Allosteric modulation and ligand binding kinetics at the Kv11.1 channel

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Summary

The superfamily of ion channels is a major focus of drug discovery and development programs in the pharmaceutical industry due to their involvement in a number of physiologic functions. However, the binding of several drugs to ion channels is also responsible for a huge number of side effects, and pharmaceutical safety is routinely required by regulatory agencies for certain ion channels, in particular cardiotoxicity mediated by the K_v11.1 channel. All these subjects have been generally discussed in **Chapter 1** of this thesis.

Chapter 2 is a literature-based review describing research progress on the K_v11.1 channel. In this chapter, we focused on kinetic studies of the channel, including the unique gating kinetics and ligand binding kinetics. In addition, biological production and degradation, structural features, physiological functions, and on- and off-target applications of the K_v11.1 channel were briefly introduced. A thorough analysis of the K_v11.1 channel is beneficial for the improvement of drug design and reduction of unwanted arrhythmic side effects caused by the blockade of this channel.

In **Chapter 3**, a previously disclosed series of compounds together with three reference compounds, were selected and evaluated for the allosteric modulation of the K_v11.1 channel in [^{3}H]astemizole and [^{3}H]dofetilide binding assays. One potent negative allosteric modulator (LUF6200) was identified in radioligand dissociation assays, while potassium ions were found to be positive allosteric modulators. These two modulators interacted with the K_v11.1 channel via separate allosteric sites with positive cooperativity towards each other. Taken together, this investigation provides direct evidence for allosteric modulation of the K_v11.1 channel.

As an extension of the research described in chapter 3, three compounds (ML-T531, VU0405601 and LUF7244) were extensively evaluated for their negative allosteric effects on the K_v11.1 channel using different [^{3}H]dofetilide binding assays in **Chapter 4**. The novel modulator LUF7244 was further assessed for its possible antiarrhythmic propensity in radioligand binding experiments and voltage sensitive optical mapping in a newly validated neonatal rat ventricular myocyte (NRVM) model. The channel affinity of three blockbuster drugs (astemizole, sertindole and cisapride) that have all been withdrawn from market due to their K_v11.1 blockade, was diminished in the presence of LUF7244. Furthermore, the heterogeneous prolongation of the action potential duration (APD) and/or a

high incidence of early afterdepolarizations (by astemizole and sertindole only) induced by these K_v11.1 drugs were normalized by pretreatment with 10 μM LUF7244 in the NRVM cultures. Intriguingly, LUF7244 *per se* did not affect the APD and the viability, excitability and contractility of the cardiomyocytes, and exerted no obvious influence on the intentional blockade of the human histamine H_1 receptor by astemizole. Accordingly, these results indicate that further development of LUF7244 raises an opportunity for antiarrhythmic drugs via combination therapy.

Subsequently, in **Chapter 5** the synthesis and allosteric evaluation of 29 compounds, which share the same chemical scaffold as LUF7244, were described. Most compounds emerged as negative allosteric modulators at the K_v11.1 channel, and the structure-activity relationships of all these ligands were established. Importantly, compounds **7f** and **7p** were more potent than LUF7244 in negatively modulating the channel, implying promising antiarrhythmic propensities superior to LUF7244.

A new [^{3}H]dofetilide competition association assay was successfully validated in **Chapter 6**, and was further utilized to determine the kinetic parameters of fifteen prototypical K_v11.1 blockers. In contrast to a classical ligand-receptor interaction mechanism, the affinity of these blockers was predominantly regulated by their association rates instead of dissociation rates, implicating the role of association rates in K_v11.1 blockade. Thereafter, membrane affinity of all these ligands was measured in an immobilized artificial membrane column using HPLC. Similar to general calculated physiochemical properties, it is shown in chapter 6 that membrane interactions of K_v11.1 blockers did not significantly impact their affinity and ligand binding kinetics.

Since dissociation rates or residence times (RTs) of all K_v11.1 blockers in chapter 6 only exhibited a 10-fold difference, a library of 46 compounds over a wide range of chemical scaffolds were tested for their affinity and kinetic data in **Chapter 7**. K_v11.1 blockers were discovered with up to a 300-fold difference in RTs (0.34 min for compound **37** versus 105 min for **38**), which enabled the investigation of the structure-kinetics relationships next to structure-affinity relationships. Afterwards, a "k_{on}-k_{off}-K_D" kinetic map was constructed based on the kinetic parameters of these compounds together with those of three reference blockers (astemizole, ranolazine and dofetilide). This kinetic map offers a probable framework for a further and more precise categorization of K_v11.1 blockers, inferring distinct proarrhythmic risks. Additionally, two representative compounds, **21** and **38**, were measured in a whole-cell patch clamp assay. It was found that the potency (IC_{50}) of these two ligands was influenced by their RTs, while the I_{Kr} inhibition rates were in line with their association rates. Therefore, association rates and RTs

of K_v11.1 blockers are strongly suggested to be incorporated next to their affinity values into the future paradigm for proarrhythmia safety assessment.

Finally, general conclusions of all these chapters were summarized and the corresponding future research directions based on all findings in this thesis were proposed in **Chapter 8**. In conclusion, allosteric modulation and ligand binding kinetics at the K_v11.1 channel have been intensively discussed in this thesis. Negative allosteric modulators of the K_v11.1 channel open a new avenue for mitigating unintentional, arrhythmic effects of drug candidates via pharmacological combination therapy. Likewise, incorporation of ligand binding kinetics, next to affinity for the K_v11.1 channel, into the new Comprehensive *in vitro* Proarrhythmia Assay (CiPA) could improve the traditional screening paradigm for assessment of K_v11.1-induced cardiotoxicity. Hopefully, considering kinetic parameters of K_v11.1 blockers at the channel will lead to the design of safer drug candidates as well as to less drug attrition in the near future.