Potassium Changing from Pro- to Anti-Convulsant in the Epileptic Juvenile Rat Hippocampus

by

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Abstract

Elevations in extracellular potassium ($K^+$) accompany seizure-like events (SLEs), but elevated $K^+$ may also participate in seizure cessation. The objective of this thesis was to investigate the possibility that $K^+$ may undergo a pro- to anti-convulsant switch in the epileptic juvenile (postnatal day 17-21) rat hippocampus.

Field recordings were performed in the CA1 pyramidal layer. SLEs and primary afterdischarges (PADs) were induced with 0.25 mM Mg/5 mM K$^+$ perfusion or tetanic stimulation of the Schaffer collaterals respectively. In these seizure models, elevating $[K^+]_e$ beyond 7.5 mM showed anticonvulsant properties. The addition of ZD7288, a blocker of the hyperpolarization activated nonspecific cationic current (Ih) and allowed SLEs to continue even in elevated $[K^+]_e$. This suggests that $[K^+]_e$ switches from being pro- to anti-convulsant, in part due to an elevated $[K^+]_e$-induced potentiation of Ih. Ih likely contributes to this anticonvulsant behavior by decreasing membrane resistance and subsequently attenuating summation of incoming EPSPs.
Acknowledgements

Many of the people that I met and worked with for the duration of my Masters of Science (MSc) degree have contributed greatly to making the past few years a very rewarding learning experience.

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<th>Description</th>
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<tbody>
<tr>
<td>aCSF</td>
<td>artificial cerebral spinal fluid</td>
</tr>
<tr>
<td>AEDs</td>
<td>antiepileptic drugs</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid</td>
</tr>
<tr>
<td>BBB</td>
<td>blood-brain barrier</td>
</tr>
<tr>
<td>CA1</td>
<td>cornu ammonis area 1</td>
</tr>
<tr>
<td>CA3</td>
<td>cornu ammonis area 3</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>cGMP</td>
<td>cyclic guanosine monophosphate</td>
</tr>
<tr>
<td>CNBD</td>
<td>cyclic nucleotide binding domain</td>
</tr>
<tr>
<td>CNG</td>
<td>cyclic nucleotide gated</td>
</tr>
<tr>
<td>DBS</td>
<td>deep brain stimulation</td>
</tr>
<tr>
<td>DS</td>
<td>depolarization shift</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalogram</td>
</tr>
<tr>
<td>fEPSP</td>
<td>field excitatory postsynaptic potential</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma aminobutyric acid</td>
</tr>
<tr>
<td>GAERs</td>
<td>genetic absence epilepsy rats</td>
</tr>
<tr>
<td>h∞</td>
<td>steady state inactivation</td>
</tr>
<tr>
<td>HCN</td>
<td>hyperpolarization-activated and cyclic nucleotide gated</td>
</tr>
<tr>
<td>If</td>
<td>funny current</td>
</tr>
<tr>
<td>Ih</td>
<td>hyperpolarization-activated nonspecific cationic current</td>
</tr>
<tr>
<td>I_{K,A}</td>
<td>transient A potassium current</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>$I_{K,DR}$</td>
<td>delayed rectifier potassium current</td>
</tr>
<tr>
<td>$I_{Na,P}$</td>
<td>persistent sodium current</td>
</tr>
<tr>
<td>$I_{Na,T}$</td>
<td>transient sodium current</td>
</tr>
<tr>
<td>IPSP</td>
<td>inhibitory postsynaptic potential</td>
</tr>
<tr>
<td>$I_q$</td>
<td>queer current</td>
</tr>
<tr>
<td>$[K^+]_e$</td>
<td>extracellular K$^+$</td>
</tr>
<tr>
<td>LTD</td>
<td>long term depression</td>
</tr>
<tr>
<td>LTP</td>
<td>long term potentiation</td>
</tr>
<tr>
<td>LVA</td>
<td>low voltage activated</td>
</tr>
<tr>
<td>NMDARs</td>
<td>N-methyl-D-aspartate receptors</td>
</tr>
<tr>
<td>NRSF</td>
<td>neuron restrictive silencing factor</td>
</tr>
<tr>
<td>O/A</td>
<td>stratum oriens and alveus</td>
</tr>
<tr>
<td>PADs</td>
<td>primary afterdischarges</td>
</tr>
<tr>
<td>PTZ</td>
<td>pentylenetetrazol</td>
</tr>
<tr>
<td>$R_m$</td>
<td>membrane resistance</td>
</tr>
<tr>
<td>RMP</td>
<td>resting membrane potential</td>
</tr>
<tr>
<td>SLEs</td>
<td>seizure-like events</td>
</tr>
<tr>
<td>SW</td>
<td>spike and wave</td>
</tr>
<tr>
<td>VDCCs</td>
<td>voltage-dependent calcium channels</td>
</tr>
<tr>
<td>VDSCs</td>
<td>voltage-dependent sodium channels</td>
</tr>
<tr>
<td>$V_{1/2}$</td>
<td>half maximal activation potential</td>
</tr>
<tr>
<td>$V_m$</td>
<td>membrane potential</td>
</tr>
<tr>
<td>ZD7288</td>
<td>4-ethylphenylamino-1,2-dimethyl-6-methylaminopyrimidinium chloride</td>
</tr>
</tbody>
</table>
1 A Brief Review of Epilepsy

1.1 Introduction

The brain is the most complex organ in the body and in order for it to function appropriately, the many billions of individual neuronal and glial cells that make up this system must work together in a coordinated fashion. Pathologies arise when this balance is disturbed, and one such condition is epilepsy. The epilepsies are a group of disorders characterized by spontaneous, recurrent seizure activity, seizures being defined as sustained periods of hyperexcitation and/or hypersynchrony associated with brain dysfunction (Hauser et al. 1993). There are approximately 1% of people who have epilepsy at any given time (Hauser et al. 1993). Epilepsy is considered one of the most serious neurological disorders, as it heavily affects the quality of life of afflicted individuals, and many epileptic patients have intractable seizures, so their condition cannot successfully be controlled by current drugs. As such, further research must be conducted to improve our understanding of the epileptic condition and to examine other treatment options. This chapter will attempt to briefly describe what is currently known regarding epilepsy, and the possible mechanisms and treatments thereof.
1.2 Clinical Types of Epilepsy

Epilepsy itself, as a group of disorders, can be most broadly categorized as symptomatic or essential (Lüders and Noachtar 2000). Symptomatic epilepsy refers to seizure disorders that are a result of an identifiable problem, such as a brain lesion. Essential, or idiopathic epilepsy, is the opposite, where the seizure disorders have an unknown origin, though many idiopathic epilepsies are now being found to have a genetic basis (Singh et al. 2002).

Seizures can further be classified as convulsive or nonconvulsive, and even more specifically, as generalized or partial, depending on the area(s) of the brain affected (Somjen 2004). Convulsive seizures are generalized tonic-clonic or “grand mal” seizures. These seizures have two phases: the initial tonic phase, involving simultaneous spasms, followed by the clonic phase, where the body undergoes rhythmic jerking movements (Engel 1989). They are considered generalized, since the seizures affect both cerebral hemispheres significantly and there is a loss of consciousness. If the seizure persists for an extended period of time or occurs so frequently that there is no recovery period in between, then it is referred to as a status epilepticus. Another form of generalized seizure is referred to as an absence or “petit mal” seizure, where there is a momentary lapse of consciousness, but no major convulsions (Cortez et al. 2001).

There can also be partial seizures, if only a limited area of the brain is affected (Engel 1989). These seizures, while limited to that area, are known as focal seizures, but seizures can propagate and evolve into secondary generalized seizures. Partial seizures can further be broken down into simple or complex partial seizures, depending on whether consciousness is retained, in the former case, or if consciousness is lost or impaired, as in the latter case. Simple partial seizures can arise from the neocortical area, and when they affect the motor cortex, they can
cause convulsions, known as Jacksonian fits, and sometimes subsequent temporary paralysis in the muscle groups associated with the affected motor area (Lüders and Noachtar 2000). On the other hand, complex partial seizures affect the conscious state and tend to originate from subcortical areas in the temporal lobe, in particular, the amygdala and hippocampus. As such, complex partial seizures are grouped under the condition known as temporal lobe epilepsy. These seizures are characterized by automatisms, which are stereotyped behaviours resulting from the mobilization of specific neuronal sequences (Somjen 2004).

In summary, there are many different classifications for the array of seizure disorders known as epilepsy, and this introduction only briefly touches upon some of the main forms of epilepsy.

1.3 Clinical Electrophysiology of Seizures

As seizures are electrical disturbances which disrupt the normal activity of the brain, seizure-like events (SLEs) can be readily recorded through the use of electrophysiological techniques. On the clinical level, seizures are studied mostly with electroencephalography (EEG) (Niedermeyer 1972). The EEG is a product of synaptic activity and represents rhythmic oscillations in voltage due to the synchronized firing of postsynaptic potentials from neurons around the electrode positions. The activity recorded in EEG recordings can be split into a number of frequency ranges: delta (0.1 – 3.5 Hz), theta (4 – 7.5 Hz), alpha (8-13 Hz), beta (14-30 Hz), and gamma (30-100 Hz), and seizures are typically dominated by high frequency activity (Niedermeyer 1972).

Seizure (ictal) events can be easily observed on EEG recordings, as they are the result of hypersynchronization of many cells. For generalized tonic seizures, the EEG recordings reveals
high-voltage, fast-discharge pattern, with a frequency typically around 15-20 Hz, though this frequency range is not always the case (Lüders and Noachtar 2000). Tonic phases are often followed by clonus, where the EEG shows large amplitude, high frequency bursting activity corresponding to the jerks seen in affected muscles. After the ictal episode, there is a characteristic period of postictal silence (Gastaut and Broughton 1972).

Absence seizures also show a standard pattern of activity, whereby there are complex waves that occur at a rhythm of about 3 Hz, and between the waves, there is a spike component (Walton 1985). As such, the standard discharge characterizing absence seizures is a 3 per second spike-and-wave (SW) pattern. The waves are due to fluctuations in membrane potentials, and the spikes are a result of the depolarizing phase of action potentials.

Apart from ictal activity, EEGs can also record interictal activity, which is the activity between seizures. These show up in the form of polyspike complexes, large-amplitude slow waves, isolated spike-wave complexes, and sharp waves. Spikes and sharp waves are differentiated on the EEG by timing, since sharp waves can last up to 200 ms, whereas spikes only last about 70 ms (Engel 1989). The role or function of interictal activity is still debated. Some have suggested that these interictal events may be suggestive of an upcoming ictal event, as some studies do show a progression of interictal activity leading to full ictal discharges (Jami 1972; Prince et al. 1983; Jensen and Yaari 1988; Amzica and Steriade 2000). Others propose that interictal activity may reflect past seizures as opposed to indicating future seizures (Gotman 1991). More recently, it has been suggested that interictal and ictal activity may actually be generated by different cell groups, and interictal activity may be inhibitory and actually protective against seizures (Swartzwelder et al. 1987; de Curtis et al. 1999; Avoli 2001).
Somjen suggests that similarities in the interictal activity electrically may not necessarily mean that all interictal activity is driven by the same mechanisms and processes, and this may explain why there is conflicting evidence for the role of interictal activity in the promotion or protection of ictal events (Somjen 2004).

1.4 Cellular Mechanisms of Epilepsy

Over the past number of decades, there has been a steady progression in technology, and this has allowed for research into the cellular and molecular mechanisms behind seizures. As mentioned in section 1.3, on the EEG, seizures can be seen as ictal events, but the presence of interictal discharges is also indicative of a hyperexcitable area that has potential for epileptogenicity. The connection between interictal discharges and seizures seems to be most closely related in focal seizure models (Dichter and Ayala 1987).

In the focal seizure models, during interictal discharges, there is a considerable depolarization shift (DS) with a burst of action potentials superimposed on the wave of depolarization, followed by a post-DS hyperpolarization where neurons are inhibited (Dichter and Spencer 1969; Lopantsev and Avoli 1998). As the seizure-like activity develops, the post-DS hyperpolarization shortens, and eventually it is replaced by depolarizing waves (Ayala et al. 1970). As the interictal discharges continue to build, there is a steady increase in extracellular $K^+$ as well as a decrease of $Ca^{2+}$ as a result of intense neuronal activity (Fertziger and Ranck 1970), but in areas surrounding the focus, there is general inhibition. On the EEG, after successive interictal discharges, afterdischarges develop and eventually grow into a full blown seizure event.
At this point, nearby areas of the brain may be recruited into the seizure process and so the seizure may spread and become generalized (Dichter and Ayala 1987). After the seizure episode stops, there is a post-ictal depression marked by hyperpolarization of the neuronal membrane.

To study the mechanisms behind epilepsy, naturally, experimental models are utilized. What adds to the complexity of epilepsy is that various agents with unrelated mechanisms of action can still provoke focal seizure-like events with the similar stereotyped DS hyperpolarization sequence (Table 1) (Dichter and Ayala 1987). These agents seem to largely have an effect on the intrinsic membrane properties and functions of cells. Thus, whether seizures occur at any focal point depends on whether the normal function of that area is changed due to alterations in the intrinsic currents of the cells in the region, or a shift in synaptic efficacy. Factors that would then have to be taken into account for that epileptogenic region include the density and location of channels in the area, possible interactions between different currents, the general synaptic organization, as well as possible neuromodulators that may affect the currents through second messenger pathways (Dichter and Ayala 1987).

Current studies support the idea that the DS is a result of excitatory synaptic currents, which may be amplified by other intrinsic currents (Dichter and Spencer 1969; Ayala et al. 1970; Johnston and Brown 1981). There are a number of proposed mechanisms for how the excitatory synaptic currents can become enhanced enough to produce SLEs.
### Convulsant Agent

<table>
<thead>
<tr>
<th>Penicillin, bicuculline, picrotoxin</th>
<th>Antagonist of GABA	extsubscript{A} receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allylglycine, 3-mercaptoproprionic acid</td>
<td>Block synthesis of GABA</td>
</tr>
<tr>
<td>Ammonium salts</td>
<td>Block Cl	extsuperscript{−} pump</td>
</tr>
<tr>
<td>Tetramethylammonium, 4-aminopyridine (4-AP)</td>
<td>Block K	extsuperscript{+} currents</td>
</tr>
<tr>
<td>Barium</td>
<td>Enhance Ca	extsuperscript{2+} currents and block K	extsuperscript{+} currents</td>
</tr>
<tr>
<td>Enkephalin</td>
<td>Inhibit inhibitory interneurons</td>
</tr>
<tr>
<td>Elevated [K	extsuperscript{+}]_e</td>
<td>Affect intrinsic membrane characteristics</td>
</tr>
<tr>
<td></td>
<td>Depolarize neurons</td>
</tr>
<tr>
<td></td>
<td>Increase emphatic coupling</td>
</tr>
<tr>
<td>Low [Ca	extsuperscript{2+}]_e</td>
<td>Increase neuronal excitability</td>
</tr>
<tr>
<td></td>
<td>Decrease neuronal threshold</td>
</tr>
<tr>
<td>Low [Mg	extsuperscript{2+}]_e</td>
<td>Reduce surface screening</td>
</tr>
<tr>
<td></td>
<td>Increase presynaptic transmitter release</td>
</tr>
<tr>
<td></td>
<td>Potentiate NMDAR activation</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>Decrease Na	extsuperscript{+} pump</td>
</tr>
<tr>
<td></td>
<td>Increase [K	extsuperscript{+}]_e</td>
</tr>
<tr>
<td></td>
<td>Increase Ca	extsuperscript{2+} spikes</td>
</tr>
<tr>
<td>Alumina Cream</td>
<td>Neuronal Loss</td>
</tr>
<tr>
<td></td>
<td>Selective decrease in inhibitory interneurons</td>
</tr>
</tbody>
</table>

**Table 1.** Agents that produce focal epilepsy and their mechanisms of action.

*Modified from Dichter and Ayala, 1987.*
There may be a decrease in inhibition, potentiation of excitation, increase in the space constant of the dendrites on the postsynaptic neuron, potentiation of neuromodulator release, and/or supplementation by other voltage-dependent receptors such as N-methyl-D-aspartate receptors (NMDARs), the slowly inactivating Na\(^+\) or Ca\(^{2+}\) currents, or the large transient Ca\(^{2+}\) current (Yamamoto et al. 1980; Connors et al. 1982; Stafstrom et al. 1982; Mayer and Westbrook 1985; Stafstrom et al. 1985; Stanfield et al. 1985; Herron et al. 1986; Derchansky et al. 2008). A viable proposal is that potentiated excitatory postsynaptic potentials (EPSPs) will summate and depolarize the neuron, progressively activating other voltage-gated channels which pass depolarizing currents such as Na\(^+\) and Ca\(^{2+}\) currents. These will further depolarize the neuron until the high threshold currents such as the NMDA-mediated current, are activated. This intense activation of depolarizing currents will thus push the cell to DS and fire a burst of action potentials (Herron et al. 1985).

Another possible mechanism for DS and burst firing is through the inhibition of K\(^+\) currents and subsequent activation of slower Ca\(^{2+}\) currents (Johnston et al. 1980; Stafstrom et al. 1984; Stafstrom et al. 1985). This may be possible through the contribution of neuromodulators which can reduce K\(^+\) currents (Benardo and Prince 1982; Benardo and Prince 1982; Benardo and Prince 1982; Benardo and Prince 1982; Benardo and Prince 1982; Brown et al. 1982), or redistributions or changes in the channels in the epileptogenic area due to an injury or lesion.

Following the DS, there is typically a hyperpolarization, which seems to be protective against the development of seizures, since the disappearance of the hyperpolarization period often signifies the onset of a SLE (Matsumoto and Marsan 1964; Dichter and Spencer 1969; Ayala et al. 1970). The most prominent mechanism explaining the hyperpolarizations would be the presence of inhibitory postsynaptic potentials (IPSPs), such as short latency GABA-mediated
IPSPs triggered by the opening of Cl⁻ channels, or longer latency IPSPs resulting from the opening of K⁺ channels (Alger and Nicoll 1980). However, the specific conductances involved in the hyperpolarization may largely depend on what cells are affected, and where the epileptogenic foci is.

Importantly, seizures are not simply due to hyperexcitability, but it is also necessary to have hypersynchrony (Wong et al. 1986). Positive feedback can come from recurrent synaptic excitation, as excitatory recurrent collaterals have been found anatomically in the cortex and hippocampus, and this recurrent excitation may also help in the amplification of the DS (Lebovitz et al. 1971; MacVicar and Dudek 1980; MacVicar and Dudek 1980; Knowles and Schwartzkroin 1981). The intense discharges from neurons can also result in changes in the ionic environment, with K⁺ becoming elevated extracellularly, which will cause cell swelling and a decrease in extracellular space, therefore promoting ephaptic interactions and synchrony among cells (Konnerth and Heinemann 1983; Yaari et al. 1986). One increasingly prominent mechanism for synchrony exists in the form of electronic coupling via gap junctions, formed by connexin proteins of adjacent cells forming a direct channel through which electrical signals can pass (MacVicar and Dudek 1980; Gutnick and Prince 1981). In fact, it has been shown that gap junctional blockers can actually block SLEs in seizure models, thus emphasizing the significance of electrotonic coupling and synchrony in the development of seizure events (Jahromi et al. 2002).

Summarily, the cellular mechanisms involved in epilepsy are complicated and diverse. There does not seem to be a single unifying theory for all different forms of epilepsies, but on the most basic level, cellularly, seizures occur as a result of hyperexcitation and hypersynchrony of a population of neurons in the epileptic focus.
1.5 Anticonvulsant Treatment Options

Thus far, no cure for epilepsy exists, and treatments focus on controlling the seizures as a symptom (Meldrum 2002). The most common treatment is through drug therapy, although there are some patients who require alternative therapy due to pharmacoresistance. Most antiepileptic drugs (AEDs) have their effect through modulating or antagonizing channels, in essence, balancing the hyperexcitable malfunction with another malfunction, and as such, many AEDs have significant side effects (Meldrum 2002). AEDs can be classified according to their mechanism of action, and there are five general classifications: ion channel modulators, enhancers of synaptic inhibition, depressors of synaptic excitation, inducers of cerebral acidosis, and AEDs with an unknown mechanism of action (Somjen 2004). AEDs often have multiple actions, and if synergistic, this can be beneficial, but it can also be detrimental if the drug action affects other channels and other areas of the brain besides the target focus.

AEDs that are ion channel modulators can work on different channels. AEDs acting on the voltage-dependent Na\(^+\) channels (VDSCs) include phenytoin, lamotrigine, valproate, carbamazepine, and topiramate (Somjen 2004). These drugs act as antagonists of VDSCs, the primary target being the inactivation of the transient Na\(^+\) current (I\(_{\text{Na,T}}\)), and the main effect is the limiting of sustained high frequency firing that occurs during SLEs (Macdonald and Kelly 1995). Some AEDs also inhibit the persistent Na\(^+\) current (I\(_{\text{Na,P}}\)), which enhances their usefulness in blocking seizure activity since the I\(_{\text{Na,P}}\) plays a significant role in the repolarization of the cells after each action potential. Recovery from firing is also slowed and the steady-state inactivation (h\(_\infty\)) is shifted to a more negative voltage. The main advantage of AEDs that antagonize VDSCs is that therapeutic dose levels can curb excessive high frequency firing without affecting the threshold for normal action potentials, so baseline levels of excitability are intact.
Some AEDs such as ethosuximide and the methadiones work on voltage-dependent Ca\textsuperscript{2+} channels (VDCCs). They inhibit low voltage-activated (LVA) Ca\textsuperscript{2+} currents, and this seems to inhibit the thalamocortical burst complexes which are characteristic of absence epilepsy (Zhang et al. 1996). A newer drug, retigabine, also affects ion channels, but it acts by increasing K\textsuperscript{+} conductance, thus hyperpolarizing neurons and reducing hyperexcitability (Armand et al. 2000).

Beyond acting on ion channels, some AEDs can have an effect by modifying synaptic transmission. Many AEDs, including phenobarbital, benzodiazepines, gabapentin, felbamate, topiramate, and also valproate, work by enhancing GABAergic inhibition (Macdonald and Kelly 1995; Macdonald and Greenfield 1997). GABAergic inhibition is enhanced by these drugs either by inhibiting the reuptake of GABA so the effect of GABA is increased and prolonged at the synapses, or by increasing the open time or the opening frequency of GABA\textsubscript{A} receptors. Other drugs can modify synaptic transmission by antagonizing NMDA receptors, thereby decreasing levels of excitation (Bialer et al. 2001).

Certain AEDs such as acetazolamide can act primarily through an inhibition of carbonic anhydrase, and the effect of this is an accumulation of CO\textsubscript{2} and corresponding cerebral acidosis (Heuser et al. 1975; Reiss and Oles 1996). It has been shown that acidosis, whether by a drug such as acetazolamide or inhaled CO\textsubscript{2}, can depress activity and result in a depression in cerebral excitability (Tombaugh and Somjen 1996; Kaila and Ransom 1998). Some other compounds such as amiloride can cause intracellular acidification by blocking transmembrane acid extrusion, and this has been shown to successfully ameliorate spontaneous bursting in some hippocampal seizure models (Bonnet et al. 2000).

An alternative to drug treatment is epilepsy surgery, however, the conditions for a viable surgical resection are stringent (Awad et al. 1991; Boon et al. 1991). The epileptic focus must be
narrowed down to a specific area of the brain, and the patient must also be untreatable with anticonvulsant medication. The surgery itself must also not involve areas which will result in a significant neurological deficit if resected (Kwan and Brodie 2000). It is generally understood that resection of the brain area will disrupt the abnormal activity from propagating from the area of onset to surrounding regions. Also if the epileptic focus is actually removed, then perhaps the hyperexcitability itself would no longer pose a problem for the patient.

Another more recent form of treatment involves electrical stimulation. Deep brain stimulation (DBS) involves the implantation of an electrical stimulator into the brain (Awan et al. 2009). It is now being used as a treatment paradigm for many neurological disorders, but the precise mechanisms which underlie this treatment are still unclear. Stimulation can be directly at the epileptogenic zone (direct control), or indirectly through an anatomical relay of the cortico-subcortical networks (remote control) since several other structures in the cortico-subcortical network play supporting roles in epileptic behaviour (Pollo and Villemure 2007).

The first study using DBS for epilepsy involved cerebellar subdural stimulation and there was a reduction in seizure frequency observed (Cooper et al. 1973). Since then, various other structures have been targeted including the anterior thalamus, centromedian thalamic nucleus, caudate nucleus, mamillary body, subthalamic nucleus, and the amygdalohippocampal complex (see review in (Pollo and Villemure 2007). Experiments *in vitro* have been done to examine possible mechanisms for stimulation impinging on seizure activity. In picrotoxin-induced epileptiform activity in the hippocampus, it has been shown that stimulation can eliminate the epileptiform discharges. The mechanism is not clear cut, however, since the effects of stimulation change depending on the frequency and duration of stimulation (Pollo and Villemure 2007). The effect also depends highly on the type of epilepsy, as well as the location of
stimulation. Stimulation at remote sites such as the mammillothalamic tract has been shown to modulate seizure activity by increasing the threshold for producing seizures (Mirski and Fisher 1994). What is more confusing is that in the kainic acid rat model, direct stimulation to the hippocampus reduces interictal spiking while leaving ictal activity intact (Bragin et al. 2002). Similar to deep brain stimulation, vagal nerve stimulation also has an effect in some epilepsy models such as pentylenetetrazol (PTZ)-induced seizures (Woodbury and Woodbury 1990), but not others, such as the genetic absence epilepsy rats (GAERs) (Dedeurwaerdere et al. 2004).

Ultimately, the mechanisms behind how electrical stimulation can treat seizures are still largely unknown, and in some cases, stimulation may actually provoke, as opposed to inhibit, seizures (Pollo and Villemure 2007). There are few main hypotheses for how electrical stimulation can work. The first is the electrical hypothesis, which suggests that neurons are inactivated because stimulation induces a large increase in $K^+$, thus shutting down neuronal activity through a depolarization block where $Na^+$ channels are too depolarized to fire. The second hypothesis is the neurochemical hypothesis, which proposes that stimulation may preferentially trigger the release of inhibitory neurotransmitters, thus blocking seizure activity. Another possibility is that high frequency stimulation may deplete excitatory neurotransmitter pools and result in a termination of hyperexcitability.
2 Potassium and Epilepsy

2.1 Introduction

In order for the brain to function properly, there must be a strict and regulated balance of ionic concentrations. There are many mechanisms in place to regulate ion concentrations intra- and extracellularly. Furthermore, the blood brain barrier (BBB) acts as a safeguard to protect the brain from fluctuations in the blood (Davson and Segal 1996). This chapter will first provide background information regarding basic membrane physiology and the role of potassium under standard conditions. Then the focus of the chapter will be on the specific role of potassium and seizures.

2.2 Basic Membrane Physiology

The membrane potential of a cell is the voltage difference between the interior and exterior of the cell. The membrane potential ($V_m$) relation to specific ion concentrations and permeabilities can be generally expressed through the Goldman-Hodgkin-Katz (GHK) equation:

$$V_m = \frac{RT}{F} \ln \left( \frac{p_K[K]_o + p_{Na}[Na]_o + p_{Cl}[Cl]_o}{p_K[K]_i + p_{Na}[Na]_i + p_{Cl}[Cl]_o} \right)$$

Where $R$ is the gas constant, $T$ is the absolute temperature, $F$ is Faraday`s number, and $P$ is the permeability of each ion. This equation leaves out other ions that contribute such as $Ca^{2+}$, and for more accurate approximations, these would have to be taken into account as well. However, the GHK equation has shown to be adequate and is widely accepted and used for computer simulations and data analysis.
The resting \( V_m \) of neurons is a result of the selective permeability of the cell membranes for specific ions, and it typically rests at approximately -60 to -75 mV, depending on the type of neuron (Somjen 2004). This is a result of \( K^+ \) being far more permeable than \( Na^+ \) at rest, and since \([K^+]_e < [K^+]_i\), there is a net gradient of \( K^+ \) moving outward, thereby pushing the cell closer to the equilibrium potential of \( K^+ \) (\(-80 \) mV). To maintain the electrochemical gradient between the interior and exterior of the cells, active transport processes are required, and the most important pump is the \( Na^+/K^+ \) ATPase, which uses energy from ATP to pump 3 \( Na^+ \) out and 2 \( K^+ \) into the cell.

When there is excitatory synaptic input to the dendrites, EPSPs summate in the soma and when the membrane potential is depolarized beyond threshold (\(-45 \) mV), \( Na^+ \) channels are opened and the permeability of \( Na^+ \) becomes much greater than that of potassium (Hodgkin et al. 1952). The transient sodium current \( I_{Na,T} \) then depolarizes the cell closer to the equilibrium potential of \( Na^+ \) (\( +40 \) mV) resulting in sharp depolarization that is the action potential. The action potential is very brief however, due to the \( Na^+ \) channels becoming inactivated from depolarization. Following the action potential, there is typically a hyperpolarization due to the slower kinetics of the voltage-dependent \( K^+ \) channels. This allows \( K^+ \) currents, particularly the delayed rectifier and smaller A currents (\( I_{K,DR} \) and \( I_{K,A} \) respectively) to drive the \( V_m \) to hyperpolarized levels. When these \( K^+ \) channels close, the cell repolarizes back to its resting \( V_m \), aided by the small inward persistent \( Na^+ \) current (\( I_{Na,P} \)). With the cell repolarized, the \( Na^+ \) channels are un-inactivated and the refractory period ends.

It is clear then, that each time a cell fires, \( K^+ \) is released and the ratio of \([K^+]_e / [K^+]_i\) increases. This elevation in extracellular \( K^+ \) can have major consequences on cellular function,
and as a result, there are certain safeguards in place for buffering $K^+$ elevations. During low frequency firing, the $Na^+/K^+$ ATPase is sufficient to return released $K^+$ back to the cell, and it ensures that the elevation in $K^+$ is only minimal and also transient (Connors et al. 1979). In situations of focal high-frequency firing, the elevation in $K^+$ will be very concentrated in a small area, so following the concentration gradient, the $K^+$ can diffuse to neighboring areas temporarily, and as a result, after firing abates and the $Na^+/K^+$ pump works to pump $K^+$ back into cells, there is a noticeable postexcitation undershoot prior to $K^+$ returning to baseline levels (Heinemann and Lux 1975). Lastly and most powerfully, glial cells, particularly astrocytes, work both passively and actively to buffer elevations in $K^+$ by taking the excess $K^+$ and transporting it through the glial network through gap junctions, thereby dispersing the $K^+$ (Ballanyi et al. 1987).

### 2.3 The Role of Potassium in Seizure Activity

The connection between $K^+$ and seizure activity has been studied for many decades. It was a widely debated issue whether the elevated $K^+$ is a cause or an effect of seizures.

The potassium hypothesis of seizure generation proposes that there may be a positive feedback loop involving hyperexcited neurons and excess $K^+$ release and this would thus, reinforce the excitation and encourage further seizure activity until depolarization reached levels at which VDSCs would be kept in an inactivated state and be unable to fire until repolarized. At this level, seizures would terminate and $K^+$ levels would return to normal (Figure 1) (Green 1964; Fertziger and Ranck 1970). Feldberg and Sherwood showed in cats that KCl injections into the lateral ventricle would induce epileptic convulsions in the cats (Feldberg and Sherwood 1957).
Figure 1. The Potassium Hypothesis of Seizure Generation.

This hypothesis proposes that there is a positive feedback between hyperexcited neurons releasing K\(^+\) and the elevated K\(^+\) further exciting the neurons until there is a depolarization block, at which point both K\(^+\) elevation and neuronal activity ceases, marking the termination of SLEs. Modified from Fetziger and Ranck, 1970.
It was later demonstrated that if artificial CSF (aCSF) with elevated K\(^+\) was perfused through the lateral ventricle, seizure activity would be seen in the hippocampal region (Zuckermann and Glaser 1968; Zuckermann and Glaser 1968; Zuckermann and Glaser 1968). Most labs have [K\(^+\)] of ~2.5-3.5 mM in their aCSF solution, and it has been found that even slight elevations up to 5 mM K\(^+\), can greatly facilitate paroxysmal firing triggered by repetitive electrical stimulation to afferent pathways (Somjen et al. 1985). With further elevations in K\(^+\), up to ~8 mM, spontaneous activity can be triggered in the form of interictal discharges or prolonged SLEs (Rutecki et al. 1985; Korn et al. 1987; Traynelis and Dingledine 1988; Jensen and Yaari 1997). There are many reasons why elevated K\(^+\) may promote SLEs, some of which are summarized in Figure 2 (Traynelis and Dingledine 1988). Elevated [K\(^+\)]\(_e\) depolarizes cells, moving the potential closer to firing threshold and increasing NMDAR activation. It causes the cell to swell, which decreases the extracellular space and increases ephaptic coupling, which aids in synchronization of the network. It also depolarizes presynaptic terminals and cause activation of VDCCs and inappropriate transmitter release (Hablitz and Lundervold 1981). GABA\(_A\)-mediated synaptic inhibition may also be rendered less effective with elevated K\(^+\), as it has been shown that this elevation can force the uptake of Cl\(^-\), possibly through activation of an inward KCl pump, and shift the reversal potential of GABAergic IPSPs to more depolarized levels (Korn et al. 1987; Chamberlin and Dingledine 1988). A further possibility is that K\(^+\) can have secondary effects through the potentiation of other currents such as the I\(_{Na,P}\) (Somjen and Muller 2000).

The potassium hypothesis has been widely debated for many years, but by now, it is largely rejected due to observations made using K\(^+\)-sensitive electrodes (Futamachi et al. 1974; Lux 1974; Pedley et al. 1976; Benninger et al. 1980; Somjen et al. 1986).
Figure 2. Proposed Effects by which Elevated $K^+$ Triggers SLEs.

The elevation in $[K^+]$ can have many effects, which all work together in a positive feedback loop. These effects are then proposed to promote the initiation of SLEs. Modified from Traynelis and Dingledine, 1988.
The potassium hypothesis predicts that there is a threshold \([K^+]_c\) for seizure initiation, but no set threshold has been found (Lux 1974). The elevation in \(K^+\) also seems to lag behind seizures as opposed to leading them. This suggests that the elevated \(K^+\) may be a consequence of the seizures as opposed to a cause. Another argument against the hypothesis is that electrical stimulation in healthy tissue can increase \(K^+\) levels to that seen during seizures without triggering seizure activity. A possible defense for the potassium hypothesis is that \(K^+\)-sensitive electrodes, since they are double-bored, may create a liquid-filled cavity that dilutes or delays the \(K^+\) measurements made (Somjen 2004). Furthermore, it has been seen that electrical stimulation can elevate \(K^+\) to a ceiling level, but upon initiation of SLEs, the \(K^+\) further increases to an even higher ceiling level (Somjen and Giacchino 1985; Stringer and Lothman 1989; Stringer et al. 1989).

In any case, it is without dispute that the elevation in \(K^+\), regardless of whether it is cause or consequence, will have a major impact on neuronal function. It has been proposed that interictal discharges may not be caused by elevated \(K^+\), but the elevated \(K^+\) resulting from these discharges may push the activity from interictal to ictal (Dichter et al. 1972; Jensen and Yaari 1997; Borck and Jefferys 1999). Preceding ictal onset, it was observed that \(K^+\) would increase and the SLE would occur from a set threshold \(K^+\) of \(\sim 7.5\) mM. During the SLE, \(K^+\) levels would reach the documented ceiling of 12 mM. The \(K^+\) may be contributing to the interictal to ictal transition not only by increasing the firing frequency of spontaneously active cells, but also by recruiting otherwise inactive cells (Cohen and Miles 2000).
3 The Ih Current

3.1 Introduction

The hyperpolarization-activated (Ih) current is a voltage-activated inward cationic current that was first studied in the heart (Noma and Irisawa 1976; Brown et al. 1979; DiFrancesco 1986; DiFrancesco et al. 1986). It was first termed the “funny” current (I_f) because of its activation by hyperpolarization as opposed to the characteristic depolarization. This current is known as a pacemaker current which allows for the sino-atrial cells to maintain spontaneous activity. It works by activating during the repolarization after action potentials, thus helping depolarize the cell again and, along with VDCCs, pushing the cells to generate an action potential again. Since then, this current has been found in neurons, including in the hippocampus (Halliwell and Adams 1982). First termed “queer” current (I_q) in central neurons, both the I_f and I_q were later grouped and termed as Ih to prevent confusion. For a review on the many factors that regulate the Ih current, see review by Wahl-Schott and Biel, 2009, but for the purposes of this thesis, these factors will not be discussed in detail (Wahl-Schott and Biel 2009). This chapter will focus on the characteristics of the Ih, its functional properties, and its role in seizures.

3.2 HCN Channel Structure

As reviewed by Wahl-Schott and Biel, 2009, the Ih flows through hyperpolarization-activated and cyclic-nucleotide-gated (HCN) channels (Wahl-Schott and Biel 2009). The HCN channels are part of a superfamily of voltage-gated pore loop channels, and are encoded by four genes: HCN1-4.
HCN channels are tetrameric and, in mammals, there are four different homotetramers each with distinct biophysical properties (Wahl-Schott and Biel 2009). In vivo, heterotetramers can form and, as such, there are more HCN channel types (Ulen and Tytgat 2001). HCN channels have two modules which cooperate allosterically during channel activation: the transmembrane core and the cytosolic C-terminal domain. The transmembrane core is composed of six α-helices (S1-S6) and an ion-conducting pore loop between the S5-S6 segment. The voltage sensor, as is characteristic in all voltage-dependent members of this family, is found on the positively charged S4 segment (Yu and Catterall 2004). As opposed to conventional depolarization-activated channels, however, it is inward movement of the S4 segment that triggers opening of HCN channels (Mannikko et al. 2002). The selectivity filter of HCN channels have with a glycine-tyrosine-glycine (GYG) motif characteristic of K⁺ selective channels, and as such, HCN channels are most permeable to K⁺. They are also highly sensitive to K⁺ fluctuations, and it has been shown that even slight elevations in K⁺ can result in a very marked increase in Ih conductance as well as a depolarizing shift in reversal potential (Spain et al. 1987; Funahashi et al. 2003; Shin and Carlen 2008). However, these channels are also permeable to Na⁺ and on a lesser level, Ca²⁺ (Pape 1996; Yu et al. 2004; Yu et al. 2007). The reversal potential lies between -25 mV and -40 mV and the cations driving the inward current depend on the membrane potential and the corresponding reversal potentials of the cations (Robinson and Siegelbaum 2003).

The cytosolic C-terminal domain is important largely because of the cyclic nucleotide-binding domain (CNBD). Unlike cyclic nucleotide-gated (CNG) channels, HCN channel opening is largely voltage-dependent and CNs act as modulators (DiFrancesco and Tortora 1991). Another difference is that HCN channels are modulated by CNs in a phosphorylation-
independent manner. The HCN channels can be modulated by both cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), though the affinity for cAMP is reportedly ~10-100-fold higher than for cGMP (Ludwig et al. 1998). When CN levels are elevated, typically by hormones or neurotransmitters, there is a shift in the half-maximal activation ($V_{1/2}$) from ~ -70 mV to more positive values (DiFrancesco and Tortora 1991). The opening kinetics are also accelerated by shifting the voltage dependence of activation. This may be because when CNs are not bound, the β-roll subdomain of the CNBD inhibits channel activation and shifts it to more negative potentials, but when CNs are bound, this inhibition may be relieved, thereby shifting the gating to more positive values and accelerating opening kinetics (Wainger et al. 2001). Beyond being a modulatory domain, it has been proposed that the CNBD may also be important for normal cell surface expression of the protein subunits (Proenza et al. 2002).

### 3.3 HCN Channel Expression

As the functional properties of each of the subunits differ, so do their levels of expression based on the region, the cell type, and the age (Ludwig et al. 1998; Moosmang et al. 1999; Bender et al. 2001; Moosmang et al. 2001). In the hippocampus, through mRNA expression analysis, it has been found that all of the HCN subunits are present, although HCN1 and HCN2 seem to predominate. Bender et al., 2001 found that during development, HCN1 was largely present in the hippocampal pyramidal cell layers for both CA1 and CA3 regions (Bender et al. 2001). The presence of HCN1 in the CA3 principal cells seems to be important for the gamma frequency oscillations observed early in development (Palva et al. 2000). By the third postnatal week, HCN1 expression is absent in the CA3 principal cells, and instead expression becomes
focused in the CA1 principal cells, as well as in the interneurons in both CA1 and CA3 pyramidal layers, specifically the basket and chandelier cells (Bender et al. 2001). It is in this third postnatal week, the hippocampus is seen to undergo a functional remodeling which reduces the excitability of the adult brain, and the corresponding absence of HCN1 in the CA3 pyramids may contribute to this (Smith et al. 1995). The basket cell and chandelier cell interneurons of the hippocampus display a high frequency spiking pattern, and the fast activating HCN1 channels expressed on these interneurons may play a role in this (van Hooft et al. 2000). It is proposed that HCN1 in these interneurons may participate in frequency regulation of the tonic inhibitory input to the principal cells, similar to its role in cerebellar basket cells (Saitow and Konishi 2000).

HCN4 generally follows the expression pattern of HCN2, which is homogenously expressed throughout the principal cell layers and expressed more copiously in the interneuronal populations in the stratum oriens and alveus (O/A) (Bender et al. 2001). O/A interneurons have been shown to exhibit rhythmic firing activity, and this has been attributed partly to Ih (Maccaferri and McBain 1996). It is likely that the slow-activating HCN2 and HCN4 contribute to this rhythmic firing activity and that this activity may provide feedback inhibition to pyramidal cells, thereby modulating synchronous activity in the pyramidal layers (Katona et al. 1999). HCN3 is not significantly expressed in the hippocampus (Bender et al. 2001).

The density, properties, and subcellular distribution of HCN channels are subject to activity-dependent regulation (Dyhrfjeld-Johnsen et al. 2009). In the hippocampal CA1 pyramidal cells, the increasing distribution along the apical dendrites is dependent on excitatory input from the entorhinal cortex (Shin and Chetkovich 2007). It has been proposed that TPR-containing Rab8b interacting chaperone proteins (TRIP8b) may co-localize with HCN1 subunits
in CA1 pyramidal neurons and work to quickly insert or remove HCN channel proteins in an activity-dependent manner (Santoro et al. 2004).

### 3.4 Functional Properties of Ih

It is known that Ih contributes to two major physiological roles: it helps set the RMP, and it helps provide negative feedback for membrane potential stabilization (Pape 1996; Doan and Kunze 1999; Wahl-Schott and Biel 2009). First, Ih is constitutively open at rest and it helps set where the RMP sits by passing a depolarizing noninactivating inward current. Ih also decreases the membrane resistance ($R_m$), which functions to stabilize the $V_m$, suppress low frequency fluctuations, and attenuate the amplitude of incoming EPSPs (Nolan et al. 2007). In the presence of Ih, the kinetic filtering of EPSPs is also affected, so EPSP rise and decay time is accelerated. Secondly, Ih provides negative feedback. With the reversal potential of Ih sitting at around -25 mV to -40 mV, depending on the cell-type and region, when the $V_m$ is at more hyperpolarized voltages, the Ih provides a depolarizing inward current. On the other hand, at more depolarized potentials, the Ih shuts off and the withdrawal of this tonic depolarizing inward current results in a hyperpolarization.

Ih has been shown to be involved in many aspects of physiology, including working memory, hippocampal LTP, motor learning, and pacemaking oscillations (Wahl-Schott and Biel 2009). Ih has also been found to be present in the presynaptic terminals of the crustacean neuromuscular junction (Beaumont and Zucker 2000), avian ciliary ganglion (Fletcher and Chiappinelli 1992), cerebellar basket cells and the calyx of Held (Southan et al. 2000), but what function presynaptic Ih has is still debated. Some have suggested that presynaptic Ih may be involved in hippocampal long term potentiation (LTP) (Mellor et al. 2002), but other studies in
the same system show contradictory results (Chevaleyre and Castillo 2002). Importantly, Ih is involved in signal processing since it plays a major role in dendritic integration (Magee 1999; Magee and Carruth 1999). Passive cable theory will suggest that distal synaptic input should generate slower EPSPs in the soma as compared to proximal input. However, dendrites are not voltage-independent and are not passive since they express a multitude of voltage-dependent channels, including HCN channels. As mentioned earlier then, experiments have since shown that Ih decreases the $R_m$ and increases local membrane conductance, thereby accelerating the speed of EPSP rise and decay (Magee 1999; Magee 2000; Nolan et al. 2007).

### 3.5 Ih and Epilepsy

The role of Ih in epilepsy has been examined in many seizure models, including the perinatal seizure-inducing hypoxia model (Zhang et al. 2006), kainate model (Shah et al. 2004), pilocarpine model (Jung et al. 2007; Shin et al. 2008; Marcelin et al. 2009), fluid percussion model (Howard et al. 2007), and the febrile seizure model (Chen et al. 2001; Dyhrfjeld-Johnsen et al. 2008). However, the role of Ih is still highly debated, as some studies have shown increased Ih promoting seizures, while others shown an attenuation of seizures with increased Ih. In the pilocarpine, kainate, and perinatal hypoxia models, there was a reduction in Ih, and it seemed that the resulting hyperexcitability was due to an increase in neuronal input resistance (Shah et al. 2004; Zhang et al. 2006; Jung et al. 2007; Shin et al. 2008; Marcelin et al. 2009). A recent paper also showed that in human epileptogenic neocortex, a deficit of HCN1 subunits may be responsible for the increase in excitability and probability of seizure activity (Wierschke et al. 2009). On the other hand, a recent study on febrile seizures showed an increase in Ih current, along with a depolarized $V_{1/2}$ (Dyhrfjeld-Johnsen et al. 2008). In studies of synaptic plasticity, it has been shown that LTP induction can increase HCN channel protein synthesis and decrease
excitability (Fan et al. 2005) while long term depression (LTD) induction downregulates Ih and increases neuronal excitability (Brager and Johnston 2007).

In examining the kainate model for temporal lobe epilepsy, the downregulation of HCN1 channels has been suggested to be linked to a corresponding seizure-induced upregulation of neuron-restrictive silencing factor (NRSF), which restricts transcription of HCN1 (Dyhrfjeld-Johnsen et al. 2009). Another possibility is that the interaction between the TRIP8b chaperone protein and the HCN1 subunits is disrupted, so there is a channelopathic mislocation of h-channels, leading to the observed downregulation (Santoro et al. 2004). In the febrile seizure model, there is also a downregulation of HCN1 subunit expression, but the Ih is increased (Chen et al. 2001; Dyhrfjeld-Johnsen et al. 2008). It is suggested that this may be a result of the seizures causing a glycosylation of HCN1 subunits, prompting the formation of HCN1/HCN2 heteromeric channels which have different properties than the former HCN1 homomeric channels (Brewster et al. 2005). These heteromeric channels may then explain the increase in Ih and the changes in the properties of Ih, including the more depolarized activation voltage.

In summary, there is a delicate balance between Ih-induced depolarization, which brings the $V_m$ closer to the threshold of firing, making it more excitable, and an Ih-induced decrease in input resistance, which decreases summation of EPSPs and making the cells less excitable. Different models may affect the interplay of these parameters in different ways. Adding to the complication is that Ih exerts influence through the interaction of other voltage-gated channels. It has been proposed that, in the febrile seizure model, part of the increased excitability from the upregulated Ih may also be due to a concomittent downregulation of a persistent K$^+$ current (Dyhrfjeld-Johnsen et al. 2008). Ih may also interact with inhibitory inputs by causing a post-inhibitory rebound depolarization (Kitayama et al. 2003).
4 Research Rationale, Objectives, and Hypotheses

Research Rationale and Objectives:

It is well established that there is a strong connection between \([K^+]_e\) and seizures, however, the role of \([K^+]_e\) in seizure cessation has not been thoroughly studied. It has been postulated that elevated \([K^+]_e\) may stop seizures through a depolarization block, whereby VDSCs are inactivated (Green 1964; Fertziger and Ranck 1970), however, this postulate has not been thoroughly characterized and the role of ionic currents affected by elevations in \([K^+]_e\) thus far have not been studied in detail.

The main objective of this thesis is to expand upon the hypothesis that \([K^+]_e\) may become anticonvulsant, and to examine the cellular mechanisms involved in this switch in the activity of \([K^+]_e\). Here I characterize the range at which \([K^+]_e\) switches from being pro- to anticonvulsant in the in vitro low Mg\(^{2+}\)/high K\(^+\) and tetanization models, and I also examine whether the anti-convulsant properties are simply a result of Goldman-Hodgkin-Katz (GHK)-mediated depolarization, or if Ih is involved in this switch in the activity of \([K^+]_e\) in epilepsy.

Hypotheses:

My hypothesis is threefold:

1. That in epileptic tissue, further elevations in \([K^+]_e\) can switch its activity from pro-convulsant to anti-convulsant

2. This switch in the action of K\(^+\) cannot be accounted for strictly by Goldman-Hodgkin-Katz (GHK)-mediated depolarization

3. That there is an ionic current, Ih, responsible for the switch from pro- to anti-convulsant in K\(^+\)
5 Materials and Methods

Animals and dissecting protocol:

Experiments were conducted on juvenile male Sprague-Dawley rats post natal day 17-21 in compliance with the guidelines of the University Health Network Animal Care Committee. Rats were anaesthetized with halothane and quickly decapitated. The brain was rapidly removed and transferred to ice cold artificial cerebral spinal fluid (aCSF) dissecting solution containing, in mM: 75 sucrose, 87 NaCl, 25 NaHCO₃, 7 MgCl₂, 1.25 NaH₂PO₄, 2.5 KCl, 25 D-Glucose, 0.5 CaCl₂, pH adjusted to 7.4 with 95% O₂/5% CO₂ (carbogen). Hippocampal slices were prepared as follows: the brain was hemisected along the midsagittal line and the cerebellum and the forebrain were removed. The dorsal cortex was cut parallel to the longitudinal axis and the brain was secured, ventral side up, into a vibratome (Leica VT1200, Leica Microsystems, Heidelberg, Germany) and 400 µm horizontal oblique slices were obtained. Afterwards, slices were left to incubate in aCSF heated to 30°C for 1 h to allow for tissue stabilization and recovery before recording. This incubation solution or aCSF contained in mM: 125 NaCl, 2.5 KCl, 25 D-Glucose, 25 NaHCO₃, 1.25 NaH₂PO₄, 1 MgCl₂, 1 CaCl₂. It was continually aerated with 95% O₂/5% CO₂.

Field recording protocol:

Transverse brain slices were placed in an interface-type chamber for recording extracellular field responses and perfused with aCSF at 2-3 mL/min, aerated and pH balanced with 95% O₂/ 5% CO₂ at 34.0°C. While the slice was present in the interface chamber, humidified oxygen was constantly gassed on top of the slice. Schaffer collaterals were
stimulated by a bipolar electrode (125 µm in diameter, platinum/iridium biconcentric, FHC, ME, USA) placed in the stratum radiatum of the CA1. Orthodromic extracellular responses were recorded largely from CA1 pyramidal neurons with borosilicate glass pipettes filled with aCSF with a tip resistance of 2-4 MΩ in the bridge balance mode. A few recordings were also performed with the recording electrode in the stratum radiation of the CA1 to examine the presynaptic volley. Constant current stimulating square pulses of 100 µs duration were delivered via a constant current isolation unit connected to a Grass S88 stimulator (Grass Instruments, Quincy, MA, USA). Responses were sampled at 2 KHz and low pass filtered at 5 KHz with an Axoclamp 2A amplifier (Molecular Devices, Sunnyvale, CA, USA). Various stimulating pulses were applied to evoke subthreshold and maximal suprathreshold potentials to construct an input-output curve. The stimulus strength required to elicit the maximal field excitatory postsynaptic potential (fEPSP) and population spike was determined from the input-output curve. This stimulus strength was typically ~3 mA and was kept constant throughout the experiment during the various treatment paradigms. To quantify field potentials in the CA1 pyramidal layer, the most positive potential was subtracted from baseline while population spikes were measured from the leading positive edge of the population spike to the most negative potential. Data acquisition and analyses were performed using pClamp 9.2 (Molecular devices, Forest City, CA, USA). For gap-free recordings, an offline Bessel (8-pole) high pass filter set at 0.2 Hz cutoff was applied to filter out background fluctuations.

Low Mg²⁺/5 mM K⁺ seizure induction protocol:

SLEs were induced by perfusing slices for 30 min. with low Mg²⁺ (0.25 mM)/high K⁺ (5 mM) after an initial 5 minute aCSF baseline. Input and output (I/O) relations were recorded prior
to, and after perfusion with different solutions, where the $[\text{K}^+]$ was altered in the range of 6 mM to 12.5 mM, while maintaining low Mg$^{2+}$.

**Tetanic stimulation-induced afterdischarge protocol:**

Primary afterdischarges (PADs) were evoked in CA1 pyramidal neurons by tetanic stimulation of Schaffer collaterals with repetitive pulses of 100-µs duration at 100 Hz for 2 seconds (Rafiq et al. 1993; Jahromi et al. 2002). Tetanization of slices was repeated once every 10 minutes, 6-10 times, depending on when robust PADs are evoked. Tetanization experiments carried out in 2.5 mM K$^+$ were performed separately. After 6-10 tetanizations in 5 mM K$^+$, K$^+$ was further elevated to 7.5 mM K$^+$ to see the effect of the further elevation of K$^+$ on the PADs.

**Treatment with Ih Blocker**

In the low Mg$^{2+}$/5 mM K$^+$ seizure induction model, after SLEs had been reliably induced, 50 µM of the hyperpolarization-activated nonspecific cationic current (Ih) blocker, 4-ethylphenylamino-1,2-dimethyl-6-methylaminopyrimidinium chloride (ZD7288) was included in the perfusion for concentrations at 5 mM K$^+$ and 7.5 mM K$^+$. ZD7288 is well-used for blockade of Ih and its effect has also been studied with respect to seizure activity. Dose-response tests have been shown in literature, from a dose of 10 µM to 100 µM, and at the higher end of these concentrations, it seems to block synaptic transmission (Inaba et al. 2006). 50 µM has been previously tested in our lab, and has been shown to effectively block Ih, while synaptic transmission is still preserved (Shin and Carlen 2008), so 50 µM was used as our testing dose.
Drugs:

All chemicals were purchased from Sigma-Aldrich (Burlington, ON, Canada), except ZD7288, which was purchased from Tocris Bioscience (Ellisville, MO).

Statistical Analysis:

For analysis of data in the low Mg$^{2+}$/high K$^+$ model, all evoked field potentials and population spikes in the various treatment groups were normalized to values obtained in aCSF. To analyze seizure amplitude and duration during perfusion of low Mg$^{2+}$/high K$^+$ (in the range of 6 mM to 12.5 mM), all SLE amplitudes and durations were normalized to values recorded in low Mg$^{2+}$/5 mM K$^+$. Prior to running any statistical test, normalized data were converted to a normal distribution using a root arcsine conversion. For analysis of data in the tetanization model, mean amplitudes of evoked population spikes and fEPSPs, as well as mean amplitudes and durations of PADs were compared. Significance levels were determined at P<0.05. Each sample size (n) equated to a single slice. All data are expressed as mean ± SEM. Statistical analysis was carried out using one-way analysis of variance (One way-ANOVA) with a Dunnett’s post-hoc (against control) analysis using Sigmastat 3.5 software program (Systat Software Inc, San Jose, CA, USA).
6 Results

6.1 The Effect of Elevating [K+]e to Ceiling Levels Observed During SLEs

To induce SLEs, brain slices containing the hippocampal formation were bath perfused with low Mg\(^{2+}\)/high K\(^{+}\) for 30 minutes. For this study, SLEs were defined as spontaneous high amplitude, high frequency events lasting a minimum duration of 5 seconds. Field recordings were obtained from the CA1 hippocampal pyramidal layer during aCSF perfusion. No SLEs were observed during this treatment (Figure 3C). Single stimulus-evoked field potentials produced stereotypical responses in the pyramidal layer – a population spike of 1.62 ± 0.15 mV (n=23) preceding a fEPSP of 1.02 ± 0.10 mV (n=25) (Figure 3A). During a 30 minute perfusion of low Mg\(^{2+}\)/5 mM K\(^{+}\), neither the population spike or fEPSP amplitudes (84.9 ± 17.8% and 117.6 ± 15.8% respectively) were significantly different from the control levels (Figures 3B, 6A,B). With low Mg\(^{2+}\)/5 mM K\(^{+}\) perfusion, SLEs were observed to spontaneously occur at 7-10 minute intervals (Figures 3D,6C).

After spontaneous SLEs were reliably recurring, the [K\(^{+}\)]\(_{e}\) was elevated in the bath up to ceiling levels (10-12 mM) typically observed with seizures. At 12.5 mM [K\(^{+}\)]\(_{e}\), the highest level of [K\(^{+}\)]\(_{e}\) tested, single stimuli were unable to evoke population spikes or fEPSPs (n=4) (Figures 4A, 6A,B), whereas at 10 mM [K\(^{+}\)]\(_{e}\) (n=3), the evoked population spikes and fEPSPs were significantly blunted to 31.5 ± 25.8% and 28.0 ± 14.1% of aCSF values respectively (n=3) (P<0.05) (Figures 4B, 6A,B). After perfusion for 30 mins at both of these [K\(^{+}\)]\(_{e}\), SLEs were completely blocked (Figure 4C,D,6C). At 10 mM [K\(^{+}\)]\(_{e}\), there was tonic low amplitude background activity (Figure 4D). Upon switching back to 5 mM [K\(^{+}\)]\(_{e}\), SLEs returned and upon washout back to aCSF, SLEs stopped and the evoked population spike and fEPSP amplitudes were not significantly different from the initial values.
Figure 3. Evoked responses and spontaneous potentials in aCSF and low Mg\textsuperscript{2+}/5 mM K\textsuperscript{+}. The stimulus strength for evoked responses was determined by an input-output curve and the maximal field and population spike responses are shown for aCSF (A) and in low Mg\textsuperscript{2+}/5 mM K\textsuperscript{+} (B). In low Mg\textsuperscript{2+}/5 mM K\textsuperscript{+}, multiple population spikes could be seen, indicating hyperexcitability. Spontaneous field potentials are shown for aCSF (C) and low Mg\textsuperscript{2+}/5 mM K\textsuperscript{+} (D). There was no observable activity seen during aCSF perfusion, whereas during low Mg\textsuperscript{2+}/5 mM K\textsuperscript{+}, spontaneous recurrent SLEs would occur at regular intervals after the initial SLE. The scale bar (1 mV/20 ms) represents all evoked responses. The scale bar (1 mV/50 s) represents the spontaneous field potential recordings in aCSF. The scale bar (2 mV/2 min) represents the spontaneous field potential recordings in low Mg\textsuperscript{2+}/5 mM K\textsuperscript{+}.
Figure 4. Evoked responses and spontaneous potentials in low Mg$^{2+}$/12.5 mM K$^+$ and low Mg$^{2+}$/10 mM K$^+$.

The stimulus strength for evoked responses was determined by an input-output curve and the maximal field and population spike responses are shown for low Mg$^{2+}$/12.5 mM K$^+$ (A) and low Mg$^{2+}$/10 mM K$^+$ (B). In low Mg$^{2+}$/12.5 mM K$^+$, no population spike or fEPSP could be measured, and in low Mg$^{2+}$/10 mM K$^+$, the evoked population spike and fEPSP is significantly attenuated (P<0.05). Spontaneous field potentials are shown for low Mg$^{2+}$/12.5 mM K$^+$ (C) and low Mg$^{2+}$/10 mM K$^+$ (D). After the transition into low Mg$^{2+}$/12.5 mM K$^+$ is stabilized, no activity is seen. After stabilization in low Mg$^{2+}$/10 mM K$^+$, tonic low amplitude background activity is seen. (E) shows an expanded trace of this background activity. The scale bar (1 mV/20 ms) represents all evoked responses. The scale bar (2 mV/100 s) represents all spontaneous field potential recordings not expanded. The scale bar (1 mV/5 s) represents the expanded trace in (E).
6.2 Finding the Anticonvulsant Range of $[K^+]_e$ in the Low Mg$^{2+}$/5 mM K$^+$ Model

After spontaneous, recurrent SLEs have been elicited through perfusion of low Mg$^{2+}$/5 mM K$^+$, $[K^+]_e$ was elevated in the bath to a concentration of 7.5 mM. In this study, in low Mg$^{2+}$/7.5 mM K$^+$, the population spike and fEPSP amplitudes were significantly reduced to 46.3 ± 23.4% and 30.3 ± 15.2% of the control aCSF values (n=4) (P<0.05) (Figures 5A,6A,B). SLEs were also attenuated and replaced with tonic low amplitude background activity, similar to what was observed in low Mg$^{2+}$/10 mM K$^+$ (Figures 5D,6C). At this concentration, some recordings were also performed in the stratum radiatum to observe the presynaptic volley, and no significant difference in the amplitude of the synaptic volley was seen (data not shown) (n=4). All other recordings were performed in the stratum pyramidal, and the presynaptic volley cannot be clearly discerned from the stimulus artifact.

To narrow down the anticonvulsant range of $[K^+]_e$, another set of experiments was carried out where $[K^+]_e$ in the low Mg$^{2+}$/5 mM K$^+$ bath was increased to 6 mM. The population spike and fEPSP amplitudes (66.6 ± 12.5% and 133 ± 66.5% of control aCSF values respectively) were not significantly changed (n=3) (Figures 5C,6A,B). With low Mg$^{2+}$/6 mM K$^+$ perfusion, SLEs were not attenuated, and the SLEs had similar amplitudes and durations as compared to SLEs seen in low Mg$^{2+}$/5 mM K$^+$ (80.5 ± 10.4% and 106.0 ± 26.0% respectively) (Figures 5G,6C).

Since low Mg$^{2+}$/6 mM K$^+$ allowed SLEs to continue unhindered, while low Mg$^{2+}$/7.5 mM K$^+$ attenuated SLEs, we hypothesized that the lower boundary for the anticonvulsant properties of elevated $[K^+]_e$ would lie between those $[K^+]_e$ values. We increased the $[K^+]_e$ in the low Mg$^{2+}$/5 mM K$^+$ solution to 6.5 mM K$^+$. At this $[K^+]_e$, the amplitude of population spikes and fEPSPs (94.3 ± 24.8% and 72.3 ± 17.8% respectively) were not significantly different than in
control aCSF (Figures 5B,6A,B). At 6.5 mM K⁺, 50% of the slices (n=3) continued to seize in the elevated [K⁺]e (Figure 5E), whereas in the other 50% (n=3), seizures were attenuated and tonic low amplitude background activity was observed (n=3) (Figure 5F). Of the slices that seized, the seizure amplitudes and durations (85.8 ± 8.0% and 104.5 ± 16.4% respectively compared to low Mg²⁺/5 mM K⁺ seizures) were similar to those seen in low Mg²⁺/5 mM K⁺ but there was also an increase in interictal discharge between SLEs (Figures 5E,6C).
Figure 5. Evoked responses and spontaneous potentials in low Mg\(^{2+}/7.5\) mM K\(^{+}\), low Mg\(^{2+}/6.5\) mM K\(^{+}\), and low Mg\(^{2+}/6\) mM K\(^{+}\).

The stimulus strength for evoked responses was determined by an input-output curve and the maximal field and population spike responses are shown for low Mg\(^{2+}/7.5\) mM K\(^{+}\) (A), low Mg\(^{2+}/6.5\) mM K\(^{+}\) (B) and low Mg\(^{2+}/6\) mM K\(^{+}\) (C). In low Mg\(^{2+}/7.5\) mM K\(^{+}\), the evoked population spike and fEPSP was significantly attenuated (P<0.05) whereas there was no significant change in the population spike and fEPSP amplitudes in low Mg\(^{2+}/6.5\) mM K\(^{+}\) and low Mg\(^{2+}/6\) mM K\(^{+}\). Spontaneous field potentials are shown for low Mg\(^{2+}/7.5\) mM K\(^{+}\) (D), low Mg\(^{2+}/6.5\) mM K\(^{+}\) with SLEs (E), low Mg\(^{2+}/6.5\) mM K\(^{+}\) when SLEs are attenuated (F), and low Mg\(^{2+}/6\) mM K\(^{+}\) (G). For low Mg\(^{2+}/7.5\) mM K\(^{+}\) and low Mg\(^{2+}/6.5\) mM K\(^{+}\) when SLEs are attenuated, there is tonic low amplitude activity seen throughout the trace (D&F). For low Mg\(^{2+}/6.5\) mM K\(^{+}\) when SLEs persist, there is increased interictal firing between SLEs (E). In low Mg\(^{2+}/6\) mM K\(^{+}\), there is no significant difference in the amplitude or duration of the SLEs (G). The scale bar (1 mV/20 ms) represents all evoked responses. The scale bar (2 mV/2 min) represents all spontaneous field potential recordings.
Figure 6. Normalized group data showing the effects of elevating $[\text{K}^+]_e$ on the population spike and fEPSP amplitude, and the SLE amplitude and duration in the low $\text{Mg}^{2+}/5$ mM K$^+$ model.

The dotted line represents the values the data is normalized to. In A and B, it represents the aCSF value and in C it represents the low $\text{Mg}^{2+}/5$ mM K$^+$ value. Asterisks denote significant changes against the control reference values ($P<0.05$). In all parameters, a significant decrease is found at 7.5, 10, and 12.5 mM $[\text{K}^+]_e$. In C, the values for 6.5 mM K$^+$ are based only on the 50% of slices that had seizures.
6.3 The Effects of Elevating [K\textsuperscript{+}]\textsubscript{e} in the Tetanization Model

In control aCSF containing 2.5 mM K\textsuperscript{+}, 100 Hz tetanizations for 2 sec periods every 10 minutes elicited PADs after 6-10 tetanizations. In 2.5 mM K\textsuperscript{+}, the population spikes had an amplitude of 1.57 ± 0.27 mV and the fEPSPs had an amplitude of 1.0 ± 0.13 mV (n=4) (Figure 7A). After 6-10 tetanizations, the measured PAD amplitude was 0.31 ± 0.07 mV and the PAD lasted for a duration of 13.93 ± 2.80 seconds (Figure 7D,G,H).

When the same experimental paradigm was run in aCSF with 5 mM K\textsuperscript{+}, the population spike amplitude (1.26 ± 0.02 mV) and the fEPSP amplitude (1.55 ± 0.29 mV) did not change significantly from values in control aCSF (n=4) (Figure 7B). However, the PADs evoked by tetanization in the 5 mM K\textsuperscript{+} were significantly higher amplitude (0.68 ± 0.13 mV) and longer duration (24.53 ± 3.47 seconds) (n=4) (P<0.05) compared to PADs evoked in control aCSF (Figure 7E,G,H). Further experiments were conducted where the [K\textsuperscript{+}]\textsubscript{e} in the bath was further elevated to 7.5 mM to study the effect of elevated K\textsuperscript{+} on tetanization-induced PADs in 5 mM K\textsuperscript{+}. This is because the PADs were much more pronounced at 5 mM K\textsuperscript{+}, and in previous papers, tetanization experiments are carried in aCSF with a baseline of 5 mM K\textsuperscript{+} (Jahromi et al. 2002).

When the [K\textsuperscript{+}]\textsubscript{e} in the bath was increased from 5 mM K\textsuperscript{+} up to 7.5 mM K\textsuperscript{+}, there was no significant difference in the amplitude of population spikes and fEPSPs (Figure 7C). However, in 7.5 mM K\textsuperscript{+}, PADs could not be evoked by tetanization (n=4) (Figure 7F,G,H). Instead, tonic background activity resembling interictal spikes was observed throughout the recording. The tonic interictal spiking was no longer observed during washout, and PADs could again be evoked.
Figure 7. Evoked responses and tetanization-induced PADs in 2.5 mM K⁺, 5 mM K⁺, and 7.5 mM K⁺.

The stimulus strength for evoked responses was determined by an input-output curve and the maximal field and population spike responses are shown in 2.5 mM K⁺ (A), 5 mM K⁺ (B) and 7.5 mM K⁺ (C). There was no significant difference in the observed evoked population spike and fEPSP amplitude among the different groups. Tetanization-induced PADs were seen at both 2.5 mM K⁺ (D) and 5 mM K⁺ (E). The PADs observed in 5 mM K⁺ were significantly larger and longer-lasting than the PADs in 2.5 mM K⁺ (G,H). When [K⁺]ₑ was further elevated to 7.5 mM, no PAD could be induced by tetanization (F,G,H), but there was tonic background activity resembling interictal spiking (F). The scale bar (1 mV/50 ms) represents all evoked responses. The scale bar (1 mV/10 s) represents all tetanization-induced PADs. * represents a significant difference from 2.5 mM K⁺ and ** represents a significant difference from 5 mM K⁺ (P<0.05).
6.4 The Effect of Blocking Ih on K⁺-induced attenuation of SLEs

After SLEs were spontaneously recurring in the low Mg²⁺/5 mM K⁺ solution, adding 50 µM ZD7288 without an additional elevation in [K⁺]ₑ did not significantly alter any of the parameters. When ZD7288 was not included in the low Mg²⁺/7.5 mM K⁺ solution, the population spike and fEPSP amplitudes had significantly decreased to 46.3 ± 23.4% and 30.3 ± 15.2% of the control aCSF values (n=4) (P<0.05) (Figures 5A, 6A,B,8A,E). Furthermore, SLEs were attenuated in the low Mg²⁺/7.5 mM K⁺ (n=4) (Figures 5D,6C,8C,F). When 50 µM ZD7288 is included in the low Mg²⁺/7.5 mM K⁺, recordings in the stratum radiatum revealed no significant difference in the amplitude of the presynaptic volley (n=4). Recordings in the pyramidal layer showed population spike and fEPSP amplitudes (105.2 ± 49.8% and 104.5 ± 19.2% respectively) similar to those recorded in control aCSF (n=4) (Figures 8B,E). Also, distinct SLEs occurred quite frequently and were separated by short periods characterized by interictal spiking activity (Figure 8D). The SLEs had amplitudes and durations (117.2 ± 43.1% and 91.2 ± 32.5% respectively) similar to what was observed in low Mg²⁺/5 mM K⁺ (Figure 8D,F).
Figure 8. The Effect of Blocking Ih on K\(^+\)-induced Attenuation of SLEs.

The stimulus strength for evoked responses was determined by an input-output curve and the maximal field and population spike responses are shown for low Mg\(^2+\)/7.5 mM K\(^+\) (A) and low Mg\(^2+\)/7.5 mM K\(^+\)/ZD7288 (B). Including ZD7288 resulted in a recovery of the population spike and fEPSP amplitude, back to levels seen in control aCSF (E). Low Mg\(^2+\)/7.5 mM K\(^+\) attenuated SLEs (C), but with 50 µM ZD7288, SLEs occurred frequently, and were separated by brief periods marked by interictal activity (D). The SLEs had a similar duration and amplitude to values seen in low Mg\(^2+\)/5 mM K\(^+\) (F). The scale bar (2 mV/50 ms) represents all evoked responses. The scale bar (2 mV/5 min) represents all spontaneous field recording potentials. Asterisks denote a significant difference compared to low Mg\(^2+\)/7.5 mM K\(^+\) (P<0.05). In all parameters, the addition of ZD7288 resulted in a significant increase in amplitude and duration (E,F).
7 Discussion

The most common form of epilepsy is complex partial epilepsy, which typically originates in the temporal lobe (McNamara 1994). These seizures have been most commonly studied in the hippocampal region, both in vitro and in vivo. It has been found that the seizure-like events (SLEs) seen in the hippocampus are typically accompanied by an elevation in extracellular potassium (K\textsubscript{e}). In healthy nervous tissue, [K\textsuperscript{+}]\textsubscript{e} levels rarely reach 4.0 mM (Somjen 1979), but during SLEs, [K\textsuperscript{+}]\textsubscript{e} levels have been recorded to be up to 12 mM (Krnjevic et al. 1982; Yaari et al. 1986). The main source of this [K\textsuperscript{+}]\textsubscript{e} is assumed to be neuronal, and it has been shown that neurons do release K\textsuperscript{+} with activity (Somjen 1979) but some have suggested that there may be a large contribution of [K\textsuperscript{+}]\textsubscript{e} from the astrocytic network (Bragin et al. 1997). Many studies have since demonstrated that exposing tissue to elevated [K\textsuperscript{+}]\textsubscript{e} (~8.5 mM) is sufficient to induce SLEs in the hippocampus in vitro (Ogata 1975; Ogata 1976; Ogata et al. 1976; Traynelis and Dingledine 1988). There are various proposals as to why this may be. The elevated [K\textsuperscript{+}]\textsubscript{e} partially depolarizes the membrane and brings it closer to threshold (Alger et al. 1983). Since elevated [K\textsuperscript{+}]\textsubscript{e} would decrease K\textsuperscript{+} efflux, it has also been proposed that GABA-B inhibitory postsynaptic potentials (IPSPs) would be decreased (Dutar and Nicoll 1988).

Furthermore, the burst afterhyperpolarization (AHP) which typically limits the amount of repetitive firing possible is likewise decreased due to the diminished K\textsuperscript{+} efflux (Alger and Nicoll 1980). The decreased K\textsuperscript{+} efflux also will induce neuronal swelling, thereby reducing the extracellular space, thus increasing the possibility of neuronal synchronization due to ephaptic interactions (Dietzel et al. 1980).

The idea that accumulations in [K\textsuperscript{+}]\textsubscript{e} may be what terminate seizures is something that has been suggested, but at what point the K\textsuperscript{+} switches from being pro- to anti-convulsant has not
been examined before. In this study, the main objective was to examine whether elevated $[K^+]_e$ could attenuate *in vitro* epileptic activity in the rat hippocampus, and more specifically, what is the range of $[K^+]_e$ that would shift the action of $K^+$ from being pro- to anti-convulsant. We then investigated whether this shift was strictly due to a depolarization block, as proposed in past literature (Green 1964; Fertziger and Ranck 1970; Herreras and Somjen 1993; Bragin et al. 1997), and also examined what other currents might be involved in this switch in the activity of $K^+$.

Here we used extracellular electrophysiological recordings in the rat hippocampus *in vitro* during low $\text{Mg}^{2+}/5 \text{mM K}^+$-induced SLEs or tetanization-evoked PADs to show that elevated $[K^+]_e$ can indeed have anticonvulsant properties if elevated in epileptic tissue. We also showed that this anticonvulsant behaviour is not simply a result of a depolarization block, but other ionic currents, specifically the $I_h$, is involved in this switch.

*Elevated $[K^+]_e$-induced $I_h$ potentiation attenuates low $\text{Mg}^{2+}/5 \text{mM K}^+$ SLEs*

Our study first confirmed that low $\text{Mg}^{2+}/5 \text{mM K}^+$ does indeed produce robust seizure activity, as is well characterized, including from various studies in our lab (Mody et al. 1987; Armand et al. 2000; Derchansky et al. 2004; Isaev et al. 2005; Derchansky et al. 2006; Derchansky et al. 2008). We then utilized this epileptic model to test the effects of elevated $[K^+]_e$ on neuronal excitability, as observed through the amplitude of the population spikes and fEPSPs. Our results showed that there is a significant decrease in both population spike and fEPSP amplitude when $[K^+]_e$ is elevated to 7.5 mM and higher. However, contrary to the depolarization block hypothesis, population spikes and fEPSPs, though attenuated, could still be evoked, and recordings of the spontaneous potential revealed tonic background activity. This suggests that the cells are still able to fire, so not all of the $\text{Na}^+$ channels have been inactivated.
To assess whether this attenuation of the population spikes, fEPSPs, and SLEs is due to axonal conduction failure, recordings were performed in the stratum radiatum to examine the effect of the elevated $[K^+]_e$ on the presynaptic volley, which would give an indication of the strength of the afferent input. We found no significant difference in the presynaptic volley, which is in line with observations made by Hablitz and Lundervold (1981), where they showed increasing $[K^+]_e$ from 3.25 to 12.25 mM in guinea pig hippocampal slices has less effect on the excitability of presynaptic fibers (Hablitz and Lundervold 1981) than on postsynaptic neurons. This suggests that the attenuation of SLEs is not a result of conduction failure down the axon.

To examine whether other ionic currents, specifically the Ih, are involved in the switch in $K^+$ activity, the Ih blocker ZD-7288 was applied to the bath solution. The idea that Ih may play a role in terminating synchronous activity has been proposed earlier by Bal and McCormick, who suggested that Ih conductance is responsible for the termination of synchronized oscillatory activity in the thalamocortical network. As such, I examined whether Ih conductance in this study may be involved in the termination of SLEs by elevated $[K^+]_e$.

The role of Ih in epilepsy has been widely debated, as different models have shown either a pro- or an anti-convulsant effect (Di Pasquale et al. 1997; Poolos et al. 2002; Kitayama et al. 2003; Surges et al. 2003; Gill et al. 2006; Inaba et al. 2006). A recent review article examining both sides and the potential for Ih to play a pro- or anti-convulsant role depending on the model system suggests that the important factor is the balance between Ih pushing for excitation by causing depolarizing, while simultaneously decreasing the input resistance of the tissue, thus attenuating the effectiveness of incoming EPSPs (Dyhrfjeld-Johnsen et al. 2009). Ih is also known to be highly influenced by $[K^+]_e$. Elevated $[K^+]_e$ has been shown to shift the reversal potential and activation voltage of HCN channels to more depolarized levels, and even minor
fluctuations in $[K^+]_e$ have been shown to drastically increase the amplitude of Ih going through HCN channels (Spain et al. 1987; Funahashi et al. 2003; Shin and Carlen 2008). With this in mind, we examined the effect of ZD7288 when $[K^+]_e$ is further elevated in this epileptic model.

ZD7288 did not seem to have a pronounced effect on the evoked population spikes or fEPSPs when added to low Mg$^{2+}$/5 mM K$^+$. It also did not have a significant effect on the SLEs. However, when ZD7288 was included in the perfusion of low Mg$^{2+}$/7.5 mM K$^+$, a recovery of population spike and fEPSP amplitude, along with SLEs, was observed. We propose, based on our observations, that in this in vitro epileptic model, the elevated $[K^+]_e$ increases the influence of Ih, and it has an inhibitory effect by increasing the membrane conductance and decreasing the membrane resistance, as others have suggested previously (Poolos et al. 2002). As such, when $[K^+]_e$ is elevated, there is a propagation failure of the signal up the dendrites due to the Ih-induced decrease in input resistance, thus resulting in less EPSP summation and the observed decrease in the evoked population spike and fEPSP amplitude. This is also in line with the expression of HCN channels, which are far more populated in the dendritic area (Jung et al. 2007). This attenuation of EPSPs could also explain the presence of the low amplitude tonic activity seen in the spontaneous potentials when $[K^+]_e$ is elevated and seizures are attenuated. With this proposal, it would suggest that when ZD7288 is applied, the Ih is blocked so input resistance increases and the dendritic shunt is no longer active. As such, there is a recovery in the population spike and fEPSP amplitude, as we observed. Also, with EPSPs no longer being shunted and attenuated due to the increase in membrane resistance, the tissue becomes hyperexcitable and SLEs occur again.
Elevated $[K^+]_e$-induced Ih potentiation attenuates tetanization-induced PADs

To examine whether our finding regarding the anticonvulsant properties of elevated $[K^+]_e$ is generalizable to other in vitro models, we shifted from the low Mg$^{2+}/5$ mM K$^+$ model and examined the effects of elevated $[K^+]_e$ on the PADs evoked by electrical stimulation, another verified in vitro seizure model with a different mechanism of action. This model using electrical stimulation is based off of the in vivo model of kindling, whereby repeated applications of subconvulsive stimulus trains in the brain of a naïve animal will eventually result in ADs and seizures (Stasheff et al. 1985). The advantage of this model over other chemically-induced seizure models is that by only utilizing electrical stimulation, presumably the model should mimic physiological communication between hyperexcitable areas of the brain, which is necessary for the development of epilepsy. We found that by elevating $[K^+]_e$ from control levels of 2.5 mM up to 5 mM, there was a significant increase in the amplitude and duration of PADs. This is in line with other literature which also uses 5 mM K$^+$ in their control solution to promote more robust PADs (Jahromi et al. 2002). The corresponding increase in amplitude and duration of PADs resulting from the elevated $[K^+]_e$ suggests that at this concentration, $[K^+]_e$ is still playing a pro-excitatory and pro-convulsant role, most likely by increasing the excitability of the neurons by depolarizing the cells so they are closer to firing threshold, among other possible mechanisms (Traynelis and Dingledine 1988). The further increase in $[K^+]_e$ up to 7.5 mM resulted in a blockade of PADs and instead, rhythmic interictal spiking activity was seen to persist throughout the recording. This may also be due to a similar mechanism as seen in the low Mg$^{2+}/5$ mM K$^+$ model. The elevated $[K^+]_e$ may increase Ih and subsequently reduce tissue resistance and attenuate EPSP summation. With the dendritic shunt, what would otherwise be
strong hyperexcitation traveling through the dendrites to the soma and producing SLEs or PADs, may be reduced to a less excitable state in the form of the observed bursting activity.

Alternatively, recent modeling studies by Bazhenov et al., showed that increases in $[\text{K}^+]_e$ can result in the onset of an intrinsic burst mechanism (Bazhenov et al. 2004; Bazhenov et al. 2008). This intrinsic bursting mechanism is proposed to be due to elevations in $[\text{K}^+]_e$ leading to activation of other depolarizing currents, including $I_h$ and $I_{Na,P}$, which pushes forward the next burst. The limiting factor of these bursts is suggested to be the increased activation of the $\text{Ca}^{2+}$-dependent $K^+$ current, due to increased intracellular $\text{Ca}^{2+}$ resulting from the bursting. This intrinsic bursting mechanism resulting from elevated $[\text{K}^+]_e$ may be linked to a proposal by Avoli, that interictal spiking activity may actually control seizure activity. He showed that interictal activity originating in the hippocampal CA3 region can promote interictal spiking in the CA1 and prevent ictal activity (Avoli 2001). When this interictal activity was stopped by lesioning the Schaffer collaterals, which are the CA3 input to the CA1, corresponding interictal spiking stopped in the CA1, but seizure events began. When rhythmic electrical stimuli mimicking the interictal activity was used, the ictal events seen in the CA1 were controlled again.

Here in this study, we show that the elevated $[\text{K}^+]_e$ is accompanied by the tonic rhythmic firing of a population of cells, and it is possible that the intrinsic bursting rhythm that accompanies elevated $[\text{K}^+]_e$ may be involved in the subsequent control of SLEs. As such, there are two possibilities proposed here: that the intrinsic bursting rhythm seen to control seizures is a consequence of the $I_h$-induced inhibition through decreasing membrane resistance and causing EPSP propagation failure, or the elevated $[\text{K}^+]_e$ potentiates the $I_h$ to further drive the bursting rhythm, and this bursting would then be the cause, rather than the consequence of seizure attenuation.
8 Conclusions

1. There is a range of \([K^+]_e\) at which the activity of K\(^+\) switches from being pro- to anti-convulsant (~6.5 mM to 12.5 mM).

2. The switch in K\(^+\) activity to being anticonvulsant is not simply due to depolarization block, but involves a more complex system of interaction which involves the Ih.

3. 7.5 mM \([K^+]_e\) appears to be an anticonvulsant \([K^+]_e\) for both the low Mg\(^2+\)/5 mM K\(^+\) and the tetanization models of epilepsy

4. The anticonvulsant activity of K\(^+\) is mediated, at least in part, by a potentiation of the Ih

5. The anticonvulsant properties of Ih is likely through a decrease in membrane resistance, resulting in attenuation of incoming EPSPs
9 Ongoing/Future Work

This thesis has laid the groundwork for future studies examining the potential for elevated [K\(^+\)]\(_e\) to exert an anticonvulsant influence through a potentiation of the Ih.

To further this project, the Ih blocker, ZD7288, needs to be tested in the tetanization model to confirm that PADs will return even in elevated [K\(^+\)]\(_e\) as long as Ih is blocked. The use of K\(^+\)-sensitive electrodes would also lend strength to the story, as currently, bath perfusion of K\(^+\) does not allow us to know what the actual [K\(^+\)]\(_e\) is in the deeper layers of the tissue. Furthermore, the [K\(^+\)]\(_e\) is dynamic, especially during hyperexcited states such as during SLEs. As such, K\(^+\)-sensitive electrodes will allow for an understanding of how the K\(^+\) dynamic is actually shifting during the experimental procedure.

Intracellular recordings need to be done in the CA1 and CA3 pyramidal layers. The resulting data will give an intracellular correlate to the extracellular field recordings we have reported in this thesis. It would also lend more strength to this study if there were whole-cell correlates in the interneurons as well, since HCN channels do become abundantly expressed in the interneurons during development and interneurons play a crucial part in mediating synchronous activity (Bender et al. 2001). Since HCN channels are differentially expressed throughout the first few weeks of life, it would be interesting to see whether these anticonvulsant properties of Ih hold throughout development.

Extracellular field recordings can also be performed in the CA3 pyramidal cell layer, since it has been shown that the CA3 is the driver for SLEs whereas the CA1 is the receiver (Dzhala and Staley 2003). This will allow for discernment with regards to whether the blockade of SLEs at elevated K\(^+\) is due to a failure in SLEs propagating from CA3 to CA1, or whether there is a failure to initiate SLEs in the CA3 region.
Other possible ion currents may be involved in this pro- to anti-convulsant switch in K⁺ activity, and one such candidate is the I_{Na,P}, as elevating [K⁺]_e does enhance its conductance, and attenuating I_{Na,P} has been shown to be anticonvulsant (Macdonald and Greenfield 1997).

On a molecular level, it has been shown that some seizure models can produce cAMP accumulation or upregulate adenosine receptors (Hattori et al. 1982; Hattori et al. 1993; Hattori et al. 1993). This elevated cAMP would greatly potentiate Ih channels, so it would be beneficial to run cAMP assays to see what levels of cAMP are seen during low Mg²⁺/5 mM K⁺ or tetanization seizure protocols and see whether the anticonvulsant properties are mediated in part by a cAMP-dependent potentiation of Ih.

Ultimately, in vitro studies are limited so this model must be translated into an in vivo model so that it has more clinical relevance. The efficacy of such a mechanism involving elevated [K⁺]_e and potentiation of Ih must then be tested, possibly through local injections of Ih blockers or perhaps even deep brain stimulation protocols, whereby elevations in [K⁺]_e would result from the direct local high frequency stimulation of the brain.
10 References


