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Kinetic versus allosteric mechanisms to explain insurmountable antagonism and delayed ligand dissociation

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Abstract

The present review addresses the theories which have been advanced to explain experimental observations dealing with insurmountable antagonism and accelerated radioligand dissociation in the presence of an excess unlabelled ligand. We came to the perception that, for each of these phenomena, the theories can be placed into two distinctive categories. The ''kinetic'' interpretations attribute these phenomena to, respectively, the ability of antagonists to form long-lasting complexes with their cognate receptor and the ability of dissociated ligands to bind again to the same or neighbouring receptors rather than to diffuse away from the cell surface. On the other hand, these observations can also be explained by negative allosteric interactions among topographically distinct ligand binding sites at the same receptor or di/multimeric receptor complex.

Keywords: Receptor; Antagonist; Radioligand; Dissociation; Kinetics; Rebinding; Non-competitivity; Negative cooperativity

From a basic science and drug development viewpoint, it is important to understand the molecular mechanisms behind the experimental observations dealing with insurmountable antagonism and accelerated radioligand dissociation in the presence of excess unlabelled ligand. The present review addresses the theories which have been advanced to explain these observations.

1. Insurmountable antagonism

Pre-clinically, receptor antagonists are often tested for their ability to affect agonist dose–response curves. Till about a decade ago, this information was obtained by performing the so-called ''organ bath'' experiments with intact tissues. In those experiments, the tissue is invariably pre-incubated with the antagonist and then challenged with increasing concentrations of agonist (Leff and Martin, 1986) (Fig. 1). Rather than simply provoking a concentration-dependent rightward shift of the agonist dose–response curve (i.e. a behaviour to be expected for competitive antagonists) quite a number of antagonists are found to depress the maximal response in those experiments. Because on this apparently ''non-conform'' behaviour, antagonists need to be divided into two categories (Vauquelin et al., 2002) (Fig. 1). Surmountable antagonists are those which produce parallel rightward shifts of the agonist dose–response curves with no alteration of the maximal response. On the other hand, insurmountable ones also depress the maximal response with or without a clear-cut rightward shift of the agonist dose–response curve. In this respect, it is not uncommon that, for the same receptor, distinct antagonists display large differences in the extent to which they are capable of depressing the maximal response (Kukkonen et al., 1997; Fierens et al., 1999a). Several theories have been put forward to explain insurmountable antagonism at the molecular level (see below). Yet, because of technical limitations that are typical to organ bath experiments (such as the necessity for consecutive cumulative dosing to produce agonist dose–response curves and the difficulty to generate such curves based on pure co-incubation experiments, Fig. 1) it was often impossible to unequivocally attribute the appropriate explanation to each individual situation.

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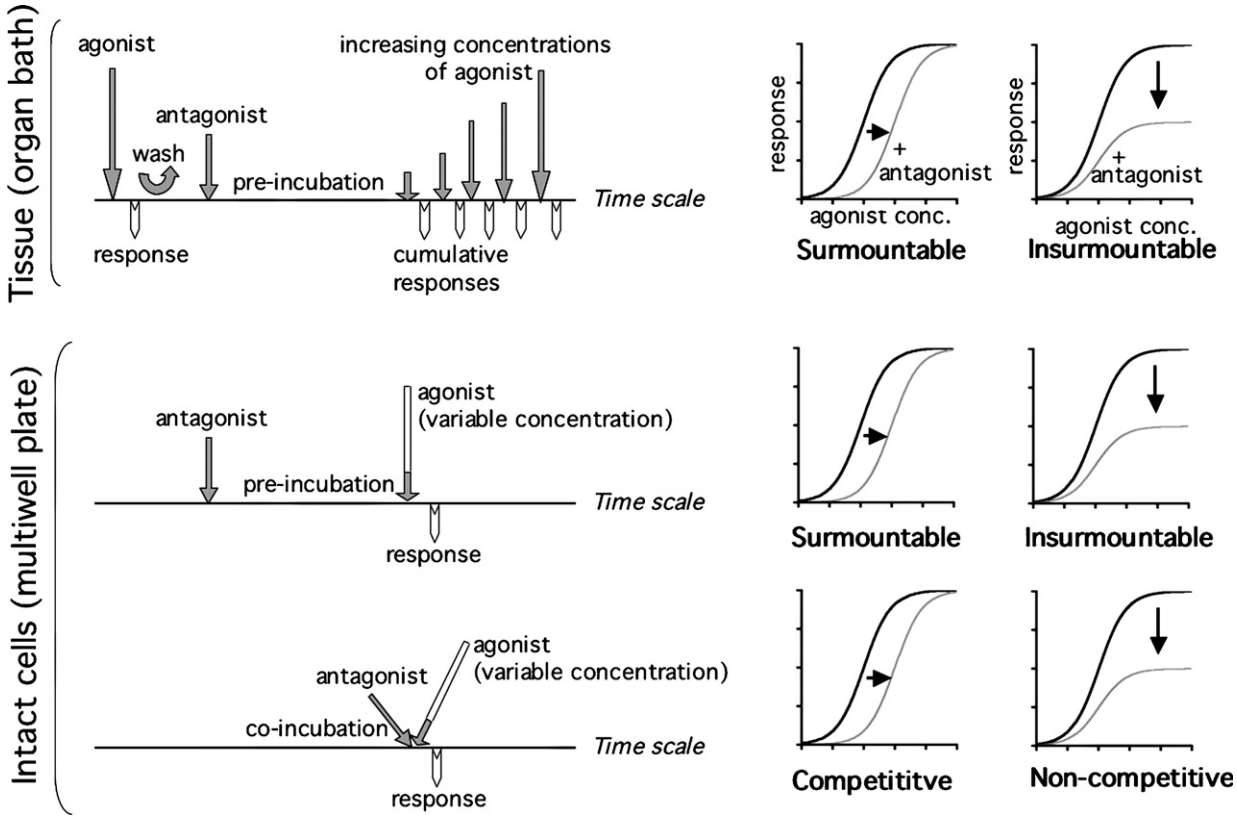

Fig. 1. *Left panel*: Timing of experimental manipulations to study the effect of antagonists on agonist concentration–response curves on intact tissues (organ bath experiments—antagonist pre-incubation only) and on intact cells (multiwell plate experiments—antagonist pre and coincubation possible). *Right panel*: Possible effects of a single antagonist concentration on the agonist concentration-response curve. Only rightward shift of the curve corresponds to surmountable inhibition in the case of antagonist pre-incubation and competitive inhibition in the case of co-incubation. A decrease in the maximal agonist response corresponds to insurmountable inhibition in the case of antagonist pre-incubation and non-competitive inhibition in the case of co-incubation.

More recently, cell lines expressing either endogenous receptors of interest or recombinant cell lines (i.e. transfected with the gene coding for such receptors), have been introduced to get further insight in the molecular mechanism of insurmountable antagonism for α_2 adrenergic (Kukkonen et al., 1997; Pihlavisto and Scheinin, 1999; Bodenstein et al., 2005) and other G protein-coupled receptors (GPCRs) (Fierens et al., 1999; Tashiro et al., 1999; Christopoulos, 2001). The physiological relevance of this approach is supported by the similarity in behaviour of the AT_1 receptors in recombinant Chinese Hamster Ovary cells (CHO cells) and in more complex *in vitro* experimental systems like contraction studies with vascular smooth muscle preparations (Vanderheyden et al., 1999). Yet, compared to isolated tissues, cell lines offer the major advantage that receptor occupancy by the antagonist (directly measured by binding studies with radiolabelled antagonists) can be directly compared to its functional consequence (i.e. the ability of the antagonist to decrease the agonist-evoked response) (Fierens et al., 1999b). This, together with the ability to perform both types of assays under a wide range of experimental conditions, greatly improves our understanding about the molecular mechanism of insurmountable antagonism.

Potential explanations for insurmountable antagonism were already accommodated into two major categories some sixty years ago (Gaddum et al., 1955). One series of explanations stipulates that insurmountable antagonism may arise from non-competititive interactions. This includes antagonists that interrupt an essential link in the chain of agonist-evoked post-receptor events by acting on cellular sites that are distinct from the receptor (Ariens et al., 1956). Such ‘‘functional’’ antagonism may, e.g. explain the insurmountable inhibition of the α adrenergic receptor evoked vasoconstriction by calcium channel antagonists (Ljung, 1985). Non-competitive antagonists could also bind to allosteric sites at the receptor (i.e. sites that are topologically distinct from the agonist binding site) to induce a conformational change in the receptor that prevents its stimulation (Christopoulos and Kenakin, 2003). In addition, whereas with the exception of metabotropic GABA receptors and other family C/class 3 receptors (Pin et al., 2005), GPCRs were previously only supposed to act as monomers, there is now increasing evidence that many of them are capable to form homodimeric and heterodimeric complexes as well (Rios et al., 2001; Pin et al., 2007). Whether or not, receptors remain side by side or swap some of their transmembrane domains during the heterodimerisation process (Gouldson et al., 1997), two ligand-binding pockets with potentially different pharmacological profile are likely to be formed. This offers the opportunity for allosteric interactions to take place, not only between both binding pockets (Urizar et al., 2005) but also between a binding