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In Rat Organotypic Hippocampal Slice Cultures, Conventional Antiepileptic Medications are Unable to Inhibit Epileptiform Activity

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ABSTRACT

Context and Goal: Prior research has shown that organotypic hippocampal slice cultures (OHSCs) can elicit tonic-clinic seizure-like events (SLEs), which resemble the electroencephalographic correlates of limbic seizures in humans and animals. We have investigated whether OHSCs can be used as in vitro models of limbic seizures to research seizures processes and vetting novel antiepileptic substances.

Experimental Strategy: The interface method was used to cultivate OHSCs. Under submerged conditions, the levels of extracellular potassium and neuronal activity were observed. Lowering magnesium concentrations or administering the potassium channel blocker 4-aminopyridine both caused SLEs. Analysis was done on the impact of common antiepileptic medications (AEDs) on SLEs, including carbamazepine, phenytoin, valproic acid, clonazepam, diazepam, and phenobarbital sodium.

Important Outcomes: AEDs did not stop the over 93% of OHSCs from happening induction of SLEs or cease current seizure activity even in the presence of hazardous dosages. The postnatal age at explanation (P2–P10), the manner of seizure provocation, and the length of in vitro cultivation (2 months) had no bearing on this chemoresistance. GABAA-agonist muscimol or glutamate antagonists were able to reversibly block SLEs.

Inferences and Conclusions: Unlike animal models of thermoresistant seizures, where responders and nonresponses may only be distinguished after an experiment, we describe an easy to set up in vitro model of tonic-clinic SLEs that is a priori thermoresistant. OHSCs may be useful for investigating the mechanisms underlying medication-resistant seizures and for the discovery of novel anticonvulsive substances that may one day be used to treat drug-resistant epilepsy.

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Abbreviations
4-AP, 4-Aminopyridine
AED-Antiepileptic Drug
CBZ-Carbamazepine
CLO-Clonazepam
DIV-Days in Vitro
DZP-Diazepam
MEM-Minimal Essential Medium
OHSCs-Organotypic Hippocampal Slice Cultures
P-Postnatal Day
PHB-Phenobarbital Sodium
PHT-Phenytoin
SLE-Seizure-Like Event
VPA-Valproic Acid
NTs-Nucleotides
Ado- Adenosine
VNUT- Vesicular Nucleotide Transporter
CNTs- Concentrative Nucleoside Transporters
ENTs- Equilibrative Nucleoside Transporters

Introduction

Drug-refractory epilepsy is found in about 70% of adult patients with mesial temporal lobe epilepsy [1]. Despite its modulator effects on seizure characteristics and incidence, antiepileptic medications (AEDs) currently on the market do not produce a sustained suppression of seizures in these patients. Only modest improvements have been seen when these patients' treatment regimens are changed to alternate monotherapy or polytherapy, which combines one of the more recent AEDs developed in the last ten years with a standard AED such as phenobarbital, phenytoin (PHT), or carbamazepine (CBZ) [2]. Childhood epilepsy has comparable challenges. More than 50% of patients with Lennox-Gastaut syndrome and between 20 and 30% of those with symptomatic/cryptogenic partial epilepsies are considered incurable [3].

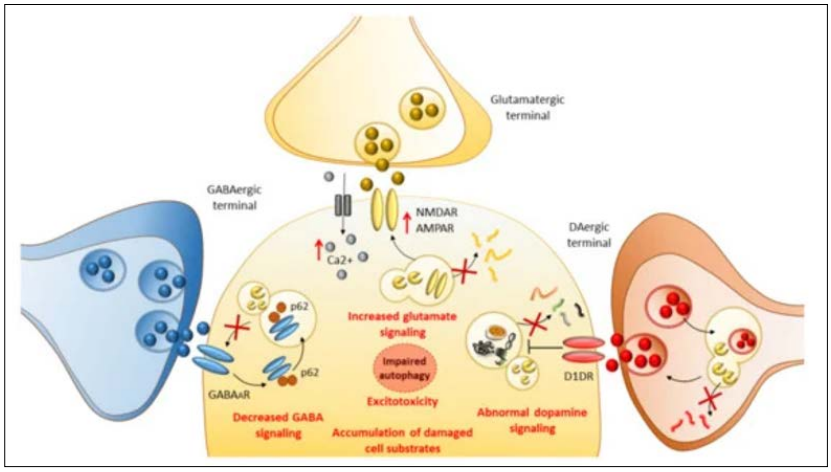


Figure 1: The role of autophagy in seizure-related molecular mechanisms. By operating at the level of gamma-aminobutyric acid (GABA), dopamine (DA) and glutamate systems autophagy are critically implicated in the molecular mechanisms underlying epileptogenesis and seizure-induced neuronal alterations. When a failure of autophagy occurs, elevated p62 hinders GABAA receptor surface presentation, leading to decreased GABA signaling. At the same time, a failure of autophagy occludes the degradation of glutamate receptors N-Methyl-D-Aspartate (NMDAR) and A-Amino-3-Hydroxy-5-Methylisoxazole-4-Propionic Acid (AMPAR), fostering abnormal glutamate signaling and Ca2+ influx. This eventually leads to imbalanced excitatory–inhibitory neurotransmission underlying epileptogenesis. At the level of DA terminals, autophagy is seminal to blunt DA release by degrading DA-filled synaptic vesicles. Autophagy failure leads to abnormal DA release and abnormal stimulation of DA D1 receptors (D1DR), which in turn exacerbate autophagy suppression via mTOR hyperactivation. This eventually leads to the accumulation of damaged cell substrates which synergize with glutamate-related excitotoxicity to produce neuronal damage.

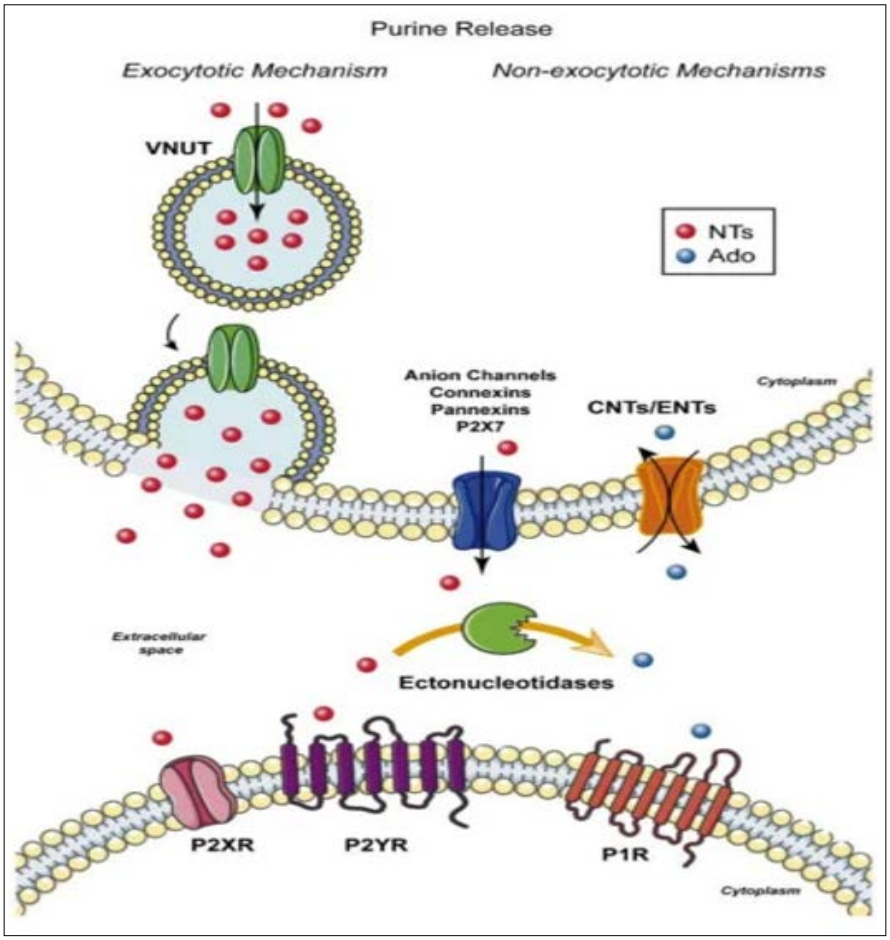


Figure 1.1: Purine release mechanisms: purines such as ATP and adenosine can be actively released from neurons and glial cells including microglia and astrocytes or passively from damaged or dying cells. Schematic showing the different release mechanisms including exocytotic and non-exocytotic mechanisms. Exocytotic mechanisms require previous storage of nucleotides via the vesicular nucleotide transporter (VNUT) in secretory/synaptic vesicles. Non-exocytotic mechanisms include the release of nucleotides by different types of channels, such as anion channels, pannexins and connexins. In contrast to ATP, adenosine can also be released

into the extracellular space via Concentrative Nucleoside Transporters (CNTs) and Equilibrative Nucleoside Transporters (ENTS). Released nucleotides activate P2X and P2Y receptors localized on neuronal or glial membranes. Simultaneously, the hydrolysis of nucleotides by ectonucleotidases produces adenosine which, in turn, activates P1 receptors.

This little example already highlights how difficult it is to handle refractory epilepsies in adults and children, making it a difficult topic for experimental and clinical research Three broad categories can be used to categorize path mechanisms that contribute to chemoresistance: genetics, disease-related mechanisms, and drug-related mechanisms [1]. There are two main theories that have been put up as disease-related mechanisms. According to Remy and Beck, the target theory suggests that pharmacological targets may change, while the transporter hypothesis suggests that the concentration of AEDs in the areas of the brain linked to epilepsy may be lowered by overexpressing multidrug transporters [4,5].

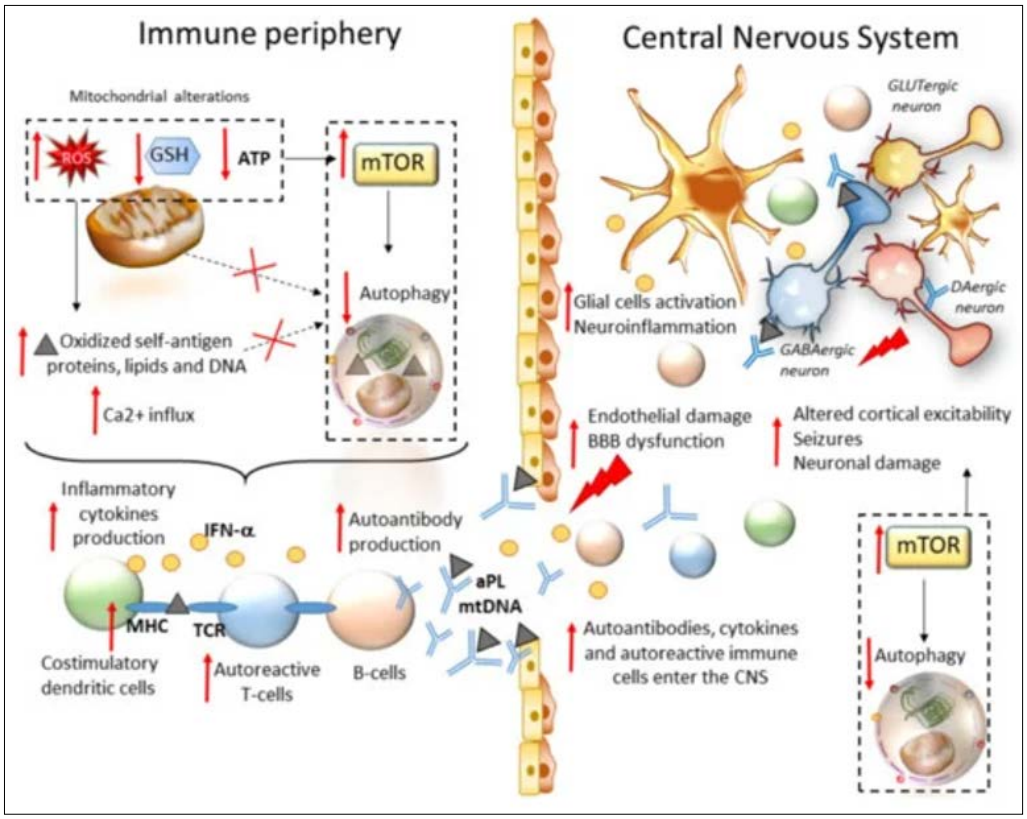


Figure 1.2: mTOR-dependent seizure development in autoimmune systemic disorders. Within peripheral immune cells, mitochondrial alterations lead to chronic oxidative stress (production of reactive oxygen species (ROS)) along with a concomitant depletion of antioxidant factors (gluthatione, GSH). This promotes oxidation of self-antigen proteins, lipids, and DNA, along with calcium (Ca2+) influx and the release of highly diffusible oxidative and inflammatory factors, which are spread to other intracellular organelles and through the bloodstream. At the same time, redox imbalance induces mTOR activation and autophagy impairment, eventually leading to impaired removal of oxidized self-antigens and mitochondria, production of inflammatory cytokines by costimulatory dendritic cells, increased stability of major histocompatibility molecules (MHC) on dendritic cells, subsequent activation of autoreactive T cells and production of autoantibodies by B cells. Autoantibodies, activated immune cells, and proinflammatory cytokines are spread through the bloodstream, and they reach the CNS where they produce endothelial damage and disruption of the blood–brain barrier (BBB). Within the brain milieu, these factors, coupled with mTOR hyperactivity and autophagy, impairment promote a chain of events consisting of neuroinflammation through activation of glial cells, altered cortical excitability, and eventually neuronal damage.

Chemoresistance may also result from genetic changes brought on, for instance, by polymorphisms in drug transporters [4]. In conclusion, tolerance as a mechanism associated to drugs could be contributing to decreased medication efficacy [6]. All of these theories still have gaps in their evidence and inconsistencies, hence further research is required to fully understand the mechanism of chemoresistance [1]. This research focuses on experimental models of drug-resistant epilepsy in addition to studies on brain tissue from individuals with drug-resistant seizures [7, 8].

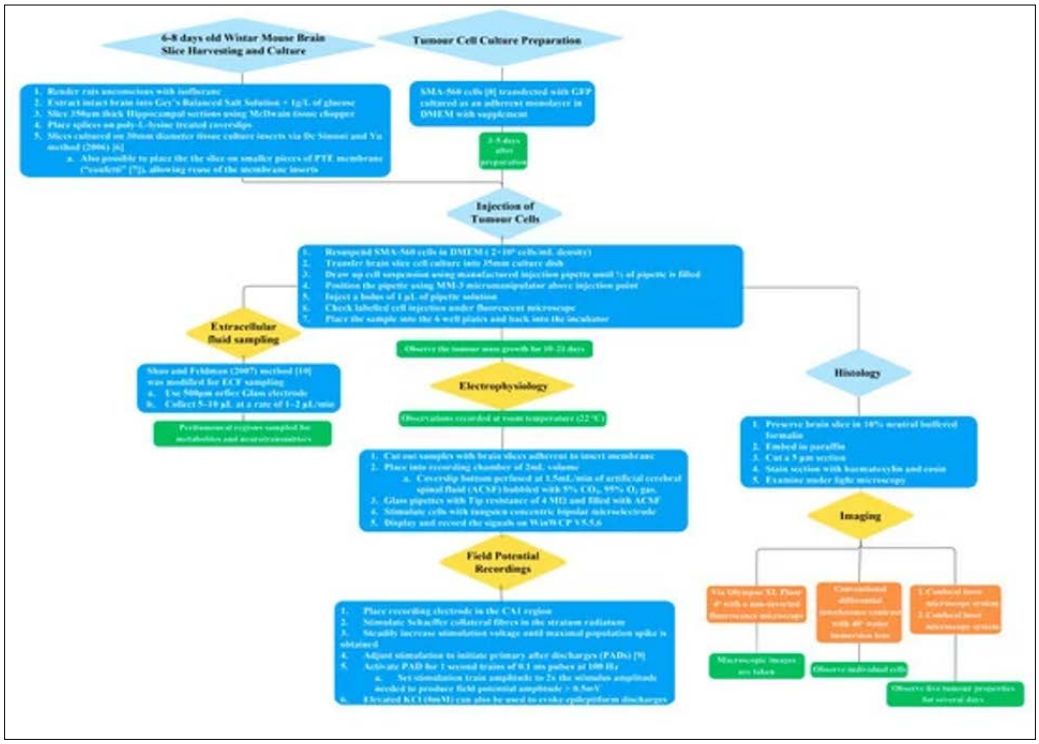
Thermoresistant subgroups of the experimental animals have been demonstrated to exhibit various forms of spontaneous seizures in epileptic dogs, spontaneous seizures in rats that have been amygdala-kindled, and post status epileptic seizures following prolonged electrical stimulation of the basolateral amygdala [9, 10]. AEDs can cause some chemical seizure models to refractory, however their predictive value for the creation of treatment plans for epilepsy that is resistant to treatment has been questioned [9]. Since it is only possible to separate responders from non-responders after the experiment is over, all of these models are a posteriori models. When considered collectively, adult patients with experimental models of resistant epilepsy are uncommon, and the circumstances surrounding pediatric epilepsy may be even more unfavorable [11].

Unlike animal models, it is possible to accurately predict in vitro chemoresistance in vivo. When analyzing in vitro models of seizure-like activity in acute slices of the entorhinal cortex, hippocampus, or neocortex, the corresponding models were found. Elevating the extracellular potassium content in these organs can cause various kinds of seizure like episodes (SLEs), decreasing the calcium or magnesium concentrations, applying toxins like or potassium channel blockers like 4-aminopyridine (4-AP) [12-17]. With the notable exception of pharmacoresistant recurrent discharges, which are brought on by the combination of bicuculline and 4-AP or by the emergence of low magnesium-induced seizure-like activity during later stages, the majority of these SLEs respond to clinically used AEDs [18,19].

In an intact immature corticohippocampal preparation of one-week-old rats, pharmacoresistant tonic-clinic SLEs have been reported recently [20]. Numerous in vitro models, such as the late-status low-magnesium model epilepticus, are increasingly included in preclinical studies assessing the anticonvulsant effectiveness of novel antiepileptic drugs. Pharmacoresistant in vitro seizure models are, however, rarely used. Only until the end of the first postnatal week is the intact corticohippocampal preparation functional. A unique instance of pharmaco resistance is seen in late recurrent discharges in acute slices of the entorhinal cortex, which mirror electroencephalogram patterns seen in the latter stages of status epilepticus. Furthermore, because both models' brain tissues are viable for short periods of time only and would require a large number of animals, they are not appropriate for screening.

Consequently, we have investigated whether organotypic hippocampal slice cultures (OHSCs) are suitable as model(s) for SLEs that are pharmaco sensitive or pharmacoresistant portions of brain tissue since they can be kept in culture for several weeks to months and because it has been demonstrated that, under the right circumstances, nerve cells can continue to differentiate and develop a tissue organization that closely resembles that seen in situ, prepared from young rodents would be ideal for screening purposes [21].

To elicit seizure-like activity in OHSCs, we have employed a reduction in magnesium concentration, a condition seen in eclampsia, and 4-AP. Using this model, we validated and expanded upon previous findings showing that it was possible to reliably produce SLEs in OHSCs that resembled electrographic correlates of limbic seizures recorded with a depth electrode in animals [22-25]. Furthermore, we discovered that in In contrast to acute slices of the entorhinal cortex, tonic-clonic SLEs and brief recurring discharges in OHSCs were resistant to conventional AEDs. An abstract of some of these findings was published by Albus et al. [26] .



Flow Diagram Overview of the in Vitro Brain Tumour Methodology

Discussion

Specifically, we chose AEDs that are clinically approved to treat focal or partial epilepsies, such as temporal lobe epilepsy (CBZ, PHT, 1,4-benzodiazepines, PHB). While gabapentin is licensed for the adjunctive care of partial seizures with or without subsequent generalization, VPA is helpful in patients with all types of seizures. Interictal discharges and RSD-SLEs were pharmacoresistant, but tonic-clonic SLEs in entorhinal-hippocampal slices taken from adult animals, whether induced by elevation of potassium, lowering of calcium, lowering of magnesium, or by application of 4-AP, generally responded to standard AEDs. In this investigation, we showed that tonic-clonic SLEs in OHSCs are resistant to clinically relevant doses, in addition to RSD-SLEs. of AEDs and imply that this preparation could be used as a drug-resistant epilepsy model [13,27,28,18].

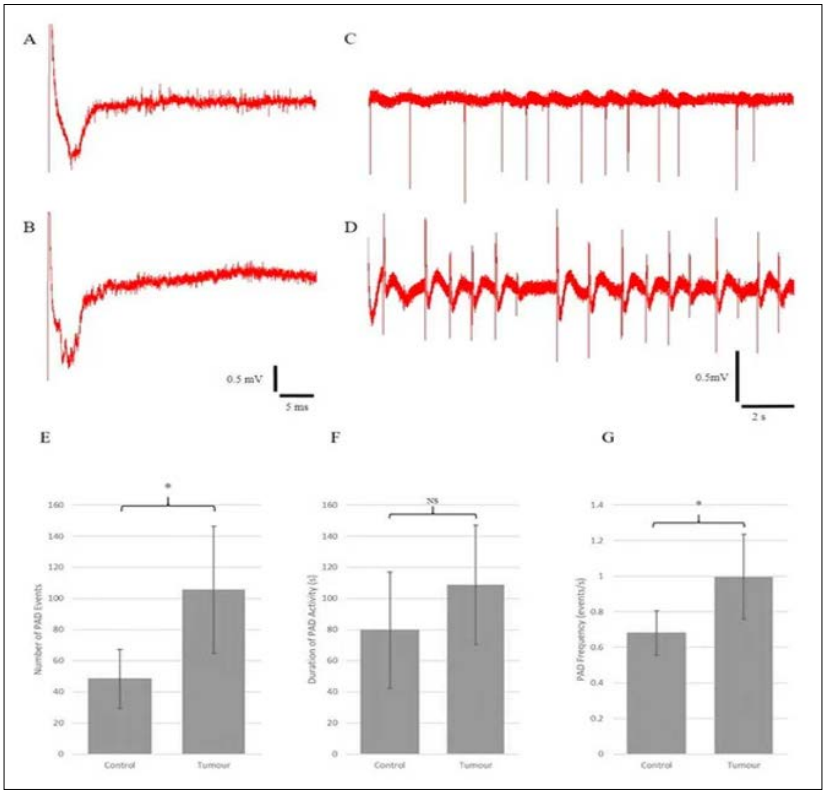


Figure 2: Organotypic hippocampal slices retain synaptic connections after SMA560 tumour cell injection and exhibit seizure-like primary after-discharges (PADs) and epileptiform discharges in a solution with 8 mM KCl or after high-frequency electrical stimulation. Recordings of evoked field potentials from control (A) and tumour-bearing (B) organotypic hippocampal slices. Representative examples of primary after-discharges from control (C) and tumour-bearing (D) organotypic hippocampal slices. Time and amplitude scales are provided for both recording groups at the bottom of the columns. Electrical activation of local field potentials shows that the tumour-bearing organotypic hippocampal slice has intact synaptic connectivity, most likely through the Schaeffer collateral pathway (A,B). (E) Tumour-bearing organotypic hippocampal slices exhibited statistically significantly more PAD events following high-frequency electrical stimulation (105.67 ± 20.43 events) compared with control organotypic hippocampal slices (48.25 ± 9.43 events). (F) No significant difference in the duration of PAD activity was found between tumour-bearing organotypic hippocampal slices (108.70 ± 19.09 s) and control organotypic hippocampal slices (79.57 ± 18.65 s). (G) Tumour-bearing organotypic hippocampal slices exhibited statistically significantly higher PAD frequency following high-frequency electrical stimulation (1.00 ± 0.12 events per second) compared with control organotypic hippocampal slices (0.68 ± 0.06 events per second). All data were normally distributed according to the Shapiro–Wilk test. Statistical analysis was conducted using Student's t-test with $\alpha = 0.05$. n = 8 for control organotypic hippocampal slices and n = 9 for tumour-bearing organotypic hippocampal slices. Data are presented as mean ± standard error of the mean * = $p < 0.05$. NS = not significant ($p > 0.05$).

In humans, complete seizure suppression is necessary for a patient to fully integrate socially and professionally. All pharmacoresistance individuals still experience seizures on a somewhat regular basis, but the frequency and severity of seizures rise when the AEDs are stopped. Therefore, in a digital sense, pharmacoresistance is characterized as either seizure-free or not. This does not rule out the possibility that antiepileptic drugs alter the course of the illness.

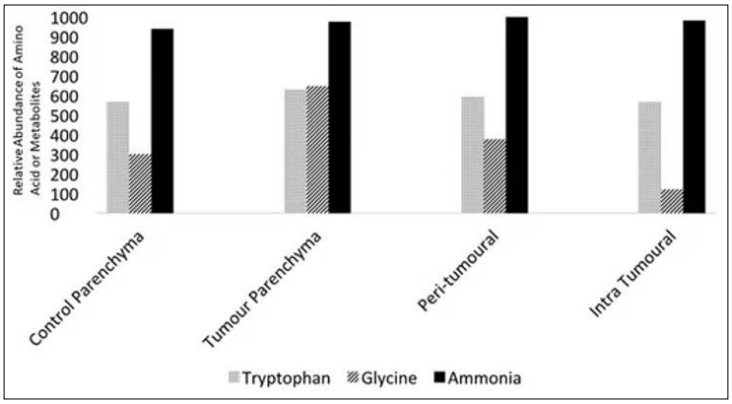


Figure 3: Micro-sampling of extracellular fluid reveals the relative abundance of amino acids and metabolites in different slice regions. Relative concentrations of tryptophan, glycine, and ammonia were derived from control brain slice parenchyma, tumour slice parenchyma, the peritumoral region, and the intra-tumoral region using 5–10 µL samples using the micro-sampling technique

described above. Concentrations are relative areas under the curve derived from the quantitative mass spectroscopic technique using a battery of reference substances, n = 5 for all samples.

In Vitro Tonic-Clonic SLEs in the Hippocampal Tissues

In this work, we employed the low magnesium model and the 4-AP model, two seizure induction models in which synaptic inhibition is maintained. 4-In certain cases, AP inhibits potassium ion channels. especially those belonging to the Kv1 and Kv3 families. It has been discovered that the presence of potassium ion channels from the Kv4 family that are less sensitive to 4-AP be decreased in a temporal lobe epilepsy animal model. Human epileptic hippocampal tissue the kainate model of temporal lobe epilepsy, and heterotopic neurons—which do not have functional A-type Kv4.2 potassium channels—have also been shown to exhibit altered potassium current characteristics [30-32]. According to Castro et al. there is a suggestion that this anomaly has a role in the increased excitability and lower seizure threshold linked to brain abnormalities.

The facilitated activation is the cause of seizure induction in the low magnesium scenario of NMDA receptors, a decrease in the screening of membrane surface charge, and an increase in transmitter release [16,33,34]. It is widely accepted that NMDA receptor activation enhances limbic palatogenesis based on pharmacological and genetic investigations of NMDA receptors in several in vivo and in vitro models [16,35]. Reduced magnesium concentrations are a major contributing factor to eclampsia; PHT and DZP, as well as normalizing magnesium levels, prevent seizures and enhance outcome [36].

It has previously been demonstrated that after more than an hour of continuous seizure-like activity, there was a reduction in both free radical-mediated cell damage and the uncoupling between neuronal and metabolic activity in OHSCs through incorporating a free radical scavenger into the mix [37]. Since we limited the administration of low magnesium MEM to 40 minutes, it was doubtful that any appreciable cell damage would occur. Between SLEs caused by low magnesium MEM paired with supplement B27 (which contains the free radical scavengers a-tocopherol and glutathione), we could not find any difference (unpublished observations). Furthermore, there was no difference in pre- and post-drug controls for the recovery and undershoot of [K Í]o, which are linked to the late phase of SLE and the subsequent suppression of neuronal activity, respectively. These findings suggest that the function of sodium/potassium-ATPase remained unaffected [38,39].

Reversible suppression of SLEs provided additional evidence of the pathophysiological significance of low magnesium-induced seizure-like activity in OHSCs using GABAA- or glutamate-agonists seizure proneness and pharmacological resistance Within 20 DIV, all major neuronal types mature in their structural characteristics. Furthermore, according to Zimmer and Gahwiler, Frotscher and Heimrich, and Gutierrez and Heinemann, the fundamental intrinsic connective arrangement found in vivo is preserved in vitro [40-43,23]. Axons will regenerate with the appropriate specificity if they are severed during the preparation [44,45].

Nonetheless, new connections are made in accordance with the principles of nerve connection rearrangement which raises network excitability [43,23,46]. Reactive colony sprouting in mouse and rat OHSCs in animals that were 7 to 10 days old began after 3-6 DIV [44,47]. The majority of OHSCs utilized in our research (explantation between P6 and P10 with at least 7 DIV) were older than 13 days. Therefore, abnormal axonal connections may be the cause of their higher susceptibility to seizure induction; these connections have also been observed in human epileptic tissue and in various animal models of temporal lobe epilepsy [48-50]. Standard AEDs prevent SLEs caused by 4-AP or low magnesium in 8-23-day-old rat hippocampal regions without aberrant connectivity this supports the idea that aberrant axonal connections in OHSCs, at least for two weeks in vitro, contribute to to the drug resistance [50,51].

In OHSCs transplanted at postnatal day (P) 2 ÷ 6–8 DIV, when aberrant connections are not yet fully formed, pharmacoresistance was also observed in those less than 10 days old. The dentate gyrus's immature gate function, the excess recurrent collateral connectivity between CA1 and CA3 pyramidal neurons that is still present in the hippocampal tissue at one week old and/or the immaturity of receptors and channels could be the mechanisms underlying this propensity [53-56]. Slowed kinetics of the hyperpolarization-activated depolarizing current (I(H)) have also been linked to an enhanced excitability of the juvenile hippocampal region; delayed postnatal development of potassium channels (Heinemann Developmental changes in voltage-operated M-type potassium functions, depolarizing GABAA receptor-mediated responses in immature neurons [57-62].

Like that According to Rohrbough and Spitzer, depolarizing GABA responses are likely brought on by an increased intracellular chloride concentration that is sustained by the action of the K ξ -Cl-cotransporter isoform 1 (NKCC1) [63]. Bumetanide, an NKCC1 blocker, has been demonstrated to decrease seizure-like activity in the high K Í model both in vivo and in vitro but not in other seizure models [64,65].

These results led us to show that bumetanide (10–20 mM; 8 OHSCs) administration explained the difference between P2–7; 6-10. DIV) had no effect on the PHT resistance of low magnesium-induced SLEs or the SLEs themselves (unpublished observations). Therefore, the unique morphological and/or functional characteristics of the early postnatal temporal cortex are most likely connected to the higher seizure susceptibility and pharmacoresistance in OHSCs younger than 14 DIV. The observation that low magnesium-induced tonic-clonic SLEs in an intact 1-week-old corticohippocampal formation in vitro are resistant to several AEDs, including gabapentin, PHT, and CBZ, lends credence to this opinion [20].

Nonetheless, a few distinctions between the two preparations have been identified. In the intact hippocampus preparation, benzodiazepines, PHB, and VPA reduced SLEs, but not in OHSCs. The PHB (300 mM) and VPA (3 mM) doses utilized were in a neurotoxic range and greater than the ones we employed in our research. Variations in the tissue's postnatal age and preparation method may also have an impact on the outcomes. It should be mentioned that OHSCs are more susceptible to seizures than an intact immature corticohippocampal formation due to their increased seizure frequency.

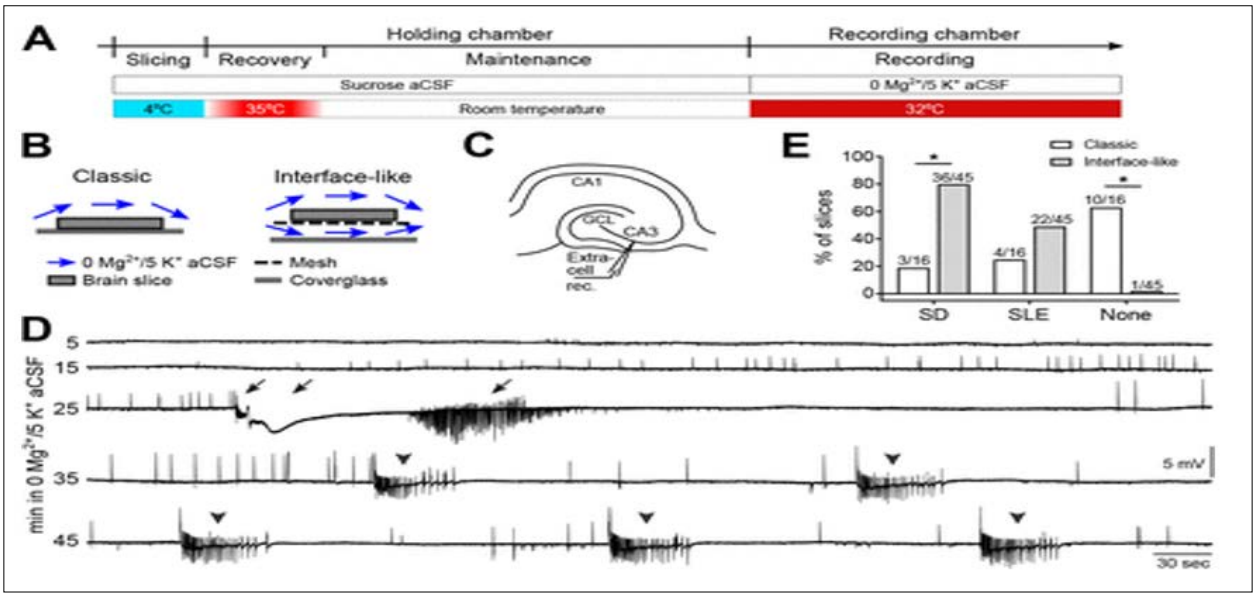


Figure 4: SD and SLEs are developed in 0 Mg2+/5 K+ aCSF in an interface-like submerged-style chamber.(A) Experimental timeline. Hippocampal slices were prepared in ice-cold sucrose-based artificial cerebrospinal fluid (sucrose aCSF). Slices were incubated at 35°C for 45 min and then maintained at room temperature in sucrose aCSF before being transferred to the recording chambers for recording at 32°C. (B) Illustration of classic (single superfusion) and interface-like (dual superfusion) recording chambers. (C) Schematic of extracellular recording in the CA3 of hippocampus. (D) A representative trace shows a continuous recording of extracellular activity in the CA3 of hippocampus in 0 mM [Mg2+]o /5 mM [K+]o aCSF (0 Mg2+/5 K+ aCSF). The slice was recorded in an interface-like recording chamber. Both spreading depolarization (SD) and seizure-like event (SLE) developed, and either one could precede the other. Arrows point to a single SD. Arrowheads point to five SLEs. (E) Percentage of SDs, SLEs, and neither event (None) developed in classic (white bars) or interface-like (gray bars) recording chambers within the first hour of 0 Mg2+/5 K+ aCSF exposure. Slices recorded in an interface-like recording chamber showed a higher percentage of SD than in a classic recording chamber (Two-sided Fisher's exact test, * $p < 0.0001$). Nighty-eight percent of slices recorded in an interface-like chamber developed SD and/or SLE.

Validity of our Pharmacoresistant Tonic-Clonic SLE in Vitro Model

We have described pharmacoresistance that may be specific to the seizure-like activity caused in vitro by low magnesium or 4-AP, which is a model of acute convulsions. It may not apply to seizures in humans and animals with epilepsy. However, our in vitro pharmacoresistant model's validity isn't based on the technique of inducing SLEs, but on characteristics of the tissue (such as the quantity of axonal recurrent connections) that are similar to those of human epileptic tissue. For the following reasons, OHSCs seem to be eligible for evaluating anticonvulsive medications that would be beneficial in pharmacoresistant epileptic tissue, in contrast to acute slices of the hippocampal tissue. First off, the greater propensity for OHSC-induced seizures and perhaps even their pharmacoresistance are likely related to the greater number of axonal recurrent connections in OHSCs compared to the normal hippocampal region. According to some research, parts of the latent period and the progressive nature of epileptogenesis may also be explained by the continual nature of axonal sprouting and the development of recurrent excitatory connections as a result of primary injuries as the degree of intractability increasing [66]. The dentate gyrus of OHSCs and the abnormal recurrent excitatory circuitry found in CA3 mimic the outcomes of similar drawn-out processes in vivo. Second, it's important to keep in mind the clinical evidence for de novo pharmacoresistance in many patients with temporal lobe epilepsy [1].

This suggests that the development of pharmacoresistance can be related to the maintenance or, alternatively, the reappearance of aberrant excitatory connections rather than a prolonged history of epileptic seizures. Thirdly, since the AEDs we have examined have distinct chemical activities, it seems improbable that a single mechanism found in epileptic tissue or the immaturity of a single neuronal or transmitter system is the exclusive cause of the OHSCs' pharmacoresistant state.

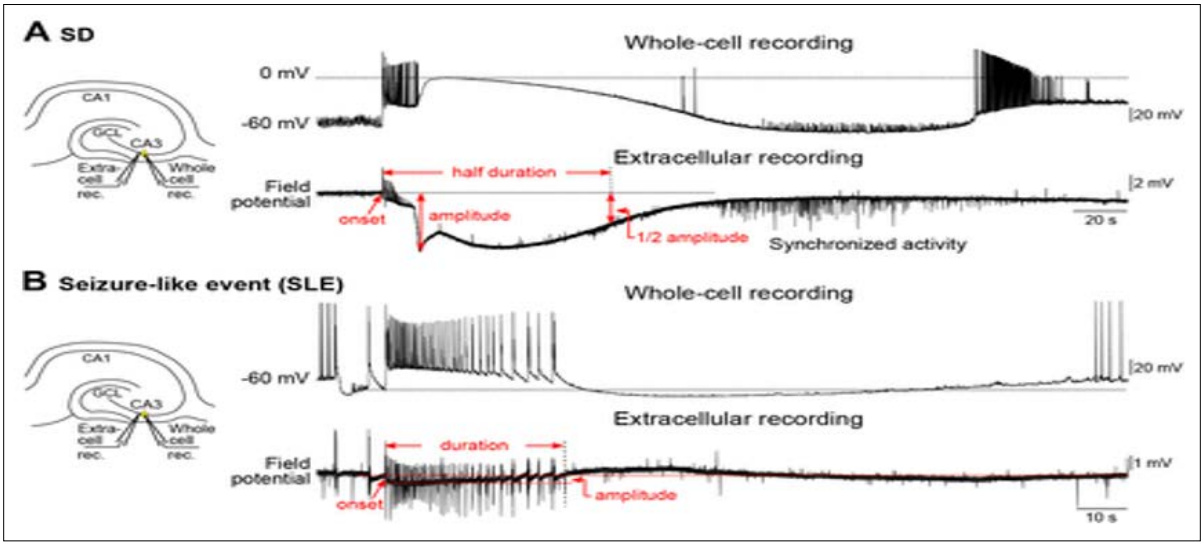


Figure 5: All measurements used field potential recordings. (A) The onset of an SD was marked by beginning of a train of bursts that was followed by a sudden, large depolarization in whole cell recording or a large negative deflection in the field recording. The amplitude of an SD was measured as the maximal negative deflection relative to baseline. The half duration of SD was from the onset of the SD to the point where the recovery of SD reached half of its maximal amplitude. (B) The onset of an SLE was the beginning of the sudden large depolarization in whole cell recording, which corresponded to the initial burst in the field recording. The duration was the difference between the onset and the point where the SLE returned to baseline. The amplitude was the maximal negative deflection during the high-frequency bursts, which corresponded to the sustained firing in the whole cell recording.

These cellular mechanisms, which include modifications in drug transporter expression that restrict AED access to epileptogenic foci and modifications in the molecular drug targets of AEDs and transmitter systems, have been identified as potential causes of pharmacoresistance in the past ten years of research [67,68,4,69]. These processes have been found in brain tissue that has been subjected to long-term epileptiform activity. However, it is impossible to rule out the possibility that these "post-status" mechanisms contribute to the de novo pharmacoresistance that is being discussed here.

AEDs' Partially Causing Tonic-Clonic SLEs

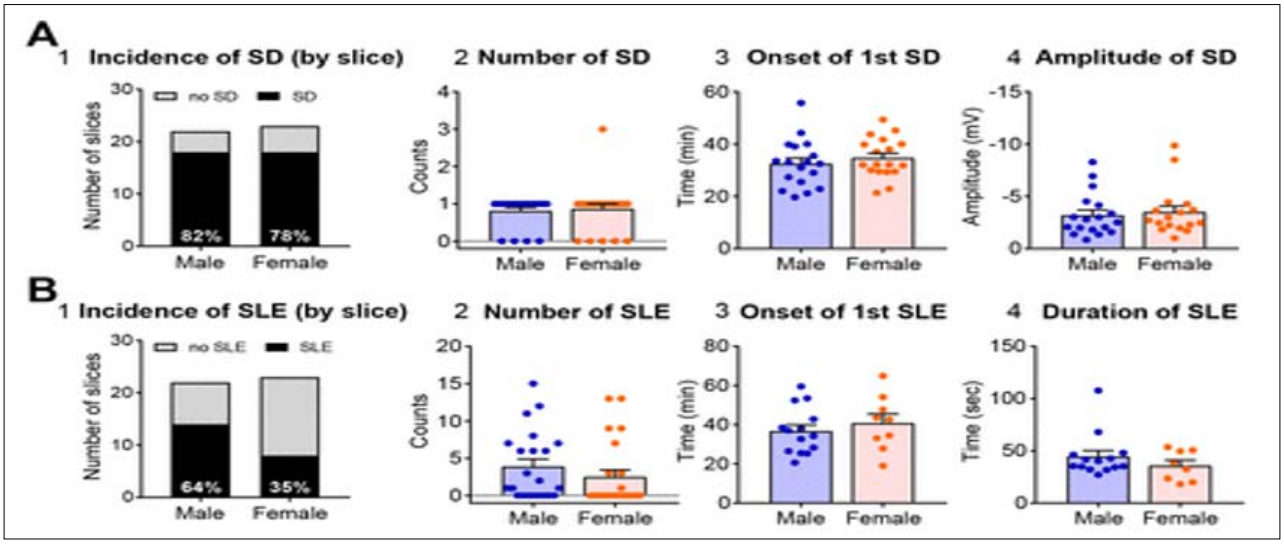


Figure 6: Lack of sex difference in characteristics of SD and SLEs in the first 60-min of 0 Mg2+/5 K+ aCSF exposure. (A) Quantification of SD. No statistical difference was found between males and females in the incidence of SD (A1, male, 22 slices/12 mice; female, 23 slices/10 mice; Fisher's exact test, $p > 0.9$), number of SDs (A2, male, 22 slices/12 mice; female, 23 slices/10 mice; Mann-Whitney test, $U = 253$, $p > 0.9$), onset of the 1st SD (A3, male, 18 slices/10 mice; female, 18 slices/9 mice; Unpaired t test, $t(34) = 0.80$, $p = 0.43$), and amplitude of SDs (A4, male, 18 slices/10 mice; female, 18 slices/9 mice; Mann Whitney test, $U = 137$, $p = 0.61$). (B) Quantification of SLEs. No statistical difference was found between males and females in the incidence (B1, male, 22 slices/12 mice; female, 23 slices/10 mice; Fisher's exact test, $p = 0.08$), the number (B2, male, 22 slices/12 mice; female, 23 slices/10 mice; Mann-Whitney test, $U = 191.5$, $p = 0.14$), the onset (B3, male, 14 slices/9 mice; female, 8 slices/5 mice; Unpaired t test, $t(20) = 0.20$, $p = 0.85$), and the duration of SLEs (B4, 14 slices/9 mice; female, 8 slices/5 mice, Mann-Whitney test, $U = 46$, $p = 0.53$).

The AEDs utilized in this investigation influence different aspects of neuronal activity and have distinct binding sites. PHT and CBZ are comparable characteristics that cause the steady state inactivation curve to shift left and encourage the removal of sodium channel inactivation, both of which lead to a decrease in the frequency of the action potential [70-72].

According to Sitges et al. both of these AEDs also lessen glutamate's presynaptic release. Consequently, the amplitudes of field potentials and [K þ]o transients were unaffected by either AED, and their suppressive effects on the temporal characteristics of SLEs were nearly equal [73]. One important distinction was that whereas field potential transient frequency dropped with CBZ during the tonic phase, it did not change with PHT. PHT operates on potassium channels and calcium channels with varying thresholds in addition to sodium channels; this might have a different impact on the OHSCs' neural networks than sodium channel blocking alone. Due to the inhibition of GABA metabolism and reuptake, VPA reduced the amplitude of tonic parameters and had no influence on the temporal structure of SLEs [4,74]. It is quite probable that PHB (200 mM) produced similar effects by directly activating GABAA receptors [75].

This is further supported by our findings that PHB had effects on SLEs comparable to low concentration GABAA-agonists and GABA uptake inhibitors, as well as influencing amplitude characteristics and concentration-dependently suppressing temporal parameters of SLEs [91]. High doses of DZP and CLO specifically shortened the clonic time of On the other hand, the amplitude parameter was unaffected by the SLE or 200 mM PHB during the tonic phase.

Phenobarbital and 1,4-benzodiazepines both modulate GABAA receptors, but they do so in different ways on GABA-activated currents. Barbiturates reduce the frequency of channel openings, while DZP increases the frequency of channel openings and lengthen the open-channel's average lifetime [76]. It is not possible to ascribe the inefficiency of CLO and DZP at inhibiting SLEs to a lack of receptors. Interneurons in the rat hippocampal region greatly influence the GABAA receptor subunits a1 and a2, which are present at P10 in concentrations only slightly greater (a1) or lower (a2) than in adults and mediate the anticonvulsive effects of DZP; express the a1 subunit [77,78]. Our findings thus show that the neural networks in OHSCs react to AEDs in distinct ways. AEDs that selectively block sodium channels, including CBZ and PHT, lessen the lengths of the clonic and tonic phases of SLEs, but high concentration AEDs that block the GABAA receptor (PHB, 1,4,-benzodiazepines) only shorten the clonic period.

The amplitudes of tonic phenomena are attenuated by increasing the local concentration of GABA (VPA); same effects are also observed at high PHB concentrations.

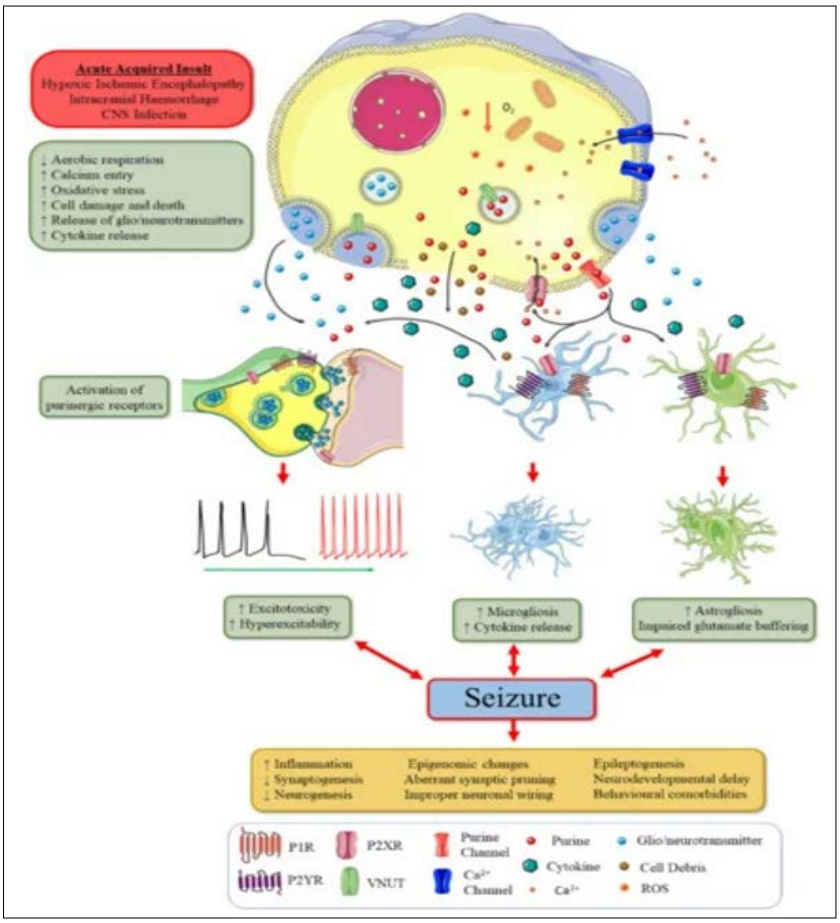


Figure 7: Cellular mechanisms of acute symptomatic neonatal seizure ictogenesis and the potential role of purinergic signalling: following an acute insult to the neonatal brain, cells are placed under high cellular stress, leading to increases in calcium entry and cell death pathways. In the case of hypoxic-ischemic encephalopathy (HIE)-induced seizures, the lack of oxygen and glucoses

limits aerobic respiration, forming radical oxygen species (ROS) causing further oxidative stress on cells. Increases in intracellular calcium and cell death can trigger the release of glio/neurotransmitters (e.g., glutamate) into the extracellular space that increases neurotransmission. Cell debris can trigger microgliosis, astrogliosis and release of proconvulsive cytokines. Purines (e.g., ATP and adenosine) are also hypothesised to be released into the extracellular space following cell death and through a combination of exocytotic and non-exocytotic mechanisms under cellular stress. ATP acts upon P2X7 to further increase intracellular calcium, contributing to cell death mechanisms and to increasing neurotransmission and, in turn, seizure severity. P2X7 activation is known to potentiate proconvulsive cytokine release following neonatal seizures, which in turn can lower seizure thresholds. Other P2 receptors are known to modulate many mechanisms of seizure ictogenesis, such as direct modulation of neurotransmission and inflammatory signalling cascades. A2A receptors may also contribute to neonatal seizures via similar mechanism to P2X7. Conversely, A1 receptor activation is anticonvulsive in neonatal seizures, acting as an endogenous compensatory mechanism. Once these outlined mechanisms create a system that favours excitatory neurotransmission, seizures are elicited. A seizure can also create further cellular stress and neuroinflammation, increasing the likelihood of recurrent seizures. Elevated neuroinflammation and hyperexcitability alter many mechanisms critical for brain development, leading to long-lasting changes of the brain. Purinergic signalling can be hypothesised to modulate this and may be targeted in the future to prevent comorbidities following neonatal seizures.

Conclusion

Organotypic slice cultures of the hippocampus could be used as an easy way to create an in vitro model of pharmacoresistant mesial temporal lobe epilepsy and to add information to existing research. proven from acute slices of the adult rat hippocampal and entorhinal cortex, an in vitro model previously shown to be a good representation of the late, pharmacoresistant stages of status epilepticus. Drugs having delayed onset activities could also be screened using OHSCs, which can be conveniently integrated with toxicological and safety research. It is now feasible to transfer such testing directly to drug-resistant human tissue because SLEs can also be induced in human tissue [48,79,7]. This could potentially hasten the development of agents that would be helpful in the treatment of those individuals who don't respond to drugs [80-100].

Conflict of Interest

The authors state no conflict of interest.

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