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A two-state model of antagonist-AT_1 receptor interaction: further support by binding studies at low temperature

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Abstract

The molecular mechanism of insurmountable antagonism was investigated to a large extent in Chinese hamster ovary cells transfected with the human angiotensin II receptor type 1 (AT_1) receptor. It was proposed that AT_1 receptor antagonists interact with their receptor according to a two-state receptor model. Briefly, this theoretical model reveals that antagonist bound AT_1 receptor can adopt a fast and a slow reversible state. The first, fast reversible state is similar for all antagonists, while the slow reversible state displays the characteristics of each antagonist. In the present study, we performed competition experiments with the AT_1 receptor antagonists candesartan, EXP3174, irbesartan, losartan and ligand [^{3}H]-angiotensin II at 0–4°. This gave the opportunity to verify the two-state model for the first time with experimental data.

Keywords: Angiotensin II; Antagonist; AT_1 receptor; CHO cells; Insurmountable; Two-state model

1. Introduction

In the past decade, several orally active nonpeptide AT_1 receptor antagonists have been developed for the treatment of hypertension. They have been referred to as surmountable or insurmountable when tested for their ability to inhibit Ang II induced contraction of rabbit aorta strips [1]. The former (e.g. losartan) only produce parallel rightward shifts of the Ang II concentration response curve while the latter also decrease the maximal response to various extent (i.e. irbesartan < EXP3174 < candesartan). The same antagonistic profile was observed when measuring the inositol phosphate accumulation in Chinese hamster ovary cells transfected with the human AT_1 receptor (CHO-AT_1 cells) [2–8]. Studies on these cells further revealed that all antagonists are competitive with Ang II and that antagonist-AT_1 receptor complexes are able to adopt two distinct states: a fast reversible (for the surmountable inhibition), and a tight binding state (for insurmountable inhibition). A close fit was found between the experimental data and computer-simulated curves according to a two-step, two-state model [9]. In the present study, this model is further supported by antagonist/[^{3}H]-Ang II competition binding experiments performed at 4°.

2. Materials and methods

2.1. Materials

[^{3}H]-Ang II (52.0 Ci/mmol, Amersham Biosciences Benelux), candesartan (CV-11974; 2-ethoxy-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]-1*H*-benzimidazoline-7-carboxylic acid), EXP 3174 (2-*n*-butyl-4-chloro-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole-5-carboxylic acid), losartan (DuP 753; 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)-methyl] imidazole) and irbesartan (SR 47436; 2-*n*-butyl-4-spirocyclopentane-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)-methyl]2-imidazolin-5-one) were obtained from AstraZeneca. All other chemicals were of the highest grade commercially available.

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Abbreviations: Ang II, angiotensin II; AT_1, angiotensin II receptor type 1; CHO-hAT_1 cells, Chinese hamster ovary cells expressing human AT_1 receptors.

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2.2. Cell culture and angiotensin II binding

CHO-AT_1 cells were obtained and cultured as described [2]. Cells were plated in 24-well plates and grown until confluence. Subsequent steps were performed at 4°. Cells were washed three times with 0.5 mL Hepes-buffered Dulbecco's modified Eagle's medium (DMEM) and 0.4 mL of this medium was added at the end. Incubations were started by adding medium containing increasing concentrations of antagonist (50 μL) and [^{3}H]-Ang II (50 μL, final concentration 1 nM). Nonspecific binding was measured in the presence of 1 μM candesartan. After the indicated time periods, cells were washed three times with phosphate buffered saline (0.9 mM $CaCl_2$, 2.7 mM KCl, 1.5 mM KH_2PO_4, 0.49 mM $MgCl_2$, 137 mM NaCl and 8 mM Na_2HPO_4, pH 7.4), solubilized with 0.5 mL Triton-X and transferred to scintillation vials. Scintillation liquid (3 mL, Optisafe, Perkin-Elmer Life Sciences) was added and the cell bound radioactivity in each well was counted in a liquid scintillation counter. Specific [^{3}H]-Ang II binding was calculated as described [2]. Data points refer to specific binding. They are the means ± SEM of at least three separate experiments with duplicate determinations each and are expressed as percentage of [^{3}H]-Ang II binding in the absence of antagonist (500 ± 16 cpm after 30 min, 1160 ± 64 cpm after 2 hr and 1753 ± 153 cpm after 24 hr at 4°).

The IC_{50} values were calculated by nonlinear regression analysis using GraphPad Prism.

3. Results and discussion

Computer assisted simulations of competition binding curves at 37° were performed by the method and with the parameters described in [9]. These simulations explicitly take account of the time as a variable and the following reaction mechanisms were used to describe angiotensin II (A)- and antagonist (I)-AT_1 receptor (R) interactions:

$$A + R <=> AR$$

$$I + R <=> IR <=> IR^*$$

In the two-step, two-state model represented above, antagonist-receptor complexes may adopt two different

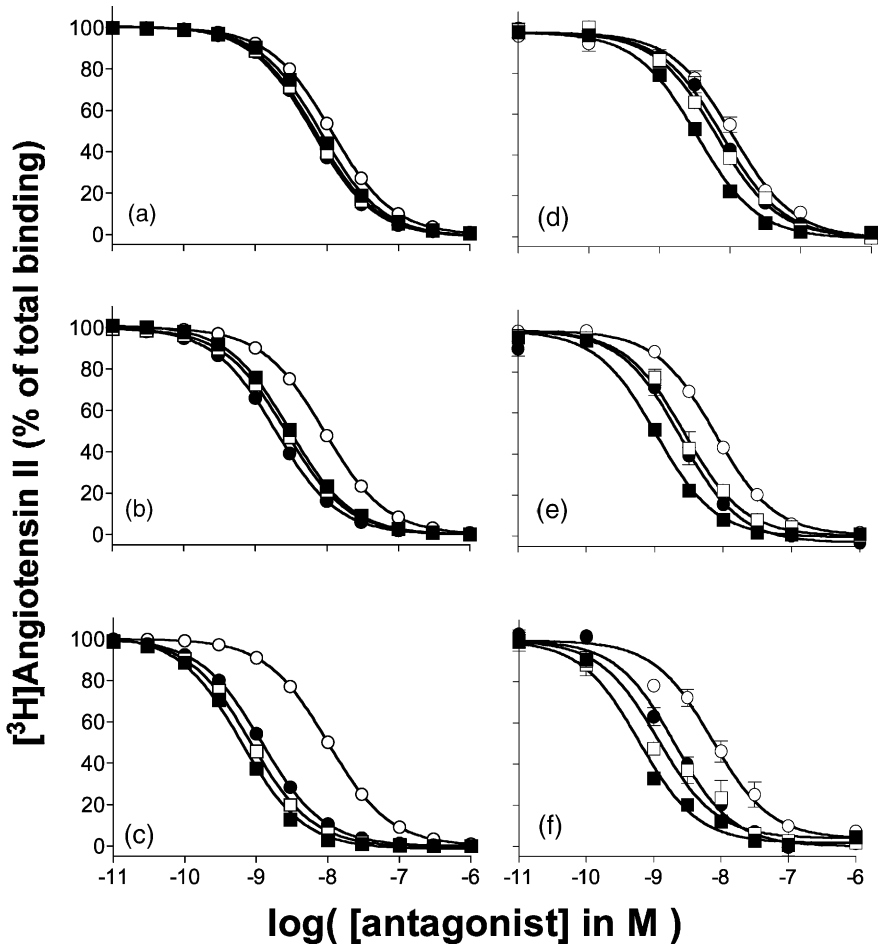

Fig. 1. [^{3}H]-Ang II binding to intact CHO-hAT_1 cells: competition with unlabelled AT_1 receptor antagonists. Computer-assisted simulations of antagonist/[^{3}H]-Ang II competition binding curves at 37° were performed according to the two-step, two-state model for 0.1 min (a), 1 min (b) and 30 min (c). Cells were incubated with increasing concentrations of antagonists (■, candesartan; □, EXP3174; ●, irbesartan; ○, losartan) and 1 nM [^{3}H]-Ang II at 4° for 30 min (d), 2 hr (e) and 24 hr (f). The relative IC_{50} values are listed in Table 1.

Table 1
Relative IC_{50} values of the antagonist/[^{3}H]-Ang II competition binding experiments at 4° (Fig. 1d-f). The IC_{50} value (11.33 ± 1.97 nM) of losartan for a 30-min incubation is taken as 1

	Candesartan	EXP3174	Irbesartan	Losartan
30 min	3.27	1.74	1.39	1.00
2 hr	10.76	3.66	4.59	1.48
24 hr	17.94	7.58	5.43	1.37

states: a fast reversible (IR) and a tight binding state (IR*). The initial binding of all antagonists is fast and reversible. In this respect, computer-assisted simulations suggest that this initial step should be quite similar for losartan, irbesartan, EXP3174 and candesartan (i.e. the antagonists used in this study). For insurmountable antagonism, IR must be further converted into the tight binding IR* state. Whereas this second state cannot be accessed with losartan, an equilibrium between IR and IR* can be achieved with the other antagonists. The degree of insurmountability, defined as the IR*/IR ratio, is specific for each antagonist and largely dictated by the stability of IR*. At 37°, this ratio is close to 0.74 for irbesartan, 3.8 for EXP3174 and 20 for candesartan [10].

Simulations according to the two-step, two-state model predict that, initially, the antagonists should inhibit [^{3}H]-Ang II binding with similar potency but that their potency should diverge with longer incubation times. In the present study, such competition binding experiments were performed at 4° to delay the process. The experimental findings comply with the prediction and add further support to this model. As shown in Fig. 1d, all antagonists inhibit the binding of [^{3}H]-Ang II with similar potency after 30 min incubation at 4°. This similarity can be explained by assuming that, during this relatively short incubation, antagonists only got the opportunity to form IR complexes. At 24 hr, the binding of [^{3}H]-Ang II to the cells expressing the AT_1 receptor was 3-fold higher than at 30 min. Among the potential explanations, the slow association of [^{3}H]-Ang II could be related to the necessity of the receptors to adopt an ''active conformation'' for optimal agonist binding (Table 1).

The potency of losartan is only slightly affected when the incubation time is increased up to 24 hr. However, the potency of the other antagonists increases time-wise and this increase is most outspoken for candesartan, followed by EXP3174 and irbesartan. At the end of 24 hr incubation, the potency order of the antagonists (i.e. candesartan > EXP3174 > irbesartan > losartan) is the same as in previous binding experiments at 37° with [^{3}H]-candesartan [4], [^{3}H]-irbesartan [6], [^{3}H]-valsartan [7] and [^{125}I]Sar^1-Ile^8 Ang II [11] and as in the corresponding simulations with [^{3}H]-Ang II. According to the presented model, the increase in potency for the insurmountable antagonists can be explained by a slow conversion of IR into tighter IR* complexes.

Acknowledgments

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References

[1] Zhang JC, Van Meel A, Pfaffendorf M, Van Zwieten P. Different types of angiotensin II receptor antagonism induced by BIBS 222 in the rat portal vein and rabbit aorta. The influence of receptor reserve. J Pharmacol Exp Ther 1993;269:509–14.
[2] Vanderheyden PML, Fierens FL, De Backer JP, Fraeyman N, Vauquelin G. Distinction between surmountable and insurmountable selective AT_1 receptor antagonists by use of CHO-K_1 cells expressing human angiotensin II-AT_1 receptors. Br J Pharmacol 1999;126:1057–65.
[3] Fierens FLP, Vanderheyden PML, De Backer JP, Vauquelin G. Insurmountable angiotensin-AT_1 receptor antagonists: the role of tight antagonist binding. Eur J Pharmacol 1999;372(2):199–206.
[4] Fierens FLP, Vanderheyden PML, De Backer JP, Vauquelin G. Binding of the antagonist [^{3}H]candesartan to angiotensin II-AT_1 receptor-transfected Chinese hamster ovary cells. Eur J Pharmacol 1999;367(2/3):413–22.
[5] Vanderheyden PML, Fierens FLP, De Backer JP, Vauquelin G. Reversible and syntopic interaction between angiotensin receptor antagonists on Chinese hamster ovary cells expressing human angiotensin II type 1 receptors. Biochem Pharmacol 2000;59:927–35.
[6] Vanderheyden PML, Verheijen I, Fierens FLP, De Backer JP, Vauquelin G. Binding characteristics of [^{3}H]-irbesartan to human recombinant angiotensin type 1 receptors. J Renin Angiotensin Aldosterone Syst 2000;1(2):159–69.
[7] Verheijen I, Fierens FLP, De Backer JP, Vauquelin G, Vanderheyden PML. Interaction between partially insurmountable antagonist valsartan and human recombinant angiotensin type 1 receptors. Fundam Clin Pharmacol 2000;14:577–85.
[8] Fierens FLP, Vanderheyden PML, Roggeman C, Vande Gucht P, De Backer JP, Vauquelin G. Distinct binding properties of the AT_1 receptor antagonist [^{3}H]-candesartan to intact cells and membrane preparations. Biochem Pharmacol 2002;63:1273–9.
[9] Vauquelin G, Morsing P, Fierens FLP, De Backer JP, Vanderheyden PML. A two-state receptor model for the interaction between angiotensin II type 1 receptors and nonpeptide antagonists. Biochem Pharmacol 2001;61:277–84.
[10] Vauquelin G, Fierens FLP, Verheijen I, Vanderheyden PML. Distinctions between nonpeptide angiotensin II-AT_1 receptor antagonists. J Renin Angiotensin Aldosterone Syst 2001;2(1):S24–31.
[11] Verheijen I, Vanderheyden PML, Bottari S, De Backer JP, Vauquelin G. Antagonist interaction with endogenous AT_1 receptors in human cell lines. Biochem Pharmacol 2002;63:1207–14.