Time is of the essence - investigating kinetic interactions between drug, endogenous neuropeptides and receptor

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Summary

In this thesis, the kinetic binding parameters of endogenous ligands and drug candidates and their effect on signal transduction are examined to provide a better understanding of drug-target interactions. While drug discovery programs are traditionally focused on equilibrium-based parameters such as affinity values, drug candidates often fail in clinical trials due to on and/or off target toxicity and/or lack of *in vivo* efficacy. In the past decade, drug-target binding kinetics, i.e. association and dissociation rate constants, are increasingly acknowledged as better predictive parameters of *in vivo* drug action and it is proposed to incorporate these parameters to decrease drug attrition rates and improve the drug discovery paradigm. To investigate the role of binding kinetics in ligand-receptor interactions, two G protein-coupled receptors (GPCRs) are used as model systems, namely the gonadotropin-releasing hormone (GnRH) receptor and the neurokinin 1 (NK1) receptor. Both receptors can be categorized in the neuropeptide receptor family and this receptor family plays a crucial role in the peripheral and central nervous system making them interesting targets in therapeutic areas such as epilepsy, pain and psychological disorders. An in-depth background on neuropeptide receptors, binding kinetics and kinetic assays is provided in **Chapter 1**.

Since drug candidates often compete with endogenous ligands in the body, more knowledge on the interactions between endogenous ligand and receptor could aid in understanding desired (kinetic) interactions of a drug candidate with that receptor. In **Chapter 2**, novel insights into the kinetic profile of endogenous neuropeptides and their receptors are considered. The binding kinetics, internalization kinetics and release kinetics of three exemplary neuropeptide-receptor pairs are reviewed and these kinetic parameters proved to be quite variable. Collectively, this review provides a perspective for future drug research to include the kinetic profile of the target receptor and its endogenous ligand(s). This will improve the understanding of desired drug-target binding kinetics and thus lead to more efficacious drugs.

One of the challenges in examining binding kinetics is the lack of robust kinetic assays suitable to study these kinetic binding parameters. To overcome this hurdle, a selection of well-known GnRH receptor drugs is used to design and validate kinetic radioligand binding and TR-FRET protocols in **Chapter 3**. A competition association assay is designed for both kinetic assays and this facilitated the determination of the kinetic binding parameters of 12 unlabeled GnRH analogs. Both affinity values and values of dissociation rate constant are highly correlated between both kinetic assays. Additionally, the association and dissociation rate constants of the tested GnRH drugs are very divergent, indicating a pivotal role of binding kinetics in drug-target interactions. This research provides new

perspectives by incorporating kinetic binding parameters in current research which could potentially improve future drug discovery targeting the GnRH receptor.

The functional effects of two kinetically diverse GnRH agonists, i.e. GnRH and buserelin, from **Chapter 3** are further studied in **Chapter 4**. A morphology-based real-time assay is found to be suitable for studying receptor-mediated responses. Persistent signal transduction profiles are observed for both agonists. However, wash-out experiments prove that the persistent signaling profile of fastly dissociating GnRH is most likely due to ligand rebinding while the persistent signaling profile of slowly dissociating buserelin is presumably due to a long-lasting receptor binding profile. This study stresses the impact of slow dissociation rates for long-lasting receptor activation and could support future research towards drugs with prolonged efficacy.

In **Chapter 5**, another well-known neuropeptide receptor is examined, namely the NK1 receptor. Considering the importance of knowledge of the binding kinetics of not only drug candidates but endogenous ligands discussed in **Chapter 2**, the association and dissociation rate constants of endogenous tachykinins and a few close analogs are examined. Interestingly, the binding kinetics of the tested tachykinins are very diverse, particularly the association rates. Furthermore, kinetic binding parameters are highly correlated to signal transduction values such as *in vitro* potency and maximal response values. Our findings demonstrate the great variability in binding kinetics of these tachykinins and underline the importance of measuring the kinetic binding parameters of not only drug candidates but also endogenous ligand(s).

Chapter 6 is focused on elucidating the missing link between binding kinetics and signal transduction to improve the understanding of drug action *in vivo*. The effects of two NK1 receptor antagonists with variable dissociation rates are examined using two endogenous tachykinins with variable association rates. We found that the divergent kinetic profiles of both antagonists and endogenous agonists resulted in different signal transduction profiles. Moreover, these findings are consistent throughout multiple assay formats, cellular backgrounds and mathematical simulations. This research emphasizes that knowledge of the relationship between drug-target binding kinetics and cellular responses is important for improved understanding of drug efficacy.

In summary, multiple kinetic binding assays (i.e. radioligand binding and TR-FRET studies) and kinetic functional assays (i.e. real-time cAMP and real-time morphology studies) are designed and validated to study binding kinetics and their role in signal transduction of numerous endogenous ligands and drug candidates. Significant differences in the kinetic profiles of endogenous neuropeptides and well-known drugs are observed and this kinetic

variability triggered differential functional effects *in vitro*. These overall conclusions are discussed in **Chapter 7**. Finally, the findings in this thesis could contribute to a larger toolbox suitable for studying kinetic ligand-receptor parameters. Moreover, knowledge of the kinetic binding parameters of drugs and endogenous ligands could play a pivotal role in understanding ligand-receptor interactions and result in an improved drug discovery paradigm.