Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 67 (2004) 397–399
Commentary
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

The two-state model of antagonist-AT_1 receptor interaction: an hypothesis defended but not tested

Michael J. Lew*, James Ziogas

Department of Pharmacology, The University of Melbourne, Victoria 3010, Australia

Abstract

A correlation between insurmountable antagonism and slow dissociation has been observed for the non-peptidic AT_1 receptor antagonists. This commentary examines the validity of conclusions regarding a two stage binding mechanism that has been proposed in order to account for both the slow dissociation and insurmountable antagonism. Support for that hypothetical mechanism is in the form of the goodness of fit between experimental data and modelled data in a number of papers from the same laboratory. We challenge the idea that a simple match of model and data is an adequate test of an hypothesis by showing that a simpler model matches the data equally well. We conclude that two stage binding is not necessary to explain the behaviour of AT_1 receptor antagonists.

We write this after reading a recent paper in this journal [1]. We are in broad agreement with Vauquelin and co-workers regarding the aetiology of the insurmountable antagonism in so far as it is a consequence of slow antagonist dissociation from the receptor. However, we do not think that their specific hypothesis regarding two-stage binding of insurmountable antagonists is well supported by experimental evidence[1] and we feel that comment on some issues raised by that particular paper is required.

Our specific concern with the paper is that the experiments are presented as a test of the authors' hypothesis concerning AT_1 receptor antagonists. In hypothesis testing it is essential to identify the critical predictions that differentiate alternative hypotheses and we contend that the experiments in question do not include any critical test. The results have been interpreted as evidence favouring a two-stage binding model (Model 2, Fig. 1), a conclusion based merely on the fact that the experimental results approximate the mathematical model. However, as we have previously suggested [2,4], such evidence needs to be interpreted with considerable caution because many different models might match the experimental data equally well. In this case we will show that the standard mass action model of competition between reversible bimolecular reactions (Model 1, Fig. 1) fits the data from the paper in question just as well as the elaborated model proposed by its authors. That leads to our secondary concern which is the authors' failure to compare their model with any other and the apparent failure of the paper's referees to insist on such a comparison.

The experiments reported in the paper [1] consist of binding studies where the radioligand (^{3}H-AngII) and antagonist were applied simultaneously (i.e. no preincubation step for either ligand) and then the amount of binding determined after various incubation times. Several different antagonists were used, including the surmountable antagonist losartan and the insurmountable antagonist candesartan. The rationale for performing those experiments as a test of their hypothesis was that the antagonists would be all similarly effective at preventing radioligand binding after brief periods of incubation, because the first stage of binding (i.e. formation of BR complexes) was assumed to proceed rapidly, whereas with longer incubations the insurmountable antagonists should become more effective due to progression of the slower second stage of binding (formation of BR′ complexes). Surmountable antagonists would not become more effective with longer incubation times because they do not undergo the second stage. The data conform well to the predictions of Model 2 with the potency of losartan being similar at all times

* Corresponding author. Tel.: +61-3-8344-8304; fax: +61-3-8344-0241.
E-mail addresses: michaell@unimelb.edu.au (M.J. Lew), jamesz@unimelb.edu.au (J. Ziogas).

[1] We have previously debated with Vauquelin and co-workers the issues relating to the underlying mechanisms to produce the variable degree of insurmountability of antagonism seen with many of the non-peptidic angiotensin AT_1 receptor antagonists. We can therefore point interested readers to some previous papers for a full discussion of our own ideas [2–4].

0006-2952/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2003.11.007

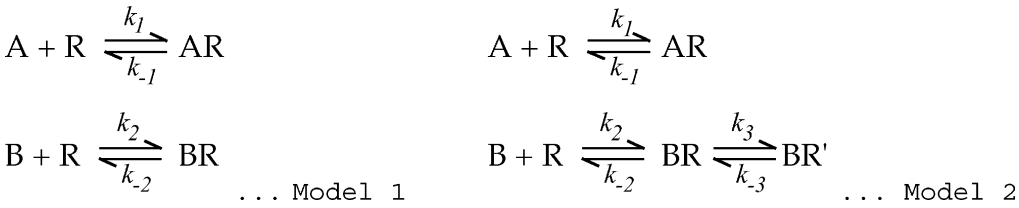

Fig. 1. Two competing models that can account for the different patterns of antagonism shown by non-peptidic angiotensin AT1 receptor antagonists. Verheijen *et al.* [1] use I, IR and IR* to define the inhibitors but we prefer the convention of A for agonists and B for antagonists. We also use BR′ to denote the putative second-stage of binding for the antagonists to distinguish from R*, routinely used to denote the active state of receptors.

whereas the potency of candesartan increased with incubation time. However, that is also exactly what is predicted by the standard (single-stage) binding model (Model 1) where the insurmountable antagonist simply needs more time to equilibrate than the surmountable antagonist.

We performed numerical simulations of Model 1 to explore its competence in predicting the patterns of binding found in the paper. Our simulations mimicked the protocol used in the real experiments and we used antagonists with both fast and slow kinetics compared to the radioligand; k_{-2} 10 or 0.1 times k_{-1}, respectively. We examined the simulation output both as time-courses of ligand binding and as antagonist concentration–inhibition curves. Figure 2 shows the time-courses of ligand binding for two ligands when they are applied simultaneously. Notably the time-course is more complex than is seen with a protocol where either ligand is pre-equilibrated, in that the binding of the faster ligand peaks quickly before declining back to the final equilibrium level. When the faster ligand is the antagonist (Fig. 2, left panel), the radioligand binding time-course is qualitatively unaltered compared to control and quickly attains its final steady, equilibrium value. However, with a slow antagonist (Fig. 2, right panel) it is the binding of the radioligand that initially overshoots, and much more time is needed for the radioligand binding to attain its final, equilibrium value.

The data in Fig. 2 represent the simulations required to obtain the values for fractional radioligand binding inhibition at all time points for a single concentration of antagonist. Equivalent time-courses were generated with a wide range of antagonist concentrations to obtain the antagonist concentration–inhibition curves shown in Fig. 3. It can be seen that the different time-courses of radioligand binding in the presence of either fast or slow antagonists result in different patterns in the concentration–inhibition curves. In the case of a fast antagonist the fractional inhibition of radioligand binding (compared to control) is fairly constant even prior to equilibrium because the time-course of radioligand binding is almost unchanged in the presence of antagonist (Fig. 2, left panel). Therefore, the location of the fast antagonist displacement curve is almost constant at all of the time-points tested, and reflects only the affinity of the antagonist for the receptors (Fig. 3, left panel). In contrast, the slow antagonist cause a marked change in the radioligand binding time-course (Fig. 2, right panel) such that the inhibition of radioligand binding is minimal at short times and increases steadily until equilibrium. This results in the slow antagonist concentration–inhibition curves moving leftwards with increasing incubation times up until the point at which full equilibration has occurred (Fig. 3, right panel). Thus, the potency of the slow antagonist does not fully reflect its affinity until equilibrium

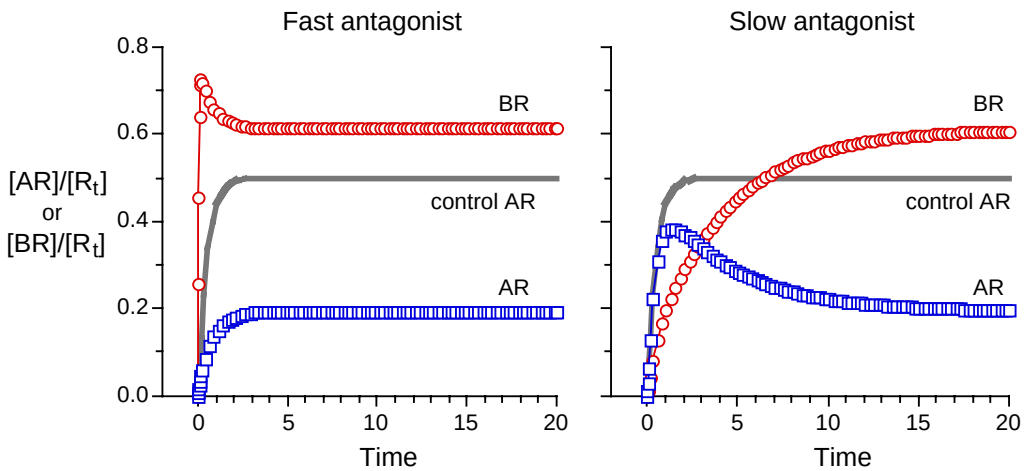

Fig. 2. Binding time-courses for ligands A and B predicted by Model 1 with both ligands applied at time = 0. Antagonist (ligand B) was either 10 times faster (left panel), or 10 times slower (right panel) than the radioligand (ligand A), or absent (grey line, both panels). Note that the binding of both ligands is shown whereas in real experiments only the radioligand binding (i.e. AR) is directly determined. Model parameters were: $k_1 = k_{-1} = 1$; [A] = 1; $k_2 = k_{-2} = 10$ or $k_2 = k_{-2} = 0.1$; [B] = 0 or [B] = 3.2 (i.e. log[B] = 0.5).

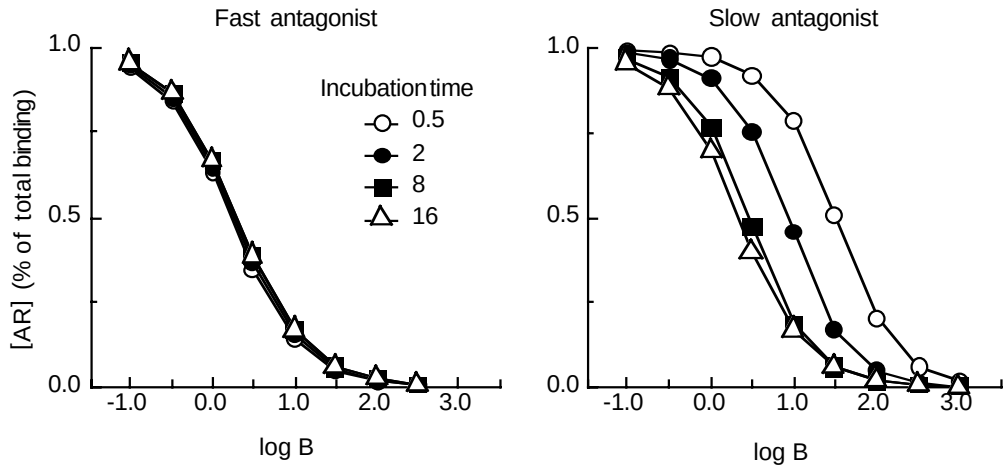

Fig. 3. Concentration–inhibition curves predicted by Model 1 for a fast antagonist ($k_2 = k_{-2} = 10$, left panel) and a slow antagonist ($k_2 = k_{-2} = 0.1$, right panel) obtained from simulations running for 0.5, 2, 8 or 16 time units. Radioligand parameters were $k_1 = k_{-1} = 1$; [A] = 1. Radioligand and antagonists were co-applied at time = 0. Note that the slow antagonist becomes more potent as incubation time increases whereas the fast antagonist does not.

conditions have been attained. It is clear from the aforegoing that Model 1 predicts exactly the behaviour found by Verheijen *et al*. in their real binding experiments and that no embellishment of the simpler model is needed to explain the data.

In our simulations we made no attempt to apply real-world values to the model parameters because we believe that too many assumptions, approximations and arbitrary values are required for such an approach to be generally fruitful [4]. Verheijen *et al*. [1] used parameter values were taken from a previous modelling paper [5] where their model was applied to response data. We note that in that previous paper the AngII association and dissociation rate constants were set arbitrarily (presumably those same arbitrary values were used in the current study) and a linear agonist occupancy–response relationship was assumed. Those factors greatly reduce the importance of a quantitative match of the model to the data. Further, because other parameter values were obtained from simulations using the same model there is a circularity in the logic of using quantitative goodness of fit as a criterion by which to judge the merits of a model. We prefer to approach the issue by concluding that if a model is able to predict the observed patterns (i.e. a qualitative match) with reasonable relative parameter values then it can be concluded only that the model meets the minimal criteria to be a valid representation of reality. Thereafter, real experiments have to be designed to critically test the model.

We should not necessarily discard the idea of multi-stage binding by antagonists just because a model without the hypothesised second stage can account for the data. However, if a simple model can explain data as well as a more complex model then we need to consider carefully whether the extra features of the complex model reflect reality, and even whether they are useful. It is unfortunate that a paper [1] whose title implies strong support for a particular hypothesis doesn't contain any critical test of that hypothesis.

References

[1] Verheijen I, De Backer J-P, Vanderheyden PML, Vauquelin G. A two-state model of antagonist-AT_1 receptor interaction: further support by binding studies at low temperature. Biochem Pharmacol 2003;65:1339–41.
[2] Lew MJ, Ziogas J, Christopoulos A. Dynamic mechanisms of non-classical antagonism by competitive AT_1 receptor antagonists. Trends Pharmacol Sci 2000;21:376–81.
[3] Lew MJ, Christopoulos A, Ziogas J. Non-surmountable antagonism: transcending steady state. Trends Pharmacol Sci 2001;22:65–6.
[4] Lew MJ, Christopoulos A, Ziogas J. Insurmountable AT_1 receptor antagonism: message in a model? Trends Pharmacol Sci 2001;22:555–7.
[5] Vauquelin G, Morsing P, Fierens FLP, De Backer J-P, Vanderheyden PML. A two-state receptor model for the interaction between angiotensin II type 1 receptors and non-peptide antagonists. Biochem Pharmacol 2001;61:277–84.