497 | Impact of zonisamide, tiagabine and pregabalin in an astrocyte-microglia co-culture model of inflammation

FS Ismail[1,2], TJ Faustmann[3], F Corvace[4], M-L Kümmel[4], E Förster[4], PM Faustmann[4]

[1]University Hospital Knappschaftskrankenhaus Bochum, Ruhr University Bochum, Department of Neurology, Bochum, Germany, [2]Klinikum Vest, Department of Neurology, Recklinghausen, Germany, [3]Medical Faculty, Heinrich Heine University, Department of Psychiatry and Psychotherapy, Düsseldorf, Germany, [4]Ruhr University Bochum, Department of Neuroanatomy and Molecular Brain Research, Bochum, Germany

Purpose: Epilepsy is a chronic neurological disorder leading to recurrent epileptic seizures. Treatment with antiseizure medication (ASMs) targeting neuronal structures is the main therapeutic approach. However, there is a strong evidence confirming the involvement of glial cells including astrocytes and microglia in the pathophysiology of epilepsy. Thus, we aimed to investigate effects of different ASMs such as tiagabine (TGB), zonisamide (ZNS) and pregabalin (PGB) in an astrocyte-microglia co-culture model of inflammation.

Method: Primary rat astrocytes co-cultures containing 5-10% (M5, "physiological" conditions) or 30-40% (M30, "pathological inflammatory" conditions) of microglia were treated with different concentrations of ZNS (10, 20, 40, 100 µg/ml), TGB (1, 10, 20, 50 µg/ml) or PGB (3, 10, 30 and 60µg/ml) for 24 h. The glial viability, microglial activation, connexin 43 (Cx43) expression and gap-junctional coupling were investigated.

Results: TGB revealed toxic effects with concentration-dependent reduction of glial cell viability under physiological and pathological conditions. In contrast, ZNS reduced the glial viability only by overdose concentration (100µg/ml) under physiological conditions. PGB did not affect the glial viability. After incubation of pathological M30 co-cultures with high concentration (20 µg/ml) of TGB, the microglial activation was significantly decreased, suggesting anti-inflammatory features. Otherwise, incubation of physiological and pathological co-cultures with different concentrations of ZNS or PGB resulted in no significant changes of microglial phenotypes. The gap-junctional coupling was significantly decreased after incubation of M5 co-cultures with 20 and 50 µg/ml TGB. A significant decrease of Cx43 expression and disruption of gap-junctional communication were found after incubation of M30 co-cultures with 10 µg/ml ZNS, contributing to anti-seizure activity.

Conclusion: TGB and ZNS revealed different effects on glial features, whereas PGB did not show effects on glia. The astrocyte-microglia co-culture model provides novel perspectives for understanding ASM effects on glia and glia-mediated inflammation and can contribute to exploring novel anti-epileptogenic targets.

514 | Characterization of vagus nerve electroneurogram (VENG) during acute kainic acid induced seizures

E Acedo Reina[1], J Chávez[2,3], R Raffoul[2], E Germany Morrison[1,4], A Nonclercq[2], R El Tahry[1,4,5]

[1]Université Catholique de Louvain, Institute of Neurosciences (IoNS), Brussels, Belgium, [2]Université Libre de Bruxelles, BEAMS (Bio- Electro- And Mechanical Systems) Department, Brussels, Belgium, [3]Synergia Medical SA, Mont-Saint-Guibert, Belgium, [4]WEL Research Institute, Walloon Excellence in Life Sciences and Biotechnology (WELBIO) Department, Wavre, Belgium, [5]Cliniques universitaires Saint-Luc, Center for Refractory Epilepsy, Department of Neurology, Brussels, Belgium

Purpose: Seizures produce autonomic symptoms, mainly sympathetic but also parasympathetic in origin. Within this context, the vagus nerve (VN) is a key player as it carries information from the different organs to the brain and vice versa. For this reason, exploiting the vagal neural traffic with respect to seizures might offer a novel way to detect seizures and develop a closed-loop Vagus Nerve Stimulation (VNS) system. Therefore, this project aims to develop a Vagus Nerve Electroneurogram (VENG)-based detection algorithm to detect seizures in an acute kainic acid rat (KA) model.

Method: Five male Wistar rats, weighing 275±75g, were used. Anaesthesia was induced using 100mg/kg Ketamine and 7mg/kg Xylazine i.p. and maintained by half concentration of the initial mixture. Three epidural stainless-steel electrodes were implanted on the frontal cortex ([+]:AP:+2mm, ML:±3mm, [-]:AP:+6mm) to record EEG. Three ECG Lab-made Tungsten electrodes were implanted according to Eindhoven's lead II to record ECG. Finally, a tripolar Micro-Cuff electrode was implanted around the left cervical portion of the VN. Rats were injected with KA (0.4µg/0.2µl saline; 0.1µl/min) in the right hippocampus ([RH]:AP:-5.6mm, ML/DV: 4.5mm) and VENG and EEG were recorded for 20 minutes.

Results: Ten seizures were recorded in 3/5 animals with a mean seizure duration of 52.38±20.98 seconds and a mean delay between the injection and the first seizure of 8.7±4.34 minutes. In all seizures, we observed a modification of VN activity characterized by the loss of respiration related rhythmicity in the signal. In addition, in 1/3 rats we observed a clear increase of the envelope of the VENG signal.

Conclusion: Our results show the occurrence of a specific change in VENG activity during acute KA induced seizures in anaesthetic conditions. Therefore, recording of VENG may be useful to detect seizures, which could in the future be used for closed-loop VNS in patients.

585 | The effect of antiseizure medications and cardioprotective drugs on cardiac injury in a post-status epilepticus rat model of temporal lobe epilepsy

Z Liu[1], E Braine[1], B Wang[1,2], N Jones[1,3,4], P Perucca[1,3,5,4], T O'Brien[1,3,5], S Sivathamboo[1,3,5], P Casillas-Espinosa[1,3,5]
[1]Monash University, Melbourne, Australia, [2]Baker Heart and Diabetes Institute, Melbourne, Australia, [3]Alfred Health, Melbourne, Australia, [4]Austin Hospital, Melbourne, Australia, [5]The University of Melbourne, Melbourne, Australia

Purpose: Building clinical and experimental evidence indicates an increased risk of cardiac abnormalities in people with chronic drug-resistant epilepsy. Postulated mechanisms include cardiac injury from repeated catecholamine surges and hypoxemia, leading to the concept of 'epileptic heart'. This study investigated acute and chronic cardiac injury in a rat model of temporal lobe epilepsy and examined whether early administration of antiseizure medication or cardioprotective drugs can alleviate such injury.
Method: Kainic acid (KA) was used to induce status epilepticus (SE) and subsequent chronic epilepsy in male Wistar rats (n=43). Levetiracetam (200mg/kg/day, n=11), atenolol (5mg/kg/day, n=12), VCP979 – a novel anti-fibrotic and anti-inflammatory agent (7mg/kg/day, n=9), and vehicle (n=11) were administered through an osmotic pump from day 0 to 12 weeks post-SE. Sham rats (n=13) were handled the same way without receiving KA. Blood samples were collected on day 2, day 7 and day 84 post-SE to analyze cardiac troponin I (cTnI) using ELISA. EEG/ECG recordings were performed continuously for 2 weeks between day 70 and day 84 to assess chronic seizure frequency.
Results: Compared to the sham controls, cTnI levels were significantly higher in vehicle-treated post-SE rats at day 7 (227.3 ± 33.5 vs. 127.0 ± 8.8 pg/mL, $p<0.0001$) and day 84 (153.0 ± 7.6 vs. 128.3 ± 8.1 pg/mL, $p=0.0069$). At day 84, there was a positive correlation between cTnI levels and spontaneous seizure frequency ($p=0.0005$, $r^2=0.8721$), and cTnI levels were significantly lower in VCP979- and atenolol-treated groups compared to the vehicle-treated group ($p<0.0001$ and $p=0.0025$, respectively). Overall, the VCP979-treated animals had the lowest cTnI levels during the development of epilepsy, which was similar to the sham controls.
Conclusion: Our findings indicate that acute SE and repeated seizures in chronic epilepsy were associated with cardiac injury, consistent with the 'epileptic heart'. Early treatment with cardioprotective drugs may decrease the risk of seizure-related cardiac injury in this population.

589 | A role for the slow AMPA receptor in generating epileptiform activity?

A Lacey[1,2], CAP Sweeney[1], KJ Sweeney[3], D O'Brien[3], M Cunningham[1,2]
[1]Trinity College, Discipline of Physiology, Dublin, Ireland, [2]FutureNeuro SFI Centre, RCSI, Dublin, Ireland, [3]Beaumont Hospital, Department of Neurosurgery, Dublin, Ireland

Purpose: Conventional knowledge suggests that fast synaptic excitation mediated by the activation of AMPA receptors (AMPAr) plays a key role in ictogenesis. Recent work has demonstrated the existence of a slow to desensitise AMPAr in cortical pyramidal neurones. The slow AMPAr has a high affinity for glutamate and can remain open for several hundreds of milliseconds (~500 ms). Thus, the presence of this slow AMPAr could bias excitatory neurons towards prolonged pathological depolarisations as observed during ictal activity. We aimed to test for a contribution of slow AMPAr to seizure activity using electrophysiological and pharmacological approaches.
Method: Extracellular local field potential (LFP) recordings were made from acute rodent (entorhinal cortex) and human (neocortex) brain slices (400μm). 4-AP or modified (0.2mM Mg^{2+}/ 8mM K^+) artificial cerebrospinal fluid (ACSF) was utilized to induce ictal discharges. Ictal activity was quantified using event duration, first spike amplitude and area power and these parameters were normalized against the baseline activity of each slice.
Results: In recordings of 4-AP induced ictal activity in the rodent entorhinal cortex, the application of sub-saturating concentrations of the AMPAr antagonist NBQX (300nM) did not significantly alter area power or first spike amplitude. Whilst the duration of ictal events was reduced ($N=8$; $p<0.05$), overall ictal activity persisted. The subsequent application of a higher concentration of NBQX (1μM) abolished ictal events ($N=9$). In separate experiments, ictal activity in the presence of sub-saturating concentration of NBQX was abolished by subsequent application of the non-competitive AMPAr antagonist GYKI 52466 (50μM). All parameters were significantly reduced ($N=10$; $p<0.0001$). In human neocortical slices obtained from patients with refractory epilepsy ($N=2$) ictal activity was unaltered by sub-saturating concentrations of NBQX