A COMPARATIVE STUDY OF SODIUM CHANNEL INHIBITORS USING ELECTROPHYSIOLOGY AND CHEMINFORMATICS

Doctoral thesis

Nóra Lenkey , MD

Semmelweis University
Szentágothai János School of PhD Studies

Supervisor: Árpád Mike, senior scientist, PhD

Official referees: Valéria Kecskeméti, professor, MD, PhD
Pál Kocsis, PhD

Committee members at the comprehensive exam:
Klára Gyires, professor, MD, PhD, DSc (chairman)
László Köles, assistant professor, MD, PhD
Ildikó Világi, associate professor, PhD

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1. Introduction

In modern drug development the approach of rational drug design is expanding, because of the increasing importance of cost- and time-effectiveness. Chemical structure and *in vitro* potency of molecules can be used to perform structure-activity relationship studies, which may serve as a basis for *in silico* drug design.

The considerable versatility of the patch-clamp technique could not be exploited because of the low throughput of the method until the recent advent of automated electrophysiology techniques. This method is especially effective in the study of voltage gated ion channels, where alternative high-throughput methods, which only yield an IC_{50} value (binding tests, ion flux assays, fluorescent methods), fail to percieve voltage-dependent gating and state-dependent affinity of drugs, which are essential in predicting therapeutic effectiveness.

We performed a comparative study on the mode of action of sodium channel inhibitors (SCIs) using „manual" and automated patch-clamp methods (Lenkey et al., 2006; Lenkey et al., 2010, Karoly et al., 2010).

1.1 Development of sodium channel inhibitors

Sodium channel inhibitor (SCI) drugs have been used in therapy for more than a century; the classic therapeutic categories are local anesthetics, class I antiarrhythmics and anticonvulsants. Novel SCIs are actively searched for the treatment of various pain syndromes, spasticity, tremor, ischemic neuronal damage, neurodegenerative diseases, and psychiatric diseases. Sodium channels are currently among the most intensively studied ion channel targets because of the attractive therapeutic effects, selective SCIs are promising. Nevertheless, despite the considerable effort, a real breakthrough has not yet been reached in this field, mostly because of manifold uncertainities regarding the binding site:

- The only established drug binding site is the „local anesthetic receptor", which is found within the pore region of the channel.
- For several SCIs it is uncertain if they indeed bind to the local anesthetic binding site.
- For those drugs which do bind to the local anesthetic binding site (based on mutagenesis data), the identity of residues involved in drug binding seems to vary from drug to drug.
- Even if drugs bind to the same residues in the pore region, they may have different modes of action, because the binding sites are supposed to be different in different conformations of the protein, and individual drugs may have different preference for specific conformations.

The complexity of the problem is also shown by the fact that many of the residues which were found to be important in drug binding are not located within the inner vestibule of the channel, but either points outward from the pore-lining alpha helices, or only accessible from the extracellular side. Furthermore, thus far it is only the pore region that has been explored by mutagenesis, it is concievable therefore that other regions may also accomodate drug binding sites.

In addition, properties of inhibition are not solely determined by drug–protein binding site interactions, but also by the interactions and reactions on the way to the binding site: partitioning into the membrane, deprotonation, entering the vestible between the alpha helices of the channel, etc (all these processes require different chemical properties).

Taking into account all these considerations, it is not at all surprising that in silico modeling of the binding site has not been successful thus far.

However, for computer aided drug development, knowledge of the binding site within the protein is not absolutely necessary. Structure-activity relationship studies may establish the required chemical structure of the ligand itself, which may indirectly give information regarding the structure of the binding site.

Several structure-activity relationship studies for SCIs have been performed within different structural classes of compounds (mostly local anesthetics, antiarrhytmics or antiepileptics). However, a generally applicable pharmacophore model for SCIs have not been established because of their extreme structural diversity. In a recent study 400 drugs have been screened for their effect on sodium channels, and ~25% were found to be potent SCIs. Structure-activity relationship studies for a chemically diverse group of SCIs have not yet been performed.

The other weakness of structure-activity relationship studies is, that they are most often based on potency (IC_{50}) values alone. This is a problem for several reasons. First of all, potency or affinity is not informative regarding therapeutical value of drugs: Many excellent SCI drugs are of low potency (lidocaine, carbamzepine, phenytoin, etc.), and several high affinity inhibitors are not known as sodium channel inhibitors, such as antidepressants, antipsychotics, neuroprotective agents (lifarizine, crobenetine, riluzole), and even eicosapentaenoic acid (Lenkey et al., 2011). The extent of state dependence is probably a much better indicator of potential therapeutic value. Most SCIs have a preferential affinity for open or inactivated states (more exactly to one of the comformational states populated upon depolarization: there are several such states; considering open and inactivated states only is a serious oversimplification). This state preference is thought to be the basis of selective inhibition of pathological activity in cells which fire at a higher frequency, or which are slightly depolarized. For this reason more thorough studies give estimations of resting and inactivated affinities (K_r , K_i). State dependence can be quantified as the K_r/K_i ratio. Beyond state dependence, other properties of inhibition (e.g. binding kinetics, aqueous phase – membrane phase partitioning, equilibrium and kinetics of protonation/deprotonation) also may be important determinants of the inhibitory mechanism of individual drugs.

For this reason, we aimed to perform a comparative study of multiple properties of inhibition for several SCIs, including drugs of various chemical stucture and therapeutic indication. Our

previous study indicated that drugs of different therapeutic indication had different mode of action. If the mode of action and therapeutic profile indeed show correlation, and if chemical properties indeed determine the mode of action, then one could predict therapeutic profile based on chemical structure.

1.2 Mode of action of sodium channel inhibitors

Classic SCIs (local anesthetics, class I. antiarrhytmics and certain anticonvulsants) are considered to share their mode of action and their binding site: the local anesthetic receptor. This binding site can be reached by either the „hydrophilic" or the „hydrophobic" route, i.e., from the cytoplasm, or from the membrane phase, respectively.

SCI drugs have been observed to show several characteristic features of inhibition in electrophysiology experiments. The inhibition was:

- Use-dependent (it became larger upon repetitive activations.)
- Frequency-dependent (the higher was the frequency of depolarizations, the larger the inhibition became).
- Holding potential-dependent (the degree of inhibition depended on the holding potential used before the test-pulse; more depolarized membrane potential meant larger inhibition).
- The steady-state availability curve was shifted to the left by the drugs (due to their inactivated state preference).

All these phenomena can be explained by state-dependnce, i.e. that the drugs have higher affinity to depolarized (inactivated) conformations of the channel.

The mode of action of sodium channels is often studied by testing these properties of inhibition. Since most SCIs behave similarly in these tests, this gives the impression that all SCIs indeed have the same mode of action. However, a thorough survey of the literature led us to conclude that although there is no irrefutable direct evidence for this, alternative binding sites and

alternative modes of action must exist, for the following reasons: 1) results of mutagenesis studies do not support a single binding site, 2) the extreme chemical diversity of potent SCIs, 3) a few insightful studies on the molecular details of drug action.

1.3 Structure-activity relationships of sodium channel inhibitors

Structure-based drug design will surely remain only a distant goal for some time for SCIs. The 3D structure of the channel has not been determined, and even when it will be, we will need to know which specific conformation is relavant for the binding of a specific drug. Ligand based approaches may give useable results sooner. In this case, structure-activity relationships have to be established first. The study of antiarrhytmics and local anesthetics during the 1980s already established some basic principles:

- More lipophilic molecules tended to be more potent.
- Because local anesthetics are predominantly charged, basic compounds, a pKa value between 7.5 and 10 was considered a requirement for being an effective SCI. (However, SCI anticonvulsants are predominantly neutral, and several very potent neutral SCIs has been discovered recently. In a recent comparative analysis of 139 compounds from 73 publications we found no correlation between pKa and potency (Lenkey et al., 2011).)
- The pKa value nevertheless is a major determinant of passable drug access pathways (accessibility was found to profoundly depend on the drugs ability for deprotonation), and therefore pKa has been shown to affect onset/offset kinetics and use-dependence.
- Molecular weight has been shown to correlate with potency (which is probably due to the correlation between molecular weight and lipophilicity).

- The geometry of the molecule (width of the molecule at the aromatic end) has been shown to correlate with kinetics of inhibition.

In summary, lipophilicity, pKa (acidic dissociation constant), and the size of the molecule were proposed to be the most important predictors of properties of inhibition.

Most of these results were based on studies of a single chemical and therapeutic class of SCIs (antiarrhytmics and local anesthetics). There is no structure-activity relationship study, in which chemically diverse SCIs were examined. Since structure-activity relationship studies must be done using drugs that bind to the same binding site, in the case of SCIs the first step must be to clarify how many different modes of action exist. In order to attempt this, we chose diverse groups of SCIs: we performed experiments using a group of 35 SCI drugs (Lenkey et al., 2010), and we used data extracted from 73 publications (Lenkey et al., 2011).

2. Aims

Our preliminary data suggested, that two antidepressants (the selective serotonin reuptake inhibitor fluoxetine and the tricyclic desipramine) inhibited sodium channels, therefore we performed detailed study on the mechanism of action of these drugs (Lenkey et al., 2006) and of three classical SCIs (lidocaine, carbamazepine, phenytoin) (Karoly et al., 2010). Our questions was:

- Do fluoxetine and desipramine inhibit sodium channels with the same mechanism of action as classical SCIs?

We concluded, that the mode of action for antidepressants and classical SCIs was different. The question arose, how many different modes of action may exist among SCIs. In order to

address this question, we conducted a comparative patch-clamp study of several CNS drugs (Lenkey et al., 2010). We aimed to examine the following questions:

- Are there additional modes of action?
- Are there chemical descriptors which specifically predict individual biophysical properties of inhibition (reversibility, use-dependence, binding kinetics)?
- Most importantly, can we find some difference between descriptors which predict K_r and K_i? Strong state-dependence is considered to be essential for good therapeutical applicability. Which chemical properties predict high state-dependence?

Our aim was to develop a method for characterizing SCIs, which can be adopted by pharmaceutical companies, therefore we used an automated patch-clamp instrument. We think that a comparative study of modes of action can only be done using electrophysiology, and if it is to be done on a large number of molecules, then it must be automated electrophysiology. Pharmaceutical companies nevertheless rarely use automated electrophysiology instruments for studying modes of action, but rather for simple screening tasks. We aimed to investigate if automated patch clamp systems were suitable for comparative mode of action studies (Lenkey et al., 2010):

- Can we use the intsrument for handling complex voltage- and perfusion protocols, which are required in such studies?
- Should we use the these protocols? The answer is not at all trivial. More information regarding the mode of action is not necessarily a gain if performing and analysis of the complex protocols turns out to be more costly. We therefore confined ourselves to protocols which require no more cost or time than a single measurement of inhibition, but which nevertheless can give radically more information regarding the mode of action of drugs.

3. Methods

3.1 Manual patch-clamp

Electrophysiological experiments were performed on hippocampal neurons cultured for 7 to 21 days. Transmembrane currents were recorded by whole-cell or outside-out patch configurations of the standard patch-clamp technique using an Axopatch 200B amplifier, Digidata 1322A digitizer and the pClamp 8.0 software. Borosilicate glass patch pipettes (1.7–4.5 MΩ) were used. Experiments were performed at room temperature. Currents were low-pass–filtered at 10 kHz and sampled at a rate of 100 kHz. Pipettes were filled with an intracellular solution of the following composition: 70 mM CsCl, 70 mM CsF, 10 mM NaCl, 10 mM HEPES, and 10 mM Cs-EGTA; the pH was adjusted to 7.3 with CsOH. The composition of the external solution was 150 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 10 mM glucose, and 5 mM HEPES; pH was adjusted to 7.3 with NaOH. Osmolality was 290-295 mOsm.

Statistical significance was determined using unpaired Student's t test or analysis of variance followed by Tukey-Kramer multiple comparisons test; $p < 0.05$ was considered significant.

3.2 Automated patch-clamp

All automated patch clamp experiments were conducted on QPatch-16X automated patch-clamp instrument (Sophion, Danmark) at Richter Gedeon Plc using HEK-293 cells stably expressing rNav1.2 sodium channels. Composition of the extracellular solution was (in mM): 140 NaCl, 3 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 0.1 $CdCl_2$, 20 TEA-Cl, 5 HEPES, adjusted to pH 7.3. Osmolality was 320 mOsm. The intracellular solution consisted of the following (in mM): 135 CsF, 10 NaCl, 1 EGTA, 10 HEPES, adjusted to pH 7.3 with CsOH. Osmolality: 320 mOsm. Data were sampled at a frequency of 25 kHz and filtered at 5 kHz. The amplifier was controlled and the data were collected by the

Sophion QPatch client software. For initial data analysis the QPatch software was used, further analysis was done using Microsoft Excel, Origin 8 (curve fitting), and Statistica 8.0 (statistical analysis) softwares. Chemical descriptors were generated using JChem for Excel 1.1.1 and Marvin 5.2 software from ChemAxon (www.chemaxon.com).

4. Results

4.1 Detailed comparative study of sodium channel inhibition by fluoxetine, desipramine, carbamazepine, phenytoin and lidocaine (Lenkey et al., 2006)

We studied seven parameters of the inhibition:

- Use-dependence
- Holding potential-dependence
- Shift of the steady-state inactivation curve
- Shift of the steady-state inactivation curve, while changing the prepulse duration (0.4-8s)
- Development of fast inactivation
- Development of slow inactivation
- Recovery from slow inactivation

The first three phenomena are well-known common properties for almost all SCI drugs examined thus far, and they were also observable with fluoxetine and desipramine as well. SCIs seem to be a homogeneous group based on these properties. However, a more detailed analysis of the modes of action revealed that the two antidepressants differ from classic SCIs.

Fluoxetine and desipramine inhibited sodium channels with high affinity, especially at depolarized (i.e physiological) membrane potentials and using repetitive stimuli. Steady-state inactivation curves were shifted by antidepressants and classical SCIs alike, however, in the case of antidepressants, the extent of the shift was

strongly dependent on prepulse duration. Fluoxetine and desipramine seemed to accelerate the development of slow inactivation and delay recovery from the slow inactivated state, while with classical SCIs recovery was unhindered. This behavior most often is considered as evidence for slow inactivated state preference. However, as our group has proven in a simulation study, the same behavior can equally well produced by slow binding kinetics and fast inactivated state preference (Karoly et al., 2010).

The different mode of action, observed with the antidepressants may suggest either alternative binding sites, or alternative access pathways to the binding site.

4.2 Comparative study of sodium channel inhibitors using automated patch-clamp and cheminformatics methods: Analysis of biophysical parameters and chemical descriptors (Lenkey et al., 2010)

With the advent of automated patch-clamp method, it is now possible to perform large-scale comparative studies of modes of action. The aim of our study was not the precise determination of properties of inhibition for specific drugs – this would have required optimization of several protocols for individual drugs – but to compare them, using multiple properties of inhibition. This allows a better assessment of similarities/differences between individual drugs.

In order to obtain comparable data, it is essential to work on the same preparation, and to use identical voltage protocols. The protocols were optimized to be quick, simple, suitable for all studied drugs, and contain as much independent descriptor regarding the mode of action as possible.

We investigated 35 SCI compounds of different chemical structures and therapeutic classes (local anesthetics, class I. antiarrhytmics, anticonvulsants, antidepressants, neuroprotective agents, spasmolitics). We wanted to investigate, if these drugs could be grouped based on the biophysical properties of inhibition

and on chemical properties; furthermore, if these properties correlate with therapeutic indication.

In the first protocol we applied 5 Hz trains of depolarizations, and we calculated potency, reversibility, time constants of onset and offset and use-dependence from the peak amplitudes of evoked currents. Resting state affinity (K_r), and affinity to depolarized states (commonly termed „inactivated state affinity"; K_i), as well as state dependence (K_r / K_i ratio) were calculated from steady-state availability curves.

Recording these 8 biophysical properties made it possible to delineate distinct groups in the multi-dimensional ''biophysical space'', which correspond with distinct types of inhibition. The identified types correlated significantly with therapeutic categories.

At least three distinct types of inhibition were identified:

- 'Type 1' drugs had high potency (K_i 0.73 to 6.1 μM; IC_{50} 14 to 43 μM), slow onset and offset kinetics (time constants between 10 and 53 s), partial reversibility (between 0.2 and 0.6) and use-dependence (1.09 to 1.66). Drugs belonging to this type were mostly antidepressants: fluoxetine, sertraline, paroxetine, amitriptyline, imipramine, desipramine and maprotiline, as well as the antipsychotic haloperidol, and the anxiolytic ritanserin.
- The properties of 'Type 2' drugs were low potency (K_i 17 to 88 μM; IC_{50} > 95 μM), fast kinetics (time constants < 27 s) and almost full reversibility (>0.75) Drugs belonging to this type were the three effective anticonvulsants (carbamazepine, lamotrigine, phenytoin), the Class IB antiarrhythmic lidocaine and mexiletine, as well as diclofenac, venlafaxine, tolperisone, bupropion, ambroxol and memantine. The group can be further divided: lidocaine, mexiletine, ambroxol and tolperisone were use-dependent (UD 1.14 to 1.39). Anticonvulsants, memantine, venlafaxine, bupropion and diclofenac, on the other hand, showed no significant use dependence (0.95 to 1.05).

- A distinct group, ‘Type 3’, was formed by the neuroprotectants flunarizine and lifarizine. These drugs had high potency, very slow kinetics, apparent irreversibility (no recovery within the 200 s of washout within this experimental environment) and no use-dependence.
- Of the remaining 13 drugs 7 were between ‘Type 1’ and ‘Type 2’ (nisoxetine, clozapine, silperisone, mianserine, mirtazapine, ranolazine and trazodone; named ‘Type 4’), 2 compounds between ‘Type 1’ and ‘Type 3’ (the antipsychotics chlorpromazine and chlorprothixene), while the remaining 4 drugs, bupivacaine, flecainide, nefazodone and riluzole seemed to have their own specific type of inhibition.

It is important to note, that some of the compounds (nefazodone, riluzole, flecainide, chlorpromazine, chlorprotixene) had especially high state-dependence (K_r/K_i) value, which is regarded as an indicator of theraputic potential.

In order to quantify differences and test the validity of our subjective classification, we performed a cluster analysis using 7 properties of inhibition (time constant of onset was excluded, because wanted to use concentration-independent properties only). The overall picture well reproduced our subjective classification. The group of tricyclic- and selective serotonin reuptake inhibitor antidepressants (Type 1), and of the two neuroprotective agents (Type 3) were clearly recognized, as well as anticonvulsants of the Type2 group.

Biophysical properties of inhibition reflect the mode of action, which in turn is determined by chemical structure. Therefore, we examined which chemical properties determine specific properties inhibition (i.e., if we can predict the type of inhibition from the chemical structure).

From the analysis we drew the following new conclusions:

- The single most important chemical property that determined potency (K_i and IC_{50}) values, as well as reversibility of drugs, was logP. The correlation values of $logD_{7.3}$ (the distribution coefficient at pH = 7.3) were somewhat lower but still highly significant with K_i, but no correlation was seen with K_r. Lipophilicity of the molecule at pH = 7.3 was the best predictor of high state-dependence. Those molecules which are positively charged at pH = 7.3, have a significantly lower $logD_{7.3}$ than logP. This means, that neutrality is in fact an advantage for inactivated state affinity (and for high state-dependence), in contrary to the view, that SCIs should be positively charged molecules.
- On the other hand, for resting state affinity it is true that positively charged molecules tend to be more potent. The most important determinant of K_r was pKa, the correlation was higher than with logP. Inactivated affinity did not correlate with pKa.
- The other important predictor of state-dependence was aromaticity (aromatic atom count, aromatic ring count, aromatic bond count). These descriptors correlated significantly with SD, K_i and IC_{50}, but not with K_r. Based on this observation (and also on data from the literature) we propose that the most important structure of the local anesthetic binding site, a phenylalanine residue is probably involved in a π-π interaction with an aromatic ring of the SCI molecule, not in a cation-π interaction as it is generally believed.

We chose seven chemical descriptors: molecular weight, minimum projection area, logP, $logD_{7.3}$, polar surface area, and aromatic atom count, in order to perform a cluster analysis of drugs based on chemical properties. The choice of descriptors was based on correlations with biophysical properties: We aimed to choose a set of chemical descriptors which predict the type of inhibition with minimal redundancy. The conspicous resemblance of the results of this cluster analysis to the one that was based on

the biophysical properties of inhibition suggests that mode of action can be predicted from chemical properties. This is remarkable, because we have also observed that therapeutic indication correlates with the type of inhibition. Therefore, a comparative study of the modes of action of SCIs may allow prediction of therapeutic usefulness from chemical properties. (Our data with a rather limited set of only 35 compounds can illustrate that this approach may be feasible, but it is too few to reliably judge its power.)

In summary, we have recorded multiple parameters of inhibition, which did not make our measurement more costly or time consuming but provided us with additional information. With this extra information, we established that SCIs are heterogeneous, delineated specific types of inhibition, and with the help of chemical descriptors identified specific predictors of certain biophysical properties. We believe that this new approach of mapping drugs in the ''biophysical space'', rather than determining a single IC_{50} value will help drug discovery, especially if we can determine the specific chemical properties which predict therapeutic usefulness.

5. Conclusions

1. A detailed study on the mode of action of two antidepressants with manual patch-clamp technique (Lenkey et al., 2006; Karoly et al., 2010)

- Fluoxetine and desipramine inhibited sodium channels with high affinity, and with a mode of action that is different from that of classical SCIs (carbamazepine, phenytoin, lidocaine).
- This special inhibitory mechanism is either due to slow inactivated state preference or to fast inactivated state preference with very slow association kinetics.

2. Comparative automated patch-clamp study of 35 SCI drugs (Lenkey et al., 2010)

- We recorded 8 biophysical properties of the inhibition. Based on these data we identified at least three distinct types of inhibition:
  - 'Type 1': high potency, slow onset and offset kinetics, partial reversibility and use-dependence.
  - 'Type 2': low potency, fast kinetics and almost full reversibility.
  - 'Type 3' high potency, very slow kinetics, apparent irreversibility, and no use-dependence.
- In addition, we identified at least four further distinct types of inhibition, each displayed by a single drug.
- The most important chemical descriptors which determined biophysical properties of inhibition were the following:
  1. logP (octanol-water partition coefficient) correlated with potency (IC_{50}) and affinities (K_r, Ki).
  2. logD (distribution coefficient) was the best predictor of inactivated state affinity and state-dependence.
  3. pKa (acidic dissociation constant) correlated with resting affinity.
  4. Aromaticity correlated with state-dependence and with binding kinetics.
- The best predictors of state-dependence were logD, pKa, and aromaticity.
- Although it is often supposed that positive charge is an essential property of SCIs, we found that inactivated state affinity did not correlate with chargedness, and therefore we propose that binding to the inactivated state does not require positively charged ligands.
- Based on the role of aromaticity we propose that the most important interaction that is responsible for inactivated state affinity is a π–π interaction between aromatic rings of compounds and aromatic residues of the channel.

3. QPatch-16X automated patch-clamp instrument as a tool for comparative studies of modes of action

- Complex voltage and perfusion protocols could be implemented on the QPatch-16X, therefore comparative studies of modes of action can be accomplished.
- For the characterization of SCIs a detailed analysis of the mode of action is not always necessary. Essential information regarding the type of inhibition can often be obtained by proper analysis of simple protocols.

4. Potential trends in SCI drug development

Based on data from our lab and from the literature, the „local anesthetic receptor" is not a universal binding site for all SCIs, but rather a dynamically changing group of overlapping, conformation-dependent binding sites. In addition, existence of alternative binding sites on other areas of the channel is also likely. For this reason, structure-activity relationship data based on potency values only are of limited value. Even until mutagenesis and structural studies will be able to resolve these issues, we believe that types of inhibition can be identified by electrophysiology. To identify distinct types of inhibition it is essential to record multiple, non-redundant parameters of inhibition. This method also allows us to identify chemical properties which determine specific properties of inhibition, and can help to explore the properties of binding sites at different conformations of the channel.

Our preliminary data indicate that SCIs that act by the same mechanism tend to have similar therapeutic action. It is concievable, therefore, that therapeutic action could be predicted indirectly from chemical structure (chemical structure predicts the type of inhibition, which predicts therapeutic indication).

6. Publications

6.1 Publications related to thesis

Lenkey N, Karoly R, Kiss JP, Szasz BK, Vizi ES and Mike A. (2006) The mechanism of activity-dependent sodium channel inhibition by the antidepressants fluoxetine and desipramine. *Mol Pharmacol. 70 (6): 2052-2063.*

Karoly R, Lenkey N, Juhasz AO, Vizi ES, Mike A. (2010) Fast- or slow-inactivated state preference of Na+ channel inhibitors: a simulation and experimental study. *PloS Comput Biol. 6(6):e1000818.*

Lenkey N, Karoly R, Lukacs P, Vizi ES, Sunesen M, Fodor L, Mike A. (2010) Classification of drugs based on properties of sodium channel inhibition: a comparative automated patch-clamp study. *PLoS One. 5(12):e15568.*

Lenkey N, Karoly R, Epresi N, Vizi ES, Mike A. (2011) Binding of sodium channel inhibitors to hyperpolarized and depolarized conformations of the channel. *Neuropharmacology. 60(1):191-200.*

6. 2 Publications independent from thesis

Szasz BK, Lenkey N, Barth AM, Mike A, Somogyvari Z, Farkas O, Lendvai B. (2008) Converging effects of Ginkgo biloba extract at the level of transmitter release, NMDA and sodium currents and dendritic spikes. *Planta Med. 74(10):1235-9.*