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Effects of irbesartan (SR 47436/BMS-186295) on angiotensin II-induced pressor responses in the pithed rat: potential mechanisms of action

Bernard Christophe [1], René Libon, Catherine Cazaubon *, Dino Nisato, Allan Manning, Pierre Chatelain

SANOFI Recherche, 371 Rue du Professeur J. Blayac, 34184 Montpellier Cedex 4, France

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Abstract

The effects of two new non-peptide angiotensin receptor antagonists, irbesartan (SR 47436/BMS-186295, (2-*n*-butyl-4-spirocyclopentane-1-[((2'-tetrazol-5-yl)biphenyl-4-yl)methyl]2-imidazolin-5-one) and SR 47155A (2-*n*-butyl-4-spirocyclopentane-1-[((2'-carboxy)biphenyl-4-yl)methyl]-2-imidazolin-5-one, trifluoroacetate), on angiotensin II-induced pressor responses were studied in the pithed rat in comparison to losartan, EXP 3174 and [Sar^1,Val^5,Ala^8]angiotensin II. SR 47155A (1–10 mg/kg i.v.) and losartan (1–10 mg/kg i.v.) shifted dose dependently the dose-response curve of angiotensin II to the right without affecting the maximal response. SR 47436 (0.3–10 mg/kg i.v.), EXP 3174 (0.03–1 mg/kg i.v.) and [Sar^1,Val^5,Ala^8]angiotensin II (0.03–1 mg/kg i.v.) induced, at least at high doses, a non-parallel shift to the right of the angiotensin II dose-response curve and this was associated with a reduction of the maximal response. During a 70 min period, the effect of [Sar^1,Val^5,Ala^8]angiotensin II (1 mg/kg i.v.) on the angiotensin II (0.3 μg/kg i.v.)-induced pressor response was shown to be reversible, the effect of SR 47155A (10 mg/kg i.v.) was partially reversible and the effect of SR 47436 (3 mg/kg i.v.), EXP 3174 (1 mg/kg i.v.) or losartan (6 mg/kg i.v.) was not reversed at the end of this 70 min period. Administration of SR 47155A (10 mg/kg i.v.) before SR 47436 (1–10 mg/kg i.v.) reversed the reduced angiotensin II-maximal response induced by SR 47436. Administration of SR 47436 (10 mg/kg i.v.) before SR 47155A (1–10 mg/kg i.v.) prevented the full development of the pressor response as observed in the absence of SR 47436. In the pithed rat, SR 47436 (30 mg/kg i.v.) and losartan (30 mg/kg i.v.) reduced the change in diastolic blood pressure induced by electrical stimulation of the spinal cord only at low stimulation rates. Taken together these results indicate that SR 47436, under in vivo conditions, is a potent non-peptide angiotensin receptor antagonist. The type of antagonism (partially insurmountable but selective) can be explained by different theoretical models which are discussed.

Keywords: Irbesartan (SR 47436/BMS-186295); SR 47155A; Losartan; EXP 3174; Pithed rat; Angiotensin II

1. Introduction

The renin-angiotensin system is one of the hormonal systems involved in the regulation of blood pressure. The primary active peptide hormone of this system, angiotensin II, is a powerful peripheral vasoconstrictor. Use of specific antagonists of the angiotensin receptor has lead to a better understanding of the physiological role of this peptide and practical importance of angiotensin receptor antagonists in various pathological situations such as hypertension or heart failure. The limited therapeutic utility of peptide antagonists such as saralasin or sarmesin has lead to the synthesis of specific, potent, long-acting and orally active non-peptide angiotensin receptor antagonists without any agonistic properties (Hodges et al., 1992; Timmermans et al., 1991, 1992, 1993; Van Meel et al., 1993). Among these compounds, it has now become clear that there are two classes of antagonists of the angiotensin AT_1 receptor: surmountable, competitive antagonists (such

* Corresponding author.

[1] Present address: Bio-Pharma – Simon, 10, Vieux Chemin du poète, B-1301 Wavre, Belgium (Tel. +32 10 421 111, fax +32 10 421 100).

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as S-8307 (Wong et al., 1988), losartan (Wong et al., 1990b), SK&F 108566 (Edwards et al., 1992), BIBS 39 (Zhang et al., 1992), SC-51316 (Olins et al., 1992), EXP6155 (Wong et al., 1989), EXP 6803 (Wong et al., 1989) and E4177 (Okunishi et al., 1993)) and insurmountable, but selective antagonists (such as DuP 532 (Wong et al., 1991), EXP 3892 (Wong and Timmermans, 1991), EXP 3174 (Wong et al., 1990a) BIBS 222 (Zhang et al., 1992), L-158,809 (Chang et al., 1992), GR 117289 (Robertson et al., 1992), TCV-116 (Shibouta et al., 1993), CV-11974 (Noda et al., 1993), BIBR 277 (Wienen et al., 1993) and CGP 48933 (Criscione et al., 1993)). The mechanism of action linked to these different classes of angiotensin receptor antagonists is not known.

Irbesartan (SR 47436/BMS-186295) is the lead compound of a new series of imidazolones which are highly specific and potent non-peptide angiotensin AT_1 receptor antagonists (Cazaubon et al., 1993; Bernhart et al., 1993). In the present study, we have assessed the effects of this inhibitor on angiotensin II-induced pressor responses in the pithed rat and we have tried to elucidate the mechanism of action of this antagonism. This study was conducted in comparison to other non-peptide angiotensin receptor antagonists such as SR 47155A, an analogue of SR 47436 (Bernhart et al., 1993), losartan, EXP 3174 and a peptide angiotensin receptor antagonist [Sar^1,Val^5,Ala^8]angiotensin II. Although definitely an in vivo preparation, as the spinal cord is destroyed, the pithed rat preparation (Shipley and Tilden, 1947) is devoid of neurogenic reflex control that might otherwise modulate the primary drug effect. Thus, this preparation gives an accurate assessment of the direct effects of drugs on the cardiovascular system under in vivo conditions.

2. Materials and methods

Normotensive male Wistar Janssen rats (approximatively 300 g body weight) were anaesthetized with pentobarbital sodium (60 mg/kg i.p.). After tracheal intubation, rats were artificially ventilated with room air using a respirator at a tidal volume of 2 ml/100 g and at a rate of 60 strokes/min. Body temperature was maintained at 37–38° C by a thermostatic heating system.

The left carotid artery and the dorsal vein of the penis were cannulated respectively for blood pressure measurement, and i.v. injection of drugs. Diastolic blood pressure was measured via a catheter attached to a pressure transducer coupled to a polygraph. Rats were pithed (Shipley and Tilden, 1947) through the orbit with a steel rod (diameter of 2 mm).

A total of 296 rats were used with 5–11 animals per group.

2.1. Angiotensin II antagonism

To test angiotensin II antagonism, the preparation was allowed to stabilize for 15 min after pithing and before i.v. administration of NaCl (0.9%) or the drug to be tested (SR 47436, SR 47155A, losartan, EXP 3174 or [Sar^1,Val^5,Ala^8]angiotensin II). After an incubation period of 15 min, a dose-pressor response curve was obtained for angiotensin II (0.003–300 μg/kg i.v.). For the lower doses of angiotensin II (those which elicited less than a 20 mm Hg rise in diastolic blood pressure) full recovery was permitted. For higher doses, angiotensin II was injected cumulatively with each successive injection given immediately after the maximal effect of the preceding dose (usually 10–20 s). Only one full dose-response curve was obtained with each animal.

2.2. Duration of angiotensin II antagonism

To determinate the duration of the drugs antagonistic effect, the preparation was allowed to stabilize for 15 min after pithing and before i.v. administration of angiotensin II 0.3 μg/kg i.v. (dose which induced at least 50% of the maximal response). This response was considered as the reference response. 10 min later, NaCl (0.9%) or the drug to be tested (SR 47436, SR 47155A, losartan, EXP 3174 or [Sar^1,Val^5, Ala^8]angiotensin II) was injected i.v. After 10 min, angiotensin II was injected 7 times every 10 min. The pressor response amplitude was measured after each injection in order to observe the duration of the effect of the different antagonists for a period of 70 min.

2.3. Interaction between SR 47436 and SR 47155A

To study the effect of a putative interaction between SR 47436 and SR 47155A on the dose-response curve for angiotensin II, the preparation was allowed to stabilize for 15 min before i.v. administration of NaCl (0.9%) or SR 47155A (10 mg/kg i.v.). 10 min later, NaCl (0.9%) or SR 47436 (10, 20 or 30 mg/kg i.v.) was injected i.v. After an incubation of 10 min, a dose-pressor response curve was constructed for angiotensin II in the same way as with group 1. In an other group, the injection order was reversed: SR 47436 (10 mg/kg i.v.) was first administered and afterwards SR 47155A (1, 3 or 10 mg/kg i.v.). Minimal active doses were chosen under these experimental conditions.

2.4. Effect of SR 47436 and losartan on spinal cord stimulation-induced pressor responses

Atropine (1 mg/kg i.v.) and *d*-tubocurarine (5 mg/kg i.v.) were administered to bivagotomized pithed

rats in order to block the parasympathetic system and to prevent skeletal muscle contraction. The pithing steel rod served as the stimulating electrode. A second steel rod, used as the neutral electrode, was inserted under the dorsal skin, parallel to the vertebral column. The spinal cord was stimulated electrically for 60 s every 5 min at 0.04–20 Hz with a rectangular pulse of 5 ms and 50 V with a stimulator coupled to an isolation unit. After allowing diastolic blood pressure to stabilize, a frequency-pressor response curve was constructed. SR 47436 or losartan (30 mg/kg i.v., maximal tested doses on the post-synaptic receptor) was then administered. 15 min later, a second frequency-pressor response curve was constructed.

2.5. Data analysis

Pressor responses were assessed by changes in diastolic blood pressure expressed in mm Hg. Since higher doses of angiotensin II induced a pressor response lower than the observed maximal effect, experimental data were adjusted by an iterative computed program fitting experimental data to the theoretical curve describing the autoinhibition activity of a drug (Ariëns et al., 1964). Adjustment of experimental data to the theoretical curve was carried out using the Gauss-Newton-Marquardt method for a non-linear model with several variables (Valkó and Vajda, 1989). The quality of the adjustment was verified by the absence of statistically significant differences between the observed results and the results calculated in accordance with the model. Maximal effect (E_{max}) and dose of the drug inducing an effect equal to 50% of the maximal effect induced by the drug (ED_{50}) were determined from the results of this fitting program.

Results were expressed as means ± S.E.M. Comparison between two means was made using a Student's *t*-test for paired variables. Comparison between several means was made using a *F*-test (variance analysis with one classification parameter).

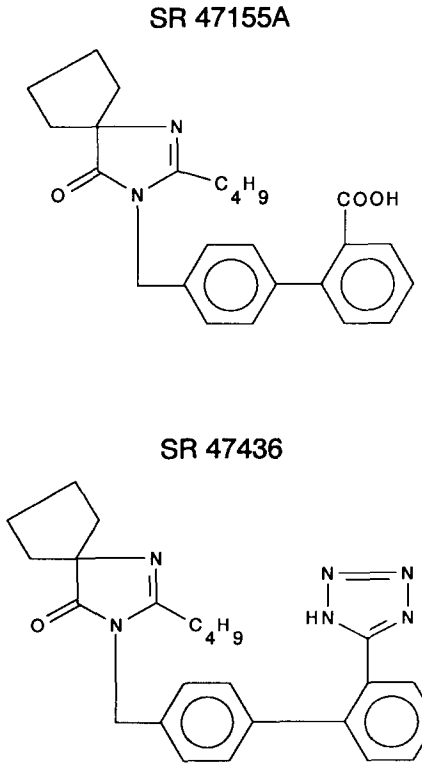


Fig. 1. Structure of SR 47436 and of SR 47155A.

Table 1
Affinities for angiotensin AT_1 receptor antagonists measured on binding studies and on rabbit aorta according to the methods described by Cazaubon et al. (1993).

	IC_{50} (nM)	
	Rat liver	Rabbit aorta
SR 47436	1.3	4.0
SR47155A	10.0	63.0
Losartan	14.0	26.0
EXP 3174	2.0	0.4
Saralasin	2.4	2.7

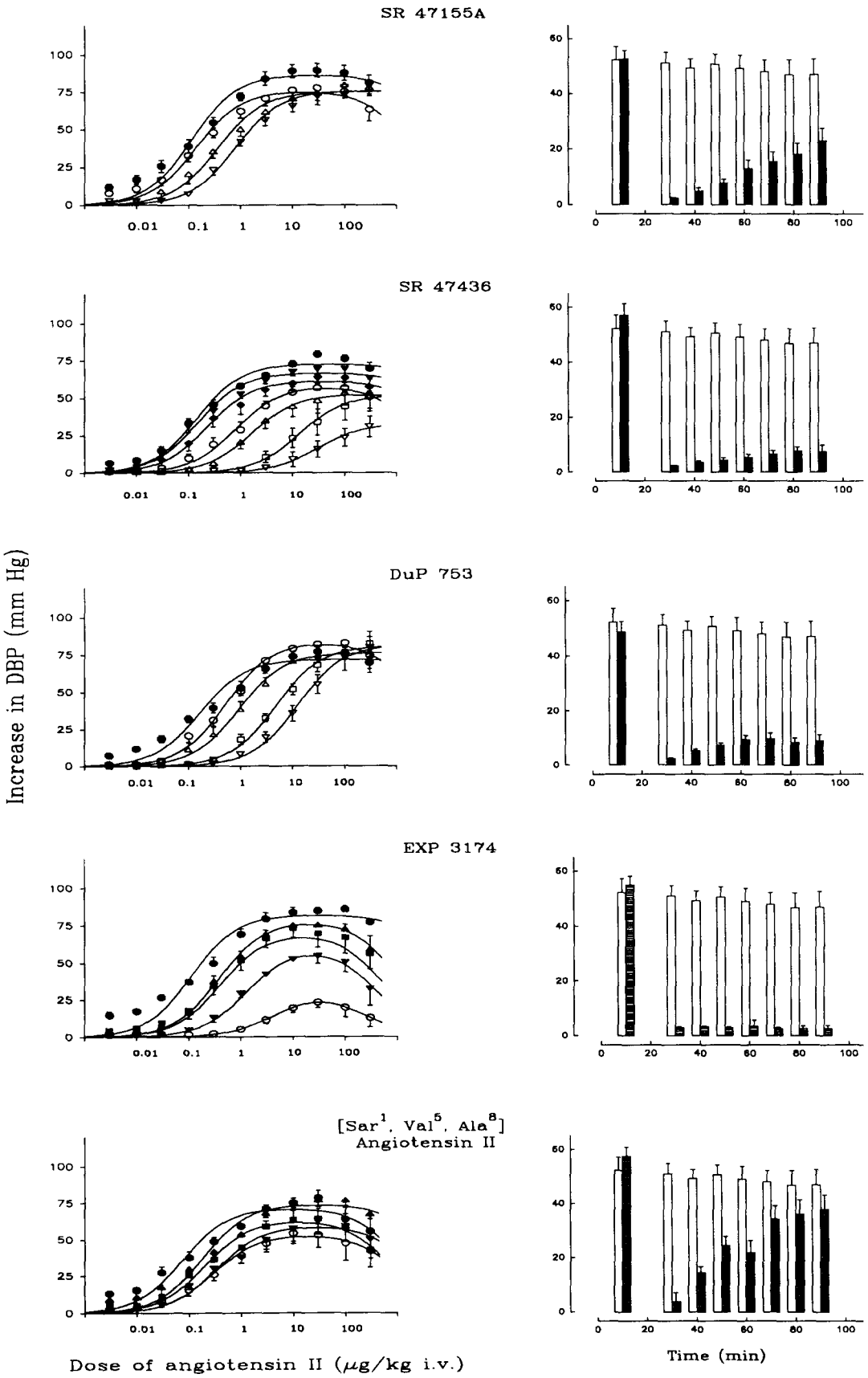
2.6. Drugs used

The following drugs were used: SR 47436 (2-*n*-butyl-4-spirocyclopentane-1-[((2′-tetrazol-5-yl)biphenyl-4-yl)-methyl]-2-imidazolin-5-one, Fig. 1), SR 47155A (2-*n*-butyl-4-spirocyclopentane-1-[((2′-carboxy)biphenyl-4-yl)methyl]-2-imidazolin-5-one,trifluoroacetate, Fig. 1), EXP 3174, losartan (all synthetized in the Chemistry Department of Sanofi Research, Montpellier and dissolved in water with free base arginine), [Sar^1, Val^5,Ala^8]angiotensin II (saralasin) and angiotensin II (Sigma, dissolved in water). The dose-response curve for angiotensin II was not modified by the presence of 12.5 mg/kg i.v. free base arginine (data not shown). Drugs were always injected at a constant volume of 0.5 ml/kg i.v. Choose of the doses to be used is based on the affinities of these drugs for angiotensin AT_1 receptors in binding studies and on rabbit aorta (Guiraudou et al., 1993; Bernhart et al., 1993; Cazaubon et al., 1993). These values are described in Table 1.

3. Results

3.1. Effect on basal pressure

After pithing, diastolic blood pressure (DBP) measured in the rat was 41.0 ± 0.4 mm Hg ($n = 296$). Injection of SR 47436 (0.3–10 mg/kg i.v.), SR 47155A


SR 47155A
SR 47436
DuP 753
EXP 3174
[Sar¹, Val⁵, Ala⁸] Angiotensin II
Increase in DBP (mm Hg)
Dose of angiotensin II (μg/kg i.v.)
Time (min)

(1–10 mg/kg i.v.), EXP 3174 (0.03–1 mg/kg i.v.) or losartan (1–10 mg/kg i.v.) induced no increase in DBP. This effect contrasted with the 20–30 mm Hg DBP phasic increase induced by injection of 0.03–1 mg/kg i.v [Sar^1,Val^5,Ala^8]angiotensin II. 15 min after the injection of [Sar^1,Val^5,Ala^8]angiotensin II, DBP returned to the control value. SR 47436 (at doses $\geq$ 3 mg/kg i.v.), losartan (at doses $\geq$ 6 mg/kg i.v.), EXP 3174 (at doses $\geq$ 1 mg/kg i.v.) and SR 47155A (at doses $\geq$ 10 mg/kg i.v.) induced a 7–10 mm Hg diastolic basal blood pressure decrease observed 15 min after the injection.

3.2. Effect on the angiotensin II dose-response curve

In the pithed rat, angiotensin II dose dependently increased DBP. The maximal pressor response observed under our experimental conditions was 79.0 $\pm$ 1.6 mm Hg ($n = 48$). The half maximal effect was observed at 0.207 $\pm$ 0.036 μg/kg i.v. ($n = 48$).

SR 47436 dose dependently inhibited the pressor response to angiotensin II. Increasing doses of SR 47436 caused a rightward shift of the angiotensin II dose-pressor response curve with a reduction of the maximum response (Fig. 2). This shift was parallel at doses lesser than 3 mg/kg i.v. and non-parallel at doses higher than 3 mg/kg i.v. The curve was shifted by a factor of 0.1, 2.3, 5.7, 12.0, 122.6 and 449.4, respectively, for 0.3, 0.6, 1, 3, 6 and 10 mg/kg i.v. At the same time, the maximum response was inhibited by 8.6, 14.0, 22.7, 27.7, 26.8 and 49.6%.

This insurmountable effect of SR 47436 contrasted with the effect of SR 47155A and losartan which dose dependently inhibited the pressor response to angiotensin II. Increasing doses of both drugs reduced neither the maximal amplitude nor the slope of angiotensin II dose-pressor response curve (Fig. 2). However, both drugs shifted the angiotensin II dose-pressor response curve to the right in a parallel manner, suggesting competitive antagonism. For SR 47155A the curve was shifted by a factor of 1.1, 3.1 and 6.7, respectively, for 1, 3 and 10 mg/kg i.v. For losartan the curve was shifted by a factor of 1.6, 3.4, 26.5 and 35.5 respectively for 1, 3, 6 and 10 mg/kg i.v.

EXP 3174 dose dependently inhibited the pressor response to angiotensin II. Increasing doses of EXP 3174 caused a non-parallel, rightward shift of the angiotensin II dose-pressor response curve with a reduction of the maximum response (Fig. 2). The curve was shifted by a factor of 3.1, 4.3, 10.7 and 23.2, respectively, for 0.03, 0.1, 0.3 and 1 mg/kg i.v. At the same time, the maximum response was inhibited by 7.2, 15.3, 30.7 and 70.3%.

[Sar^1,Val^5,Ala^8]angiotensin II dose dependently inhibited the pressor response to angiotensin II. Increasing doses of [Sar^1,Val^5,Ala^8]angiotensin II caused a non-parallel, rightward shift of the angiotensin II dose-pressor response curve with a reduction of the maximum response (Fig. 2). The curves were shifted by a factor of 1.8, 2.4, 3.4 and 2.8, respectively, for 0.03, 0.1, 0.3 and 1 mg/kg i.v. At the same time, the maximum response was inhibited by 0.4, 13.1, 18.3 and 26.3%.

3.3. Duration of the antagonistic effect

In the pithed rat, 0.3 μg/kg i.v. angiotensin II induced a phasic increase in DBP of 53.6 $\pm$ 1.5 mm Hg ($n = 39$). The presence of NaCl 0.9% did not modify this increase during the seven successive injections of angiotensin II (Fig. 2 right side). SR 47436 3 mg/kg i.v. (like losartan 6 mg/kg i.v. and EXP 3174 1 mg/kg i.v.) inhibited for at least 70 min the pressor response to angiotensin II. This effect contrasted with the reversible inhibition of SR 47155A (10 mg/kg i.v.) or [Sar^1,Val^5,Ala^8]angiotensin II (1 mg/kg i.v.) on the pressor response induced by angiotensin II. [Sar^1, Val^5,Ala^8]angiotensin II reversed the effect of angiotensin II at a faster rate than SR 47155A.

3.4. Interaction between SR 47436 and SR 47155A

In the presence of 10 mg/kg i.v. SR 47436, the dose-response curve to angiotensin II was shifted to the right (Fig. 3) by a factor of 651 and the maximal pressor response of the pithed rat was inhibited by 22.8%. Under these conditions, SR 47155A produced (Fig. 3) a parallel shift to the right of the dose-response curve for angiotensin II without further reduction of the maximum response. The curve was shifted by a factor of 3.6 for 30 mg/kg i.v. At this dose, the maximal pressor response did not return to the level observed in the absence of SR 47436.

In the presence of 10 mg/kg i.v. SR 47155A, the

Fig. 2. Left side: effect of SR 47436, SR 47155A, losartan, EXP 3174 and [Sar^1,Val^5,Ala^8]angiotensin II on the dose-response curve for angiotensin II in the pithed rat. The abscissa is the dose of angiotensin II expressed as μg/kg i.v. The ordinate is the increase in diastolic blood pressure expressed as mm Hg. Vertical bars represent S.E.M.. When S.E.M. bars are absent, the S.E.M. was smaller than the symbol. Solid lines represent the fitted curves (see Methods). Symbol: control (●), 0.03 (▲), 0.1 (■), 0.3 (▼), 0.6 (◆), 1 (○), 3 (△), 6 (□) or 10 (▽) mg/kg i.v. Right side: duration of the effects of SR 47436 (3 mg/kg i.v.), SR 47155A (10 mg/kg i.v.), losartan (6 mg/kg i.v.), EXP 3174 (1 mg/kg i.v.) and [Sar^1,Val^5,Ala^8]angiotensin II (1 mg/kg i.v.) on the angiotensin II (0.3 μg/kg i.v.) pressor response in the pithed rat. The abscissa is the time expressed in min. The ordinate is the increase in diastolic blood pressure expressed as mm Hg. Vertical bars represent S.E.M. Number of observations: six to nine.

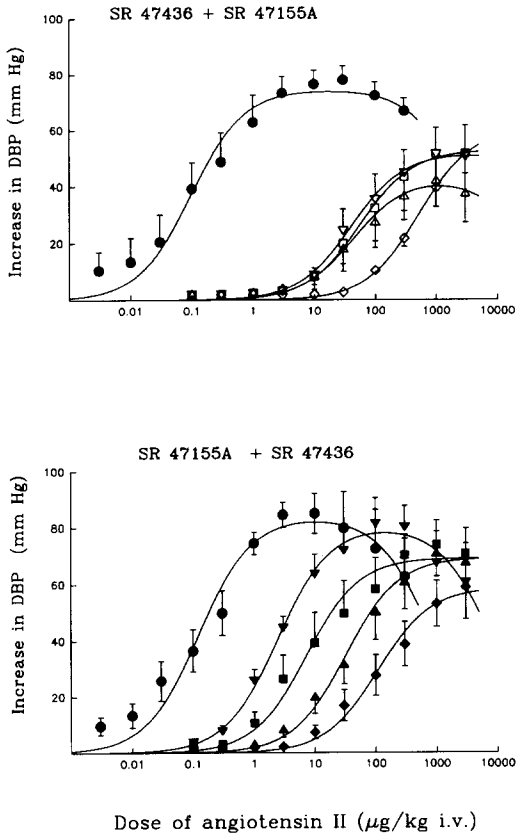


Fig. 3. Effect of SR 47155A (or SR 47436) on the dose-response curve for angiotensin II in the presence of SR 47436 (or SR 47155A) in the pithed rat. Symbols: control (●), SR 47436 10 mg/kg i.v. + SR 47155A 0 (▽), 10 (□), 20 (△) and 30 (◇) mg/kg i.v.; SR 47155A 10 mg/kg i.v. + SR 47436 0 (), 1 (■), 3 (▲) or 10 (◆) mg/kg i.v. The abscissa is the dose of angiotensin II expressed as μg/kg i.v. The ordinate is the increase in diastolic blood pressure expressed as mm Hg. Vertical bars represent S.E.M.. When S.E.M. bars are absent, the S.E.M. was smaller than the symbol. Solid lines represent the fitted curves (see methods). Number of observations: four to eight.

maximal pressor response to angiotensin II was not modified (79.7 ± 8.7 mm Hg, $n = 6$). However the dose-response curve was shifted (Fig. 3) to the right by a factor 12.3. In the presence of SR 47155A, increasing doses of SR 47436 produced (Fig. 3) a parallel shift to the right of the dose-response curve for angiotensin II without a statistical significant change in the maximal pressor effect. The curve was shifted by a factor of 16.0, 28.4 and 95.1 respectively, for 1, 3 and 10 mg/kg i.v. The maximal pressor response observed was always higher than that observed in the absence of SR 47155A.

3.5. Effect on the frequency-pressor response curve

Electrical stimulation of the spinal cord caused (Fig. 4) a frequency dependent increase in DBP, with a maximal increase of 80 mm Hg at 20 Hz. SR 47436 and losartan, both at 30 mg/kg i.v., half reduced the pressor responses to spinal cord stimulation within a stimulation rate range of 0.2–4 Hz.

4. Discussion

4.1. Effect of SR 47436 and SR 47155A on basal blood pressure

Both SR 47436 and SR 47155A decreased basal blood pressure in the pithed rat. This effect has also been observed previously with other non-peptidic angiotensin receptor antagonists such as losartan (Wong and Timmermans, 1991), EXP3892 (Wong and Timmermans, 1991), EXP3174 (Wong et al., 1990a), EXP6155 (Wong et al., 1989) or EXP6803 (Wong et al., 1989). This action has been attributed to blockade of

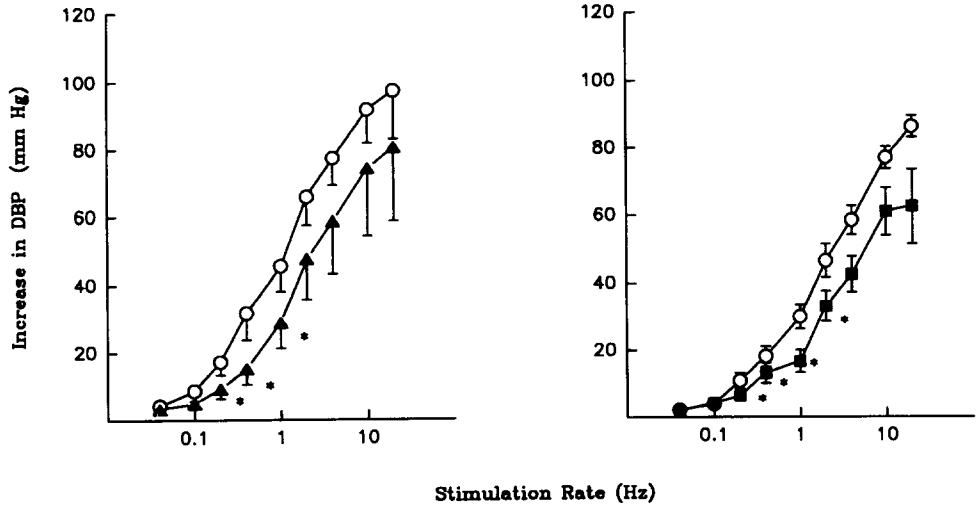


Fig. 4. Effect of vehicle (○), SR 47436 (▲) or losartan (■) on the frequency-response curve induced by electrical stimulation of the spinal cord of the pithed rat. The abscissa is the stimulation rate expressed as Hz. The ordinate is the increase in diastolic blood pressure expressed as mm Hg. Vertical bars represent S.E.M.. When S.E.M. bars are absent, the S.E.M. was smaller than the symbol. Number of observations: five to eight.

the vasoconstrictor effects of angiotensin II by these antagonists due to the high plasma renin activity in the pithed rat (Wong and Timmermans, 1991). None of these compounds showed partial agonist activity of short duration (15 min) unlike [Sar^1,Val^5,Ala^8]angiotensin II.

4.2. Post-synaptic effects of SR 47155A

SR 47155A antagonized the hypertensive action of angiotensin II in the pithed rat. This compound induced a parallel shift to the right of the dose-pressor response curve for angiotensin II without any decrease of the maximal observed effect. These results confirm the data observed in vitro with isolated rabbit aorta (Cazaubon et al., 1993; Guiraudou et al., 1993). When SR 47155A (10^{-6} M) shift the concentration-response curve by a factor of 60, the antagonism remains totally surmountable. This contrasts with the effect of SR 47436. A same factor of shift of 60 times is observed with a SR 47436 dose of 10^{-7} M and in this case, the antagonism is insurmountable (Guiraudou et al., 1993). Structure of SR 47155A and SR 47436 are very close except that SR 47155A has a carboxy group and SR 47436 has a tetrazole. The fact that a small change introduced a difference in the antagonism was not surprising because it is now demonstrated using different chemical series that subtle changes in the structure-activity relationships of a selection of non-peptide ligands can produce a whole spectrum of antagonistic effects (Noda et al., 1993).

4.3. Post-synaptic effects of SR 47436

SR 47436 antagonized the hypertensive action of angiotensin II in the pithed rat. This compound induced, at least at high doses, a non-parallel shift to the right of the dose-pressor response curve for angiotensin II and decreased the maximal observed effect. This results confirms the data observed in vitro with isolated rabbit aorta (Cazaubon et al., 1993; Guiraudou et al., 1993). This type of response can be explained by different mechanisms such as non-competitive inhibition, mixed competitive/quasi non-competitive antagonism (Moore et al., 1984), irreversible or slowly dissociable competitive antagonism (Pendleton et al., 1989), heterotropic or homotropic cooperativity (De Chaffoy de Courcelles et al., 1986; Moore and Scanlon, 1989; Scanlon et al., 1990) or reversible and syntopic action (Liu et al., 1992)

Non-competitive antagonism?

A non-parallel shift to the right of the dose-pressor response curve and a decrease of the maximal observed effect can be a typical profile of non-competitive antagonism (Ariëns et al., 1964). However, in rat liver membrane preparation, the competitive nature of SR 47436 was shown by the Scatchard plot (Cazaubon et al., 1993). In addition, no activity of SR 47436 has been demonstrated on other types of receptors than the angiotensin receptor. SR 47436 is ineffective against hypertension induced by vasopressin (data not shown) in the pithed rat or against contraction induced by noradrenaline or KCl in isolated rabbit aorta (Cazaubon et al., 1993). Binding data suggest no effect on α_1- or α_2-adrenoceptors, 5-HT$_2$ or 5-HT$_1$ serotoninergic receptors, endothelin receptors, bradykinin receptors, neurotensin receptors, V_1 or V_2 vasopressin receptors, Ca^{2+} channels, Na^+/ Ca^{2+} or Na^+/ H^+ antiports (Cazaubon et al., 1993). The existence of different subtypes of angiotensin receptors (angiotensin AT$_1$ and AT$_2$ receptors) has now been demonstrated in both different tissues and in the same tissue (Bumpus et al., 1991; Timmermans et al., 1993). However, SR 47436 displayed a specific affinity for the angiotensin AT$_1$ receptor and lacked affinity for the angiotensin AT$_2$ receptor (Cazaubon et al., 1993; Delisée et al., 1993). It has also been speculated that two subtypes of angiotensin AT$_1$ receptors (angiotensin AT$_{1A}$ and angiotensin AT$_{1B}$ receptors) can exist (Zhang et al., 1992; Timmermans et al., 1993) with a possible predominance of angiotensin AT$_{1A}$ receptor in vascular smooth muscle. These subreceptors could be non-selectively stimulated by angiotensin II but the maximal response could be lower and the slope of the dose-response curve flatter when only one subtype is stimulated. This implies that angiotensin receptor antagonists act preferentially on one or simultaneously on both receptor subtypes. From our present study, it is difficult to conclude that the decrease of the maximal effect induced by SR 47436 is linked to the action of this compound on one of these putative receptor subtypes. Binding data suggest monophasic inhibition of SR 47436 on smooth muscle cells (Delisée et al., 1993)

Mixed competitive / quasi non-competitive antagonism?

Another possible explanation for the mechanism of action of SR 47436 could be based on the model developed by Regoli et al. (1974) and Moore et al. (1984). On the basis of different substitutions made to the 8th angiotensin II amino acid, it seems that the angiotensin II active site is localized in two regions of the molecule, i.e. a catalytic site comprising Tyr^4 and His^6 residues and an accessory site comprising a Phe^8 residue and the *C*-terminal carboxyl anion. The catalytic site interacts with the receptor and permits the binding of angiotensin II to the receptor. The two sites are interdependent and a correct alignment of angiotensin II at the receptor requires information from both regions of the angiotensin II molecule. The catalytic site may be primarily responsible for the mechanism of receptor activation. The accessory site, in addi-

tion to its role in the activation process, may contribute to increasing the binding affinity. In accordance with this model (Moore et al., 1984), two types of angiotensin receptor antagonists can be distinguished. First, agents as sarmesin ([Sar^1,Tyr(Me)^4]angiotensin II) are competitive antagonists (Moore et al., 1984; Scanlon and Moore, 1988) with an antagonistic activity on the catalytic site (where activation is impossible). This antagonist binds only when in a correct alignment with the angiotensin receptor. Second, agents as [Sar^1,Ile^8]angiotensin II have an antagonistic activity due to the inability of the Ile^8 moiety to bring the Tyr^4 residue and the angiotensin receptor into correct alignment except during the first minutes of binding. These antagonists are specific for angiotensin receptors and were described by Moore et al. (1984) as mixed competitive/quasi non-competitive antagonists. Further studies by Moore et al. (Scanlon et al., 1990; Moore et al., 1991) suggest that this type of antagonist may interact with a different 'desensitizing' site on angiotensin receptor from that occupied by angiotensin II and angiotensin receptor antagonists such as sarmesin. A positive charge on the second residue and on the *N*-terminal region of the antagonist can modulate, by different binding with this 'desensitizing' site, the potency, the action duration and the type of antagonism observed with each mixed competitive/quasi non-competitve antagonist (Moore et al., 1991). The antagonistic properties of SR 47436 correspond to the properties of a competitive/quasi non-competitive antagonist of the angiotensin receptor. Nevertheless, SR 47436 is a non-peptide molecule as opposed to sarmesin or to saralasin and the alignment of SR 47436 to the angiotensin receptor is until now unknown. Using chimeric receptor constructions, Schambye et al. (1994) show that the binding mode for peptide and non-peptide ligands on the angiotensin AT_1 receptor is rather different and that non-peptide angiotensin receptor antagonist determine binding to overlapping but distinct sites located in transmembrane segments VI and VII of the angiotensin receptor. Discrimination between surmountable and insurmountable non-peptide angiotensin receptor antagonists could be based on this difference in binding but the existence of a link between in vitro binding to chimeric receptor and functional effects observed in vivo (in the pithed rat) remains to be established. Further studies of the conformational state of SR 47436 are necessary to accept or reject this competitive/quasi non-competitive antagonism of SR 47436

Irreversible or slowly dissociable competitive antagonism?

Pendleton et al. (1989) and Wienen et al. (1990) have demonstrated that an insurmountable action of different angiotensin receptor antagonists can be inhibited by a preincubation with peptide or non-peptide surmountable angiotensin receptor antagonist. This has been made possible by the existence of differences in the association and/or dissociation kinetics of the different antagonists which are in competition for the same binding site. Pendleton et al. (1989) describes antagonists demonstrating the same behaviour than SR 47436 as an irreversible or slowly dissociable competitive antagonist. Our studies have shown that competitive antagonists can have different binding kinetics: fast in the case of SR 47155A and very slow for losartan as already observed (Wong and Timmermans, 1991; Wong et al., 1990b). Thus, it seems unlikely that the kinetics of the antagonist action can be predictive for the type of antagonism to be observed.

Heterotropic or homotropic cooperativity?

An other possible explanation for the type of antagonism existing between SR 47436 and angiotensin II may be drawn from the work of De Chaffoy de Courcelles et al. (1986). These authors proposed a coupling model in order to explain an unsurmountable antagonistic effect. The receptor model depicts two binding sites on the receptor, one for the agonist and the antagonist at the outer side of the membrane and another for the transducing factor at the inner side of the membrane (heterotropic cooperativity). According to this model, the binding of the agonist to the outer site leads to a coupling of the transducer at the inner site and a subsequent activation of the signal transducing system. In this case, the fit on the binding sites of the agonist and of the transducer is perfect. Binding of a competitive or a non-competitive antagonist at the outer site induces conformational changes at the outer site and subsequently at the inner site. Addition of an agonist displaces the antagonist but the antagonist-induced conformational changes are only slowly reversible. In the case of competitive inhibition, the antagonist-induced changes at the inner site reduce the affinity of the transducer for this site (non-perfect fit). In the case of non-competitive inhibition, the antagonist-induced changes at the inner site make coupling with the transducer impossible. An additional feature of this model is that competitive antagonists can displace non-competitive antagonists and vice versa. Our results have shown that, in the pithed rat, the interaction of SR 47436 with its receptor can be modified by the presence of SR 47155A. The order of administration is important because the effect seems to be determined by the first antagonist injected. Thus, this model of De Chaffoy de Courcelles (De Chaffoy de Courcelles et al., 1986) could explain the effect observed with SR 47436 and the cross reaction existing between SR 47436 and SR 47155A. This explanation could correspond to a general feature because such an interaction has been observed with other pairs of competitive-non-competitive angiotensin receptor antagonists

such as [Sar^1,Ile^8]angiotensin II-losartan (Wienen et al., 1990) or losartan-EXP 3892 (Timmermans et al., 1991; Wong and Timmermans, 1991), [Sar^1,Ile^8]angiotensin II-losartan (Van Meel et al., 1993; Wong and Timmermans, 1991), [Sar^1,Ile^8]angiotensin II-sarmesin (Van Meel et al., 1993; Wienen et al., 1992) or BIBR277-losartan (Wienen et al., 1993). The possibility of homotropic cooperativity for the interaction of angiotensin II and its receptor can be associated to this kind of heterotropic cooperativity, (Moore and Scanlon, 1989; Scanlon et al., 1990). According to Moore and Scanlon (1989) and Scanlon et al. (1990), the action of angiotensin II is linked to a 2-site agonist binding mechanism involving receptors dimers. Binding of angiotensin II to its receptor induces an increase in its own receptor binding activity (homotropic cooperativity) and influences heterotropic cooperativity. The amplitude of this homotropic cooperativity depends on the type of tissue. The presence of a competitive or a slowly reversing competitive antagonist should only effectively decrease the number of receptors available to the agonist and then produce a decrease in the observed level of positive cooperativity. A non-competitive antagonist such as [Sar^1,Ile^8]angiotensin II (Moore and Scanlon, 1989; Scanlon et al., 1990) should produce a negative cooperativity. The type of action of SR 47436 on heterotropic and/or homotropic cooperativity could be identified by a further study of the effect of SR 47436 on different types of isolated organs.

Reversible and syntopic action?

Finally a last explanation could be based on the model of Liu et al. (1992) developed from the operational model of agonism. Liu et al. (1992) attempted to explain the apparent complexity of receptor antagonism by angiotensin receptor antagonists by a reversible and syntopic action at a single site of action of the angiotensin receptor, action linked to a saturable reduction in the apparent affinity and efficacy of angiotensin II. This change in efficacy could be linked to an internalisation of the angiotensin receptor after its occupation by angiotensin II or by the antagonist. Efficacy of expression of angiotensin II at the angiotensin AT_1 receptor could be linked to the concentration of external calcium (Whittaker, 1990). This model has been evaluated with different peptide angiotensin receptor antagonists (Liu et al., 1992) and remains to be evaluated with non-peptide angiotensin receptor antagonists. This hypothesis is difficult to assess using an in vivo preparation such as the pithed rat as the concentration of agonist and antagonist at the receptor site is not exactly known. Further quantitative studies of the effect of SR 47436 on isolated organs would be thus necessary to identify the exact mechanism of action.

4.4. Presynaptic effects

Electrical stimulation of spinal cord in the pithed rat induces a blood pressure increase linked to an increase of the cardiac output and of the total peripheral resistance (Wong et al., 1992; Kaufman and Vollmer, 1985). Blood pressure and total peripheral resistance (but not cardiac output) are inhibited by the presence of different angiotensin receptor antagonists: S-8307 (Wong et al., 1988), losartan (Wong et al., 1992), [Sar^1, Ile^8]angiotensin II (Ohlstein et al., 1992) or SK&F 108566 (Ohlstein et al., 1992). Our results also indicate that SR 47436 inhibits the increase in blood pressure induced by electrical stimulation of the spinal cord in the pithed rat. Thus SR 47436, as losartan, acts on prejunctional angiotensin receptors as already suggested by the results obtained with electrical stimulation of the spinal cord of spontaneously hypertensive rats (Moreau et al., 1993). The pre-synaptic effect of SR 47436 was observed with doses which produced a maximal effect on the post-synaptic site.

4.5. Concluding comments

SR 47436 (0.3–10 mg/kg i.v.) shows in the pithed rat an antagonistic activity against the pressor effect of angiotensin II. The action of SR 47436 is more selective for a post-synaptic site of action vs. a pre-synaptic site. The type of interaction existing between SR 47436 and angiotensin II at the post-synaptic level (partially insurmountable, but selective antagonism) can be explained with different models and further studies are necessary to discern among these models.

References

Ariëns, E.J., A.M. Simonis and J.M. Van Rossum, 1964, Drug-receptor interaction: interaction of one or more drugs with different receptor systems, in: Molecular Pharmacology. The Mode of Action of Biologically Active Compounds., ed. E.J. Ariëns (Academic Press, New York, London) p. 287.

Bernhart, C., P. Perreaut, B.P. Ferrari, Y.A. Muneaux, J.L.A. Assens, J. Clement, F. Haudricourt, C.F. Muneaux, J.E. Taillades, M.A. Vignal, J. Gougat, P. Guiraudou, C. Lacour, A. Roccon, C. Cazaubon, J.-C. Breliere, G. Le Fur and D. Nisato, 1993, A new series of imidazolones –highly specific and potent nonpeptide AT(1) angiotensin-II receptor antagonists, J. Med. Chem. 36, 3371.

Bumpus, F.M., K.J. Catt, A.T. Chiu, M. de Gasparo, T. Goodfriend, A. Husain, M.J. Peach, D.G. Taylor and P.B.M.W.M. Timmermans, 1991, Nomenclature for angiotensin receptors: a report of the nomenclature committee of the council for high blood pressure research, Hypertension 17, 720.

Cazaubon, C., J. Gougat, F. Bousquet, P. Guiraudou, R. Gayraud, C. Lacour, A. Roccon, G. Galindo, G. Barthelemy, B. Gautret, C. Bernhart, P. Perreaut, J.-C. Breliere, G. Le Fur and D. Nisato, 1993, Pharmacological characterization of SR 47436, a new non-

peptide AT_1 subtype angiotensin II receptor antagonist, J. Pharmacol. Exp. Ther. 265, 826.

Chang, R.S.L., P.K.S. Siegl, B.V. Clineschmidt, N.B. Mantlo, P.K. Chakravarty, W.J. Greenlee, A.A. Patchett and V.J. Lotti, 1992, In vitro pharmacology of L-158,809, a new highly potent and selective angiotensin II receptor antagonist, J. Pharmacol. Exp. Ther. 262, 133.

Criscione, L., M. De Gasparo, P. Buhlmayer, S. Whitebread, H.P.R. Ramjoue and J. Wood, 1993, Pharmacological profile of valsartan –a potent, orally active, nonpeptide antagonist of the angiotensin-II AT1-receptor subtype, Br. J. Pharmacol. 110, 761.

De Chaffoy de Courcelles, D., J.E. Leysen, P. Roevens and H. Van Belle, 1986, The serotonin-S_2 receptor: a receptor-transducer coupling model to explain insurmountable antagonist effects, Drug Dev. Res. 8, 173.

Delisée, C., P. Schaeffer, C. Cazaubon and P. Chatelain, 1993, Characterization of cardiac angiotensin AT1 receptors by [H-3]SR-47436, Eur. J. Pharmacol. 247, 139.

Edwards, R.M., N. Aiyar, E.H. Ohlstein, E.F. Weidley, E. Griffin, M. Ezekiel, R.M. Keenan, R.R.J. Ruffolo and J. Weinstock, 1992, Pharmacological characterization of the nonpeptide angiotensin II receptor antagonist, SK and F 108566, J. Pharmacol. Exp. Ther. 260, 175.

Guiraudou, P., C. Cazaubon, R. Gayraud, C. Bernhart, P. Perreaut and D. Nisato, 1993, SR 47436, a non-peptide angiotensin II receptor antagonist. Study of the mechanism of action on rabbit aorta, J. Mol. Cell. Cardiol. 25, S17.

Hodges, J.C., J.M. Hamby and C.J. Blankley, 1992, Angiotensin II receptor binding inhibitors, Drugs Fut. 17, 575.

Kaufman, L.J. and R.R. Vollmer, 1985, Endogenous angiotensin II facilitates sympathetically mediated hemodynamic responses in pithed rats, J. Pharmacol. Exp. Ther. 235, 128.

Liu, Y.J., N.P. Shankley, N.J. Welsh and J.W. Black, 1992, Evidence that the apparent complexity of receptor antagonism by angiotensin II analogues is due to a reversible and syntopic action, Br. J. Pharmacol. 106, 233.

Moore, G.J. and M.N. Scanlon, 1989, Methods for analyzing and interpreting cooperativity in dose-responses curves 1.Antagonist effects on angiotensin receptors in smooth muscle, Gen. Pharmacol. 20, 193.

Moore, G.J., J.M. Matsoukas, M.N. Scanlon, K.J. Franklin and M.H. Goghari, 1984, On the mechanisms of action of two classes of angiotensin antagonists at smooth muscle receptors, Proc. West. Pharmacol. Soc. 27, 377.

Moore, G.J., R.C. Ganter, M.H. Goghari and K.J. Franklin, 1991, Importance of the *N*-terminal domain of the type I angiotensin II antagonist [Sar^1,Ile^8]ANG II for receptor blockade, Int. J. Pept. Protein Res. 38, 1.

Moreau, N., C. Richer, M.P. Vincent and J.F. Giudicelli, 1993, Interaction entre le SR 47436, un nouvel antagoniste de l'angiotensine II, et le système sympathique chez le SHR amyélé, Arch. Mal. Coeur 86, 1269.

Noda, M., Y. Shibouta, Y. Inada, M. Ojima, T. Wada, T. Sanada, K. Kubo, Y. Kohara, T. Naka and K. Nishikawa, 1993, Inhibition of rabbit aortic angiotensin II (AII) receptor by CV-11974, a new nonpeptide AII antagonist, Biochem. Pharmacol. 46, 311.

Ohlstein, E.H., M. Gellai, D.P. Brooks, L. Vickery, J. Jugus, A. Sulpizio, R.R.J. Ruffolo, J. Weinstock and R.M. Edwards, 1992, The antihypertensive effect of the angiotensin II receptor antagonist DuP 753 may not be due solely to angiotensin II receptor antagonism, J. Pharmacol. Exp. Ther. 262, 595.

Okunishi, H., K. Song, Y. Oka, T. Kobayashi, T. Kawamoto, H. Ishihara, N. Mori and M. Miyazaki, 1993, In vitro pharmacology of a novel non-peptide angiotensin II-receptor antagonist, E4177, Jpn. J. Pharmacol. 62, 239.

Olins, G.M., V.M. Corpus, E.G. Mcmahon, M.A. Palomo, J.R. Schuh, D.J. Blehm, H.-C. Huang, D.B. Reitz, R.E. Manning and E.H. Blaine, 1992, In vitro pharmacology of a nonpeptidic angiotensin II receptor antagonist, SC-51316, J. Pharmacol. Exp. Ther. 261, 1037.

Pendleton, R.G., G. Gessner and E. Horner, 1989, Studies on inhibition of angiotensin II receptors in rabbit adrenal and aorta, J. Pharmacol. Exp. Ther. 248, 637.

Regoli, D., W.K. Park and F. Rioux, 1974, Pharmacology of angiotensin, Pharmacol. Rev. 26, 69.

Robertson, M.J., J.C. Barnes, G.M. Drew, K.L. Clark, F.H. Marshall, A. Michel, D. Middlemiss, B.C. Ross, D. Scopes and M.D. Dowle, 1992, Pharmacological profile of GR117289 in vitro: a novel, potent and specific non-peptide angiotensin AT_1 receptor antagonist, Br. J. Pharmacol. 107, 1173.

Scanlon, M.N. and G.J. Moore, 1988, Classification of angiotensin receptors in rat isolated uterus, portal vein, and aorta with the novel competitive antagonist sarmesin, Pharmacology 37, 137.

Scanlon, M.N., P. Koziarz and G.J. Moore, 1990, The relationship between homotropic and heterotropic cooperativity for angiotensin receptors in smooth muscle, Gen. Pharmacol. 21, 59.

Schambye, H.T., S.A. Hjorth, D.J. Bergsma, G. Sathe and T.W. Schwartz, 1994, Differenciation between binding sites for angiotensin II and nonpeptide antagonists on the angiotensin II type 1 receptors, Proc. Natl. Acad. Sci. USA 91, 7046

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

Shipley, R.E. and J.H. Tilden, 1947, A pithed rat preparation suitable for assaying pressor substances, Proc. Soc. exp. Biol. Med. 64, 453.

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

Timmermans, P.B.M.W.M., A.T. Chiu, W.F. Herblin, R.D. Smith and P.C. Wong, 1992, Multiple angiotensin receptors: selective ligands and functional correlates, Jpn. J. Pharmacol. 58, 135P.

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

Valkó, P. and S. Vajda, 1989, Parameter estimation, in: Data handling in science and technology –volume 4. Advanced scientific computing in BASIC with applications in chemistry, biology and pharmacology, eds. P. Valkó and S. Vajda (Elsevier, Amsterdam, Oxford, New York, Tokyo) p. 139.

Van Meel, J.C.A., M. Entzeroth, N. Hauel, B. Narr, U. Ries and W. Wienen, 1993, Angiotensin II receptor antagonists, Arzneim.-Forsch./Drug Res. 43, 242.

Whittaker, V.P., 1990, The contribution of drugs and toxins to understanding of cholinergic function, Trends Pharmacol. Sci. 11, 8.

Wienen, W., W. Diederen, A.B.M. Mauz and J.C.A. Van Meel, 1990, Reversal of the non-competitive behaviour of the angiotensin II-antagonist Sar^1 Ile^8-AII by a non-peptide AII-antagonist in vitro and in vivo, Eur. J. Pharmacol. 183, 1553.

Wienen, W., A.B.M. Mauz, J.C.A. Van Meel and M. Entzeroth, 1992, Different types of receptor interaction of peptide and nonpeptide angiotensin II antagonists revealed by receptor binding and functional studies, Mol. Pharmacol. 41, 1081.

Wienen, W., N. Hauel, J.C.A. Van Meel, B. Narr, U. Ries and M. Entzeroth, 1993, Pharmacological characterization of the novel nonpeptide angiotensin II receptor antagonist, BIBR 277, Br. J. Pharmacol. 110, 245.

Wong, P.C. and P.B.M.W.M. Timmermans, 1991, Nonpeptide angiotensin II receptor antagonists: insurmountable angiotensin II antagonism of EXP3892 is reversed by the surmountable antagonist DuP 753, J. Pharmacol. Exp. Ther. 258, 49.

Wong, P.C., A.T. Chiu, W.A. Price, M.J.M.C. Thoolen, D.J. Carini, A.L. Johnson, T.I. Taber and P.B.M.W.M. Timmermans, 1988, Nonpeptide angiotensin II receptor antagonists. I. Pharmacological characterization of 2-*n*-butyl-4-chloro-1-(2-chlorobenzyl)imidazole-5-acetic acid, sodium salt (S-8307), J. Pharmacol. Exp. Ther. 247, 1.

Wong, P.C., W.A. Price, A.T. Chiu, M.J.M.C. Thoolen, J.V. Duncia, A.L. Johnson and P.B.M.W.M. Timmermans, 1989, Non peptide angiotensin II receptor antagonist. IV. EXP6155 and EXP6803, Hypertension 13, 489.

Wong, P.C., W.A. Price, A.T. Chiu, J.V. Duncia, D.J. Carini, R.R. Wexler, A.L. Johnson and P.B.M.W.M. Timmermans, 1990a, Non peptide angiotensin II receptor antagonists. IX. Pharmacology of EXP3174: an active metabolite of DuP 753, an orally active antihypertensive agent., J. Pharmacol. Exp. Ther. 255, 211.

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

Wong, P.C., S.D. Hart, A.T. Chiu, W.F. Herblin, D.J. Carini, R.D. Smith, R.R. Wexler and P.B.M.W.M. Timmermans, 1991, Pharmacology of DuP 532, a selective and noncompetitive AT receptor antagonist, J. Pharmacol. Exp. Ther. 259, 861.

Wong, P.C., R. Bernard and P.B.M.W.M. Timmermans, 1992, Effect of blocking angiotensin II receptor subtype on rat sympathetic nerve function, Hypertension 19, 663.

Zhang, J., M. Entzeroth, W. Wienen and J.C.A. Van Meel, 1992, Characterization of BIBS 39 and BIBS 222: two new nonpeptide angiotensin II receptor antagonists, Eur. J. Pharmacol. 218, 35.