Projet de Recherche Doctoral Concours IPV 2021

Intitulé du Projet de Recherche Doctoral : Novel KCC2 enhancers for epilepsy and related disorders: Molecular modeling and virtual screening for drug discovery

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Intitulé : Plasticity in Cortical Networks & Epilepsy
Thématique de recherche : Neurosciences
Responsable d'équipe :
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Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant : ED3C (ED158)

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 1 (Florian Donneger, 100%, Oct. 2018)

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Intitulé : Metabolism, Pharmacochemistry and Neurochemistry
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Ou si ED non SU : MTCI (ED563)

Doctorants actuellement encadrés par le co-directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 2.5 (Alexandre Cabayé, 100%, 2018 ; Huan Xiong, 50%, 2019 ; Floriane Eshak, 100%, 2020)

Cotutelle internationale : ☒ Non ☐ Oui, précisez Pays et Université :

Précisez ici les éventuels co-encadrants (non HDR)

Co-encadrant :
NOM : Prénom :
Titre : HDR ☐
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Intitulé :
Code :
Equipe de Recherche *(au sein de l'unité)* **:**
Intitulé :
Thématique de recherche :
Responsable d'équipe :
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Ou si ED non SU :

Résumé (2 000 caractères maximum) :
Altered neuronal chloride regulation is a hallmark of many neurological disorders including epilepsy. Perturbed expression/function of the cation-chloride cotransporters KCC2 and NKCC1 impair GABA signalling and neuronal excitability. Whereas inhibiting NKCC1 function lacks CNS specificity, acting to promote KCC2 function is a highly promising therapeutic strategy in epilepsy and related disorders. Candidate *KCC2 enhancers* have been proposed based on screening of drug-like compound libraries. However, their mechanisms of action, specificity and therapeutic potential in epilepsy remain unknown. Our project aims to develop a totally novel approach based on molecular modelling and virtual screening to design new molecules with improved specificity and bioavailability. This goal will be achieved by combining advanced medical chemistry approaches (F Acher) together with cellular imaging and electrophysiological techniques (JC Poncer) in order to design and optimize specific KCC2 allosteric modulators, test their potency and specificity as well as evaluate their therapeutic potential in epilepsy.

Joindre en annexe un descriptif du PRD avec références au format pdf (« NOM_2_IPV_2021 » / 3 pages maximum, taille police 11)

AVIS et VALIDATION de l'ECOLE DOCTORALE :

AVIS FAVORABLE

ECOLE DOCTORALE 3C
CERVEAU COGNITION COMPORTEMENT
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à envoyer simultanément par e-mail à l'ED de rattachement et au programme : interfaces_pour_le_vivant@listes.upmc.fr avant le lundi 15 février minuit.

Doctoral Project

Title: Novel KCC2 enhancers for epilepsy and related disorders: Molecular modeling and virtual screening for drug discovery

Alterations in neuronal chloride regulation is emerging as a hallmark of neurological disorders[1], including epilepsy[2-6], autism spectrum disorders[7-9] and neuropathic pain[10-12]. Perturbed expression and/or function of the cation-chloride cotransporters (CCCs) KCC2 and NKCC1 are reported in these conditions and expected to impair fast inhibitory signalling mediated by the chloride-permeable GABAA receptor (GABAAR)[1]. Thus, while NKCC1 imports Cl^- and K^+ inside neurons, KCC2 usually contributes to maintain low intraneuronal [Cl^-], thereby promoting the inhibitory effect of GABAAR activation[13]. Reduced KCC2/NKCC1 functional ratio in the pathology may then depolarize the reversal potential of GABAAR currents and even promote excitatory actions of GABA[7,10,14-16]. This in turn may contribute to anomalous neuronal activities underlying the pathology. Moreover, several recent studies revealed a variety of effects of KCC2 downregulation, independent of ion transport, including altered glutamatergic synaptic transmission[17] and plasticity[18] as well as enhanced neuronal excitability[19].

Strategies targeting CCC function or expression have therefore been tested in animal models of neurological disorders, including epilepsy. For instance, intracerebral infusion of the NKCC1 antagonist bumetanide was shown to prevent epileptogenesis in an experimental model of temporal lobe epilepsy (TLE)[20]. However, bumetanide has numerous side-effects - as NKCC1 is also widely expressed in non-neuronal cells - and hardly crosses the blood/brain barrier, making it a poor candidate in CNS disorders[21]. Instead, promoting KCC2 function or expression holds many promises, as KCC2 is almost exclusively expressed in neurons. Thus, viral-based KCC2 overexpression in cortex was recently shown to prevent seizures in mice[3]. Screening of drug-like compound libraries also identified small molecules suggested to act as *KCC2 enhancers*[11,22,23]. Despite their well-documented therapeutic benefit in neuropathic pain and other disorders[11,22-24], their effect in epilepsy, however, has been overlooked[25]. In addition, their mechanism(s) of action and specificity remain unknown and even controversial[26,27]. **An advantageous alternative to the screening of chemical compound libraries consists in specifically designing ligands to target effectors by molecular modelling and virtual screening**[28-30]. Considerable progress has been made recently in understanding the molecular mechanisms regulating KCC2 function and membrane stability[31-34] as well as in deciphering the 3D structure of CCCs[6,35,36,37] and some of their key regulators[38-40]. This knowledge may then now be used to implement novel approaches of computational chemistry to design specific KCC2 ligands to promote its membrane expression and function.

We propose that **acting to promote KCC2 function represents a highly promising therapeutic strategy in epilepsy and related disorders**. Our preliminary data demonstrate the effectiveness of existing KCC2 enhancers in preventing epileptiform activity *in vitro* and *in vivo*. However, their specificity is limited and other adverse effects are also observed. Therefore, our project aims at i) designing new molecules with improved specificity and bioavailability and ii) evaluating and comparing their therapeutic potential in the context of epilepsy. To this end, an **interdisciplinary approach** will be implemented, combining **advanced computational chemistry and**

virtual ligand screening with **biochemical and super-resolution imaging techniques** as well as **electrophysiological recordings** from human brain tissue and animal models.

Specific aims

Aim 1. To model the carboxy-terminal domain of KCC2 interaction with kinases SPAK and OSR1 and identify new candidate KCC2 enhancers by virtual screening
Our modeling approach will aim at designing new molecules to more selectively enhance KCC2 function and stability than existing compounds. KCC2 membrane stability and function are primarily regulated through posttranslational modifications, in particular phosphorylation of residues of its carboxy-terminal domain[31-34]. These sites therefore represent targets of choice for designing specific KCC2 enhancers. We will focus on Thr$^{906/1007}$ residues in the carboxy-terminal domain (CTD) that we and others have shown reduce KCC2 membrane stability and function upon phosphorylation by the SPAK and OSR1 kinases[31-34]. In order to avoid the lack of selectivity inherent to the highly conserved ATP-binding site of the kinases, we will use molecular modelling and virtual screening to design ligands targeting specific allosteric sites of the kinase or substrate. The recently released cryoEM structure of KCC2[36] (PDB ID 6m23) reveals residues Thr$^{906/1007}$ to be in a solvent-exposed position. Based on available structures and homology models of SPAK and OSR1[39,40], we propose to dock these structures to the KCC2-CTD 3D-structure in order to define selective contact hotspots between them and then prevent these protein-protein interactions. These hotspots will be first validated by mutagenesis and chloride-imaging based functional assays. We will then search for small molecules/peptides with high affinity for these hotspots by virtual high-throughput screening[29] using Discovery Studio (Dassault Systemes BIOVIA), routinely used by F. Acher. Curated libraries of commercial small compounds/peptides will be virtually docked at the specific sites of the 3D structures and scored. Top-ranked molecules will then be used for pharmacological evaluation. The validated hits will be then chemically optimized for *in vivo* delivery and stability.

Aim 2. To compare the specificity and modes of action of existing vs. novel KCC2 enhancers in hippocampal neurons *in vitro*
We will test the efficacy and specificity of compounds obtained from Aim 1 and characterize their effects on KCC2 expression/function as well as GABA signaling in cultured rat hippocampal neurons. Specifically, we will test i) KCC2 membrane expression (surface biotinylation), ii) KCC2 phosphorylation state (phosphosite-specific antibodies[41]), iii) KCC2 membrane dynamics (single particle tracking[31,41]) and iv) KCC2 function (focal GABA uncaging in whole-cell and gramicidin perforated patch recordings[18,19,41]). Preliminary results from JC. Poncer suggest CLP257 and piperazine derivatives may act similarly to promote KCC2 function through reduced phosphorylation at Thr$^{906/1007}$ and enhanced clustering. We will also explore the specificity of these effects, in particular with respect to NKCC1 and GABAAR function[26].

Aim 3. To evaluate and compare the antiepileptic potential of KCC2 enhancers in human epileptic tissue *in vitro* as well as in animal models of temporal lobe epilepsy
Human cerebral tissue resected from patients with pharmaco-resistant epilepsy represent an invaluable model for exploring the effect of candidate antiepileptic drugs *in vitro*. Using multielectrode array recordings[42], in collaboration with the Neurosurgery department of Sainte-Anne Hospital, we will compare the effect of novel KCC2 enhancers on neuronal activity and spontaneous interictal discharges (IIDs)

from slices of human epileptic hippocampus. In parallel, we will test these compounds in the pilocarpine mouse model of TLE. This will let us test the effect of KCC2 enhancers both on epileptogenesis (chronic injection during 8 days post *status epilepticus* (SE)) and during the chronic phase (from day 35 post SE). Using telemetric ECoG and video recordings, we will monitor and quantify seizure frequency and duration as well as IIDs in saline *vs.* KCC2 enhancer-injected mice. This task based on assays routinely used in the Poncer lab will be performed in collaboration with trained electrophysiologists.

Justification of suitability for the *IPV* program:

This interdisciplinary project gathers two groups with complementary expertise in **computational chemistry** and **cellular neurobiology**. It is based on solid preliminary data and expertise from both partners and aims to produce the first proof-of-concept preclinical evidence for using KCC2 enhancers in epilepsy. It is also innovative and ambitious as it proposes to develop a totally novel approach to designing KCC2 enhancers with improved specificity and bioavailability, using state-of-the-art molecular modeling and virtual screening of allosteric antagonists KCC2 phosphorylation. We trust this approach, which has been successfully applied to other receptors, transporters and kinases, should let us identify the most promising molecules for optimal translation to clinical studies, both in epilepsy and other neurological disorders associated with CCC dysfunction. It will also produce invaluable data on KCC2 structure and the understanding of molecular interactions between KCC2 and its regulatory partners, with a predicted impact beyond the field of epilepsy research.

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