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Digest

Influence of the cellular environment on ligand binding kinetics at membrane-bound targets

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ABSTRACT

While historically 'in vitro' binding data were obtained by analyzing equilibrium experiments, kinetic data are increasingly appreciated to provide information on the time a particular compound remains bound to its target. This information is of biological importance to understand the molecular mechanism of a drug not only to evaluate the time a particular receptor/enzyme is blocked in the case of antagonists/inhibitors but also to investigate its contribution to the efficacy to mediate signaling in the case of agonists. There is accumulating evidence that many drugs binding to either membrane-bound receptors or enzymes are found to display long duration of action which can be ascribed to slow dissociation from their target proteins. In the present review three such examples are discussed which encompass ligands that bind to membrane-bound proteins and from which it appears that the tight binding kinetics is influenced by the cellular/membrane environment of the target protein.

Introduction

Most often the strength by which a drug binds to its target receptor/enzyme is determined on basis of equilibrium affinity measurements. However extrapolation of these data to 'in vivo' situations is certainly not straightforward principally because the reaction between a drug and its target never reaches equilibrium 'in vivo'. With this respect the 'in vivo' ligand-receptor interaction can be considered to take place in an 'open system' in which the ligand continuously flows in and out the receptor compartment.[1] Therefore and in contrast to the 'in vitro' binding assays the free ligand concentration constantly fluctuates. As a consequence the rate by which a ligand binds to a receptor cannot be determined accurately 'in vivo'. On the other hand dissociation of a ligand obeys first-order kinetics which makes its determination (at least theoretically) independent of the used methodology such as for instance 'in vitro' in cells/membranes or 'in vivo'.[2] With this respect, ligand residence time at membrane-bound receptors or enzymes is increasingly appreciated as an important parameter in the optimization and development of novel drugs.[3] Simply said ligand residence time refers to the time a particular compound remains bound to its target. This property is inversely related to the ligand dissociation rate. When studied at membrane-bound targets such as typically G-protein coupled receptors (GPCR's), having knowledge of the ligand residence time of antagonists is of great importance in understanding not only their mode of action but also their duration of action such as blocking a particular biological process. Furthermore receptor residence time of agonists is able to contribute significantly to the potency as well as the agonist's efficacy to trigger receptor-mediated signaling and thus also improves our knowledge of agonists.[3] The last decade a number of excellent reviews have appeared focusing on the definition as well as determination of ligand residence time.[2–4] Moreover these reviews put forward arguments why the concept of ligand residence time of new compounds merits attention for current pharmacologists and anyone who is involved in drug development. When studying this parameter we and several other research teams found that certain ligands display tight binding to their target receptor i.e. long receptor residence time, while this behavior was often overlooked in classical equilibrium binding studies. Evidently such a tight binding was dictated by a strong interaction between the ligand (or part of its structure) and the corresponding binding site on the target receptor. Tight binding of ligands has a profound impact on a number of 'in vitro' and likely also 'in vivo' pharmacological properties as will be discussed in particular examples discussed in the next chapters of this review. Last but not least we will, in these examples, discuss how the cellular and/or lipid environment is able to affect the formation of such tight binding complexes and thus alters the binding kinetics.

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Binding kinetics Here the basic parameters are described that characterize the binding kinetics of the non-covalent interaction of a compound with a receptor protein. A receptor can be a membrane-bound or soluble receptor, an enzyme or any protein involved in the function of this compound. For simplicity only the kinetics parameters for a bimolecular reaction are outlined:A bimolecular reaction is defined by the rate the complex is formed (binding kinetics with an on-rate constant: k_{on}) and the dissociation rate (unbinding kinetics with an off-rate constant: k_{off}) which have as unit 1/(concentration time) and 1/time respectively.

$$R + L \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} RL \quad (1)$$

In this equation R is the "receptor" protein but which can be any kind of protein with which the compound L associates with a reversible bimolecular reaction. L is a compound and RL the receptor-ligand complex. When the on- and off-rate balance each other or, in other words, when there is an equilibrium between both reactions then:

$$k_{on}[R][L] = k_{off}[RL] \quad (2)$$

From this equation the equilibrium dissociation constant K_d is defined as

$$K_d = k_{off}/k_{on} = [R][L]/[RL] \quad (3)$$

The rate of the formation of the RL complex follows a pseudo-first-order reaction with a rate constant of k_{obs}. As k_{obs} depends on the concentration of L, the true k_{on} can be calculated by:

$$k_{on} = (k_{obs} - k_{off})/[L] \quad (4)$$

The rate by which a compound dissociates from its receptor follows a first order reaction which is quantified by the off-rate constant (k_{off}; unit is min^{-1} or s^{-1}). This constant can be converted into the ligand-receptor half-life ($t_{1/2}$; unit = min or s) which is equal to $0.693/k_{off}$ or to the receptor residence time (RT; unit is min or s) which is equal to $1/k_{off}$.

Binding kinetics of insurmountable angiotensin II type 1 receptor antagonists

The renin-angiotensin system plays a crucial role in the regulation blood pressure and water volume homeostasis.[5] The major hormone of this system is the octapeptide angiotensin II (Ang II) that activates the Angiotensin II type 1 receptor (AT_1R) which is typical member of the large subfamily of rhodopsin-like GPCR, resulting in a wide range of (patho)physiological effects including increase in blood pressure, inflammation and proliferation of vascular smooth muscle cells.[6] Consequently the development of non-peptide AT_1R antagonists was and is a substantial contribution in the treatment of hypertension and congestive heart failure.[7–10] Among these antagonists we evaluated the 'in vitro' binding properties of losartan and its more active metabolite EXP3174, valsartan, irbesartan and candesartan (the active metabolite of candesartan cilexetil), all molecules that have a common biphenyl-tetrazole ring structure but with different side chains[11] (Fig. 1).

Interestingly striking differences emerged when Ang II concentration-response curves were generated in antagonist pre-incubated isolated aorta rings/strips and in CHO cells expressing human AT_1 receptors. With this respect compounds such as losartan and eprosartan produce rightward and parallel shifts of the concentration- response curves which are typical for classical competitive /surmountable antagonists. On the other hand the other investigated antagonists decrease either completely (candesartan) or partially (EXP3174, valsartan, irbesartan) the maximal response to Ang II[11,12] (Fig. 2). The underlying molecular mechanism of this particular inhibitory pattern is extensively discussed in previous review articles and it appears that all investigated antagonists are competitive towards the orthosteric ligand Ang II and that insurmountable inhibition was closely linked to the rate by which the antagonists dissociate from the receptor.[13] Consequently it was meaningful to investigate and compare the kinetic properties of these antagonists.[14] Methodologically our approach was to determine the ligand-receptor residence time (which is equivalent to the inverse of the dissociation rate) in an experimental setup that is in full measure with functional experiments. With the exception of [^{35}S]-GTPγS binding assay most functional readout systems for GPCR involve intact living cells and therefore the antagonist dissociation rate was quantified on CHO-cells stably expressing AT_1 receptors and on vascular smooth muscle cells that endogenously express AT_1 receptor. The following methodologies were used: (i) Recovery of Ang II induced IP accumulation after antagonist pre-incubation and washout (ii) Measurement of the slowing of the association of [^{3}H]-antagonist binding to intact adherent cells after antagonist pre-incubation and removal of unbound unlabeled antagonist, a method first described by Hara et al.[15] (iii) Direct measurement of [^{3}H]-labeled antagonist dissociation either after isotopic dilution or by washout of the radioligand. From these kinetic experiments it appeared that comparable dissociation rate constants for all antagonists were obtained irrespective of the used method (see a summary of the values in Table 1). Moreover these detailed kinetic experiments indicated that the different degree of insurmountable inhibition was compatible with a model in which in a first step all antagonists binding very fast and reversible (formation of ANT-R), as characterized by k_1 and k_{-1} (see Eq. (5)). Subsequently certain antagonist-receptor complexes such as for candesartan, are converted into a tight binding/slowly reversible state (Ant-R*).[16] These reactions can be represented by:

$$ANT + R \underset{k_1}{\overset{k_1}{\rightleftharpoons}} ANT - R \underset{k_2}{\overset{k_2}{\rightleftharpoons}} ANT - R^* \quad (5)$$

The equilibrium between both states (k_2 and k_{-2}) or, in other words, whether ANT-R* will be formed, is dictated by the antagonist structure. In line with this notion a strong ionic interaction between a carboxylic acid group at the benzimidazole moiety of certain of the antagonists such as candesartan and EXP3174 and the $Lysine_{199}$ residue in the transmembrane helix5 of the AT_1 receptor is proposed to be involved in the formation of the tight antagonist-receptor complex.[17] On the other hand when this carboxylic group is absent as in losartan only weak binding occurs and no tight antagonist-receptor complex can be formed (Fig. 3). In support of this model mutation of Lys_{199} mainly affects the binding affinity of carboxylic containing and insurmountable antagonists such as candesartan and EXP3174 while the binding losartan is only weakly affected. On the other hand mutation of Arg167 abolishes the binding of all antagonists.[17]

In an alternative docking model it is proposed that the benzimidazole carboxylic group of candesartan is strongly interacting with Arg_{167} in extracellular loop2 while $Lysine_{199}$ may form a salt-bridge with the tetrazole moiety of candesartan.[43] Surprisingly tight antagonist binding is influenced by the cellular context of the receptor. When the binding of [^{3}H]-candesartan was evaluated its dissociation was substantially accelerated when other unlabeled AT_1 receptor ligands were included in the washout buffers.[18] A possible explanation was that the (tight) binding of antagonists is modulated by the binding of other AT_1 receptor ligands to a distinct binding site inducing a conformational change of the receptor resulting in a faster dissociation of [^{3}H]-candesartan. Such a mod-