Thématique :   Neurosciences

Unité :   Institut du Fer à Moulin (IFM)

Directeur :   GIRAULT Jean-Antoine

Equipe :   Plasticité des réseaux corticaux et épilepsie

Responsable :   PONCER Jean-Christophe / LEVI Sabine

Encadrant(s) : PONCER Jean-Christophe (IFM) et NAVARRO Vincent (ICM/APHP)

Un autre financement est-il envisagé ? Si oui, auprès de quel organisme  non

L'HDR encadre-t-il des doctorants ?  Oui

Nombre de doctorant(s) actuellement dirigés par l'HDR :  1

Nom, Prénom, date de début de thèse et financement des doctorants dirigés :  Donneger, Florian, 10/2018, CD SU

**SUJET PROPOSE**

**Titre :   Alterations of cholesterol metabolism in refractory epilepsies**

**Projet :**

Status epilepticus (SE) is a devastating condition consisting in an uninterrupted epileptic seizure lasting over minutes. Despite initial therapeutic control, about 25% SE become refractory. Persisting epileptic seizures increase the risk of excito-toxicity, neuro-inflammation and neuronal death, leading to irreversible neurological sequels, therefore calling for intensive effort to identify novel therapeutic targets to control refractory SE.

Among the many physiopathological consequences of SE, a recently discovered pathway involving brain cholesterol homeostasis may offer novel therapeutic opportunities. Whereas cholesterol is present in all membranes, an excess of cholesterol is neurotoxic, through mechanisms that remain largely unknown. Neuronal cholesterol metabolism is controlled in part by the neuron-specific CYP46A1, which catalyzes its degradation into 24-hydroxcholestérol (24-OHc). Recent observations in animal models revealed altered brain cholesterol metabolism with a rapid decline of 24-OHc. In addition, chronic CYP46A1 knockdown results in neuronal cholesterol accumulation, neuronal death and epileptiform activity. This suggests that loss of CYP46A1 upon SE may trigger pathological neuronal cholesterol accumulation leading to excitotoxicity and neuronal death. Thus, preventing brain cholesterol accumulation using for instance statins offers a novel therapeutic perspective that may help reduce the functional consequences of SE.

We propose a transversal and multi-scale approach aiming to explore the functional consequences of a loss of CYP46A1 neuronal expression. Our goal is to better understand how CYP46A1 suppression affects neuronal activity and survival and thereby contribute to anomalous activities following SE.

1. Cholesterol is a component of plasma membrane lipid rafts, which are key organizers of intracellular signaling and membrane tethering of cytoskeletal components. In synaptic vesicles, it is responsible for vesicle membrane curvature and fusion, thereby influencing transmitter release. By perturbing membrane lipid composition, increased neuronal cholesterol may then influence vesicular traffic, transmitter release as well as membrane expression and function of ion channels and postsynaptic receptors. Using in vitro electrophysiological approaches combined with superresolution imaging techniques, we will investigate the consequences of chronic CYP46A1 knockdown by RNA interference in cortical neurons. Changes in membrane and synaptic properties will be monitored, including transmitter release probability/dynamics as well postsynaptic receptor function. We will then explore membrane expression and dynamics of ion channels and receptors using single particle tracking techniques as well as PALM-STORM microscopy, as well as lipid raft distribution and neuronal survival. Since lipid rafts also regulate membrane expression of the tyrosine kinase neurotrophin receptors Trk and p75NTR, we will explore whether altered p75NTR signaling may be engaged upon CYP46A1 knockdown and contribute to neuronal vulnerability in SE.

2. Human brain samples are available from patients with simple, refractory and super-refractory SE. Using immunohistochemical and biochemical approaches, we will test whether CYP46A1 is a reliable biomarker of the pathology and its severity. We will compare CYP46A1 expression in the LCS and brain tissue of controls vs. SE patients. Since active CYP46A1 may be specifically translocated from the RE to the plasma membrane, we will compare subcellular CYP46A1 expression using immunogold staining and electron microscopy from human brain samples.

3. We will then test whether compensating for CYP46A1 deficits may improve clinical outcome in an animal model of SE. Using the intrahippocampal kainate injection model, which mimics several key aspects of human SE and leads to CYP46A1 downregulation, we will explore and compare the effects of i) chronic overexpression recombinant CYP46A1 using viral transduction in vivo or ii) chronic administration of simvastatin, a statin with optimal penetration of the blood-brain barrier. We will monitor electrophysiological activity by chronic video-ECoG recordings and anatomical markers of the pathology, such as neuronal death, microglial activation and cholesterol accumulation. These results will let us predict the therapeutic potential of strategies aiming to restore cholesterol metabolism in SE, and possibly other neurological disorders.

Recent publications

Hanin A, Lambrecq V, Denis JA, Imbert-Bismut F, Rucheton B, Lamari F, Bonnefont-Rousselot D, Demeret S, Navarro V (2020) Cerebrospinal fluid and blood biomarkers of status epilepticus. ***Epilepsia*** *61(1):6-18.*

Goutierre M, Al Awabdh S, Donneger F, François E, Gomez-Dominguez D, Irinopoulou T, Menendez de la Prida L, Poncer JC (2019) KCC2 Regulates Neuronal Excitability and Hippocampal Activity via Interaction with Task-3 Channels. ***Cell Reports*** *28(1):91-103.e7*

Heubl M, Zhang J, Pressey JC, Al Awabdh S, Renner M, Gomez-Castro F, Moutkine I, Eugène E, Russeau M, Kahle KT, Poncer JC, Lévi S (2017) GABAA receptor dependent synaptic inhibition rapidly tunes KCC2 activity via the Cl--sensitive WNK1 kinase. ***Nature Communications*** *8:1776*

Giralt A, Brito V, Chevy Q, Simonnet C, Otsu Y, Cifuentes-Díaz C, de Pins B, Coura R, Alberch J, Ginés S, Poncer JC, Girault JA (2017) Pyk2 modulates hippocampal excitatory synapses and contributes to cognitive deficits in a Huntington's disease model. ***Nature Communications*** *8:15592*

Chevy Q, Heubl M, Goutierre M, Backer S, Moutkine I, Eugène E, Bloch-Gallego E, Lévi S, Poncer JC (2015) KCC2 Gates Activity-Driven AMPA Receptor Traffic through Cofilin Phosphorylation. ***Journal of Neuroscience*** *35(48):15772-86*

Chali F, Djelti F, Eugene E, Valderrama M, Marquer C, Aubourg P, Duykaerts C, Miles R, Cartier N, Navarro V (2015) Inhibiting cholesterol degradation induces neuronal sclerosis and epileptic activity in mouse hippocampus**. *Eur J Neurosci.*** *41(10):1345-55.*