of action of EXP3174 suggest that it may be responsible for part of the angiotensin II receptor antagonistic effect of losartan in rats (Timmermans et al., 1991b). UP 269-6 at 30 mg/kg inhibited significantly the angiotensin II pressor response 24 h after oral administration, evidencing the long duration of action of the angiotensin II receptor antagonistic properties of this compound.

Administered orally in dogs, UP 269-6 (0.1–30 mg/kg) induced a dose-related significant inhibition of angiotensin II pressor responses. UP 269-6 presented a rapid onset of action and its maximum effect was reached approximately 1 h after dosing. UP 269-6 (3–30 mg/kg) maximally inhibited the angiotensin II-induced blood pressure increase and the angiotensin II pressor response was still significantly inhibited 24 h after treatment.

The results of the present study demonstrate that UP 269-6 administered orally possesses similar angiotensin II antagonistic activity in rats and dogs but the duration of the angiotensin II antagonistic effect of UP 269-6 is greater in dogs than in rats. UP 269-6 inhibited the pressor response to angiotensin II with an ID_{50} of 0.0045 mg/kg, i.v. and approximately 0.3 mg/kg p.o. in conscious dogs. The difference between these ID_{50} values may be due to the poor oral resorption of UP 269-6 since these data are in accordance with the absolute bioavailibity (20–26%) determined during pharmacokinetic studies with dogs.

Angiotensin III is a heptapeptide metabolite of angiotensin II. Although angiotensin III is less potent than angiotensin II as a vasoconstrictor, it was of interest to examine whether UP 269-6 was able to antagonize the pressor effect of angiotensin III in a similar degree as it did the angiotensin II pressor response. With a dose of angiotensin III (0.2 μg/kg, i.v.) that induced a vasopressor effect equipotent to that observed after administration of angiotensin II (0.1 μg/kg), UP 269-6 was shown to be at least equally potent in blocking the pressor responses to angiotensin II and angiotensin III in conscious dogs.

Pressor actions of vasopressin were also evaluated in conscious normotensive dogs in order to assess the specificity of the angiotensin II receptor antagonist. UP 269-6 was devoid of inhibitory effect on pressor response to vasopressin, evidencing its specificity of action. The specificity of UP 269-6 for angiotensin II receptors was evidenced in many radioligand binding assays and isolated aortic preparations, demonstrating that UP 269-6 lacked any affinity or activity (IC_{50} > 10000 nM) for peptide and nonpeptide receptors, ion channels and uptake sites relevant for cardiovascular regulation (Caussade et al., 1995).

UP 269-6, up to 30 mg/kg, did not induce a modification of blood pressure or alterations in ECG waveforms and heart rate in the conscious normotensive dog. Thus, following oral administration, UP 269-6 is devoid of agonistic properties.

In conclusion, the present results indicate that UP 269-6 is a highly potent and specific noncompetitive angiotensin AT_1 receptor antagonist and is orally active in rats and dogs. Furthermore, UP 269-6 has a long-acting angiotensin II antagonistic activity and is devoid of angiotensin II agonistic property.

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