

Dissertação – Artigo de Revisão Bibliográfica  
Mestrado Integrado em Medicina – 2013/2014

## **MESIAL TEMPORAL LOBE EPILEPSY: NEW EMERGING THERAPEUTIC APPROACHES**

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Porto 2014

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Porto 2014

## Acknowledgements

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To Prof. Graça Lobo for the remarkable support, countless help and enthusiasm. A special thanks for introducing me to the fascinating world of neuropharmacology.

To Drs. Miguel Cordeiro and Pedro Coelho for the usefull coments to the manuscript.

To my family and friends for the notable presence, countless help and patience and everlasting encouragement throughout the years. A huge thanks for believing in me most of all.

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## Abstract

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Epilepsy is one of the most prevalent neurological disorders, and mesial temporal lobe epilepsy (MTLE) is the most pharmacoresistant form of epilepsy. About 30% of these patients are not controlled by antiepileptic drugs (AED) therapy, being necessary the use of surgical intervention to control the seizures, although only less than 10% of patients are eligible for surgery. Current AED only treat symptoms with no reduction or even cure of epilepsy. The high prevalence and percentage of refractory epilepsy have motivated extensive research concerning new regulatory systems of neurotransmitters or neuromodulators, in order to reduce this pathology.

Adenosine (ADO) modulates neuronal activity, being considered an endogenous anticonvulsant. ADO levels increase dramatically during seizures leading to activation of inhibitory A1 receptors, decreasing excitatory neurotransmission contributing in this way to the cessation of seizures and to the post-ictal refractoriness. Being ADO an ubiquitary molecule, its administration *per se* would lead to numerous adverse effects, mainly cardiovascular. ADO's formation and inactivation mechanisms in the brain are spotlighted by researchers aiming new therapeutic targets, with focal adenosine augmentation in the hippocampus as one as the most feasible approaches in the near future. Implants applied in the hippocampus of experimental models regulate or release ADO *in situ*. These implants contained release-control ADO polymers, histaminergic cells manipulated to release ADO or "anti-sense" genes against the adk gene of dysregulated adenosine kinase (ADK), which controls the extracellular levels of ADO. The ketogenic diet, used mainly in the control of refractory epilepsy in children, has also been studied.

The domain of these mechanisms will allow the development of completely innovative conceptual strategies for the treatment and possible cure of a syndrome as complex as epilepsy, in a broader sense that goes beyond mere symptomatic suppression.

**Keywords:** Epilepsy; MTLE; Adenosine; ADK, ADO-based therapies.

## Resumo

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A epilepsia é uma das doenças neurológicas mais prevalentes, e a epilepsia do lobo mesial temporal (MTLE) a mais frequente das epilepsias resistentes a fármacos. Cerca de 30% dos pacientes com MTLE não são controlados por fármacos anti-epilépticos (AED), sendo a cirúrgica a única alternativa para controlar as convulsões, em que apenas 10% dos pacientes são elegíveis. A terapia actual com AED apenas trata os sintomas, sem redução ou possível cura da epilepsia. A prevalência e percentagem elevadas de doentes com epilepsia farmacorresistente justificam a vasta investigação sobre os sistemas reguladores e/ou neuromoduladores da hiperexcitabilidade neuronal.

A adenosina (ADO) modula a atividade neuronal, sendo considerada um anticonvulsivante endógeno. Os níveis da ADO aumentam dramaticamente durante as convulsões. A activação dos receptores inibitórios A1 da ADO, reduz a hiperexcitabilidade, controla a crise e assegura a refractoriedade pós-convulsiva. Sendo a ADO uma molécula ubiquitária, a administração sistémica conduziria a inúmeros efeitos adversos, nomeadamente cardiovasculares.

O domínio dos mecanismos de formação e inactivação da ADO no cérebro está na base da intensa investigação visando novos alvos terapêuticos. O aumento da adenosina focal no hipocampo é uma das abordagens mais viáveis num futuro próximo: implantes aplicados no hipocampo de modelos experimentais regulam ou libertam ADO *in situ*. Esses implantes podem conter polímeros de libertação controlada de ADO, células histaminais manipuladas que libertam ADO ou genes "anti-sense" contra o gene *adk* da enzima adenosina cinase (ADK) desregulada, que controla os níveis extracelulares de ADO. A dieta cetogénica, usada sobretudo no controlo de epilepsia refractária em crianças, tem sido também estudada.

Estratégias conceptuais inovadoras que vão além da supressão dos sintomas, são a esperança para o tratamento de uma das doenças neurológicas mais prevalentes no mundo.

**Palavras-chave:** Epilepsia; MTLE; Adenosina; ADK; terapias baseadas na adenosina.

## Abbreviation List

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**ADK** – Adenosine Kinase

**Adk gene** – Adenosine Kinase gene

**ADO** – Adenosine

**AMP** – Adenosine 5'-monophosphate

**AR** – Adenosine Receptor

**ATP** – Adenosine 5'-triphosphate

**BDNF** – Brain-derived Neurotrophic Factor

**CNS** – Central Nervous System

**COX** - Cyclooxygenase

**COXIBE** – Selective COX2-inhibitor

**GABA** –  $\gamma$ -Aminobutyric Acid

**HS** - Hippocampal Sclerosis

**KA** – Kainic Acid

**K<sub>ATP</sub>** – inwardly rectifying potassium channels

**MTLE** - Mesial Temporal Lobe Epilepsy

**mTOR** - Mammalian Target of Rapamycin Protein

**NADH** – Nicotinamide Adenine Dinucleotide

**RNA** - Ribonucleic Acid

**SE** – *Status Epilepticus*

**VGLUT2** - Vesicular Glutamate Transporter

## Introduction

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Epilepsy is a chronic neurological disease, the most common worldwide. According to the World Health Organization ([WHO, 2009](#)), about 50 million people are affected by this disease.

Partial epilepsies occurring in adulthood are most likely to be mesial temporal lobe epilepsies (MTLE), the most frequent form of pharmacoresistant epilepsy ([Theodore, 1991](#)). This neurological disorder cannot be controlled with medication in 30% of cases. Therefore, surgical intervention is necessary, in order to control the seizures ([Spencer et al., 1984](#)).

Adenosine (ADO) is an extracellular signaling molecule, able to coordinate homeostasis in cells, with effects on tissue's protection and repair ([Linden, 2005](#)). In the nervous system ADO also exerts a rather specific neuromodulatory role, controlling synaptic transmission and synaptic plasticity ([Gomes et al., 2011](#)), as well as coordinating neuronal networks ([Sperlagh and Vizi, 2011](#)). This system's double action allows considering ADO as a therapeutic target in the management of neurologic disorders. However, part of the ADO's role seems to be related to the pathophysiology of epilepsy because ADO acts as an endogenous anticonvulsant ([Boison, 2006](#)). Subsequently impaired adenosinergic modulation is thought to be involved in the epileptogenic process. Those mechanisms' apprehension in health and disease will, eventually, allow researchers to develop entirely new conceptual strategies, in order reverse and eventually cure epilepsy as a whole and not only suppressing its symptoms.

## Objectives

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This present review focuses on the relevance of the ADO-mediated mechanisms in the pathophysiology of epilepsy, in particular on MTLE. It aims to report the most relevant therapeutic strategies developed in recent years, and which will provide pioneering drugs in the treatment of epilepsy.

Therefore, a short overview of epileptogenesis, epilepsy and MTLE will be made. The role of ADO in brain function and its clinical implications in epilepsy will be highlighted. Describing the various approaches designed to increase ADO brain levels will be the core of this work.

## Epilepsy

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As proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE) in 2005, epilepsy is defined as a brain disorder characterized by an enduring predisposition to generate epileptic seizures and by the neurobiologic, cognitive, psychological, and social consequences of this condition ([Fisher et al, 2005](#)). Traditionally, the diagnosis of epilepsy requires the occurrence of at least two unprovoked seizures. Some clinicians also diagnose epilepsy when one unprovoked seizure occurs in the setting of a predisposing cause (such as a focal cortical injury) or if a generalized interictal discharge occurs suggestive of a genetic predisposition.

Seizures are the manifestation of abnormal hypersynchronous or hyperexcitable discharges of cortical neurons. The clinical signs or symptoms of seizures depend on the location of the epileptic discharges in the cerebral cortex and the extent and pattern of the propagation of the epileptic discharge in the brain. The probability of having at least one epileptic seizure in lifetime is about 9% and the probability of being diagnosed as epileptic is almost 3%, while the prevalence of active epilepsy is only about 0.8% ([Pugliatti et al., 2007](#)).

In a substantial number of cases, the cause of epilepsy remains unknown. Identified causes tend to vary with patient age: Inherited syndromes, congenital brain malformations, infection, and head trauma are primary causes in children; head trauma is the most common known cause in young adults; strokes, tumors, and head trauma become more frequent in middle age adults; stroke is the main cause in the elderly, along with Alzheimer disease and other degenerative conditions.

Anti-epileptic drugs (AEDs) are the frontline treatment for epilepsy ([Wiebe and Jette, 2012](#)). There are over twenty AEDs in clinical use, including phenytoin, sodium valproate and carbamazepine, and newer generation drugs such as levetiracetam and lacosamide. The introduction of newer generation AEDs has provided a wider range of treatment options that can reduce potential side effects and allow better tailoring of therapies to an individual's specific syndrome. However, the proportion of patients with pharmaco-resistant epilepsy has changed relatively little, remaining at ~30% ([Wiebe and Jette, 2012](#)). Existing AEDs target a relatively small number of proteins. These include: enhancement of inhibitory (GABAergic) transmission; reduction of excitatory (glutamatergic) neurotransmission; modulation of neurotransmitter release and targeting voltage-gated ion channels ([MacDonald and Rogawski, 2008](#)). Patients with poorly-controlled seizures suffer additional reductions in quality of life, severe limitations on work and personal activities, and are at increased risk of neurological deficits, accidents and death ([Pugliatti et al., 2007](#)).

## Mesial Temporal Lobe Epilepsy

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Mesial Temporal Lobe Epilepsy (MTLE), the most common form of pharmacoresistant epilepsy ([Wieser, 2004](#)), is often associated with previous injuries, including trauma, *status epilepticus* (SE), febrile seizures, and infection ([Kharatishvili and Pitkanen, 2010](#); [Yang et al., 2010](#)).

Typically, a standard course of events leads to epileptic status. After the initial insult, a latency period of 5–10 years occurs without any symptoms or complications ([Wieser, 2004](#); [Boison, 2008](#)). The latency period ends when the patient begins to suffer from spontaneous seizures. At the beginning of spontaneous seizure activity, seizures are often controllable with medication. This period is known as the silent period. As the disease progresses, patients commonly develop intractable symptoms that cannot be managed with the current antiepileptic drugs ([Wieser, 2004](#)). The latency period associated with epileptogenesis is thought to involve structural and biochemical changes resulting in spontaneous seizures. These changes, presumably, are initiated by the primary aggression and occur over an extended time course ([Sharma et al., 2007](#)).

A plethora of changes have been observed in epileptic tissue. Among which, astrogliosis (astroglial scar), neuronal death and aberrant mossy fiber sprouting are observed both in animal models (e.g. [Pitkanen et al., 2009](#); [Hayashi et al., 2011](#); [Zheng et al., 2011](#)) and in resected human tissue (e.g. [de Lanerolle et al., 2003](#); [Bae et al., 2010](#); [Yang et al., 2010](#)). Although the underlying biochemical pathways remain unclear, the first structural changes are caused by the primary insult (e.g. [Sloviter, 1996](#); [Sharma et al., 2007](#)). Other authors have proposed that said modifications continue to accumulate together with disease progression ([Pitkanen and Sutula, 2002](#); [Wieser, 2004](#); [Yang et al., 2010](#)).

Upregulation of inflammatory indicators was observed ([Crespel et al., 2002](#); [Yang et al., 2010](#); [Ravizza et al., 2011](#)) with an increase of proinflammatory cytokines, namely: I-calcain, interleukin (IL)-1b, IL-6, and transforming growth factor (TGF)-b1 in resected human anterior temporal lobe specimens ([Feng et al., 2011](#)). Supporting the epilepsy's inflammatory hypothesis even further, downstream cyclooxygenase-2 (COX-2) inhibition in epileptic rats, by selective COX-2 inhibitors (COXIBE's) ([Jung et al., 2006](#); [Polascheck et al., 2010](#); [Holtman et al., 2009](#)) is found to reduce seizure initiation, severity, and frequency while preserving neurons. The inflammatory hypotheses should

be treated with caution because although brain inflammation during childhood often precedes adult epilepsy, adult-onset epilepsy may emerge when no inflammatory event happened in childhood (Ravizza et al., 2011).

## Morphological Changes

### Neuronal Degeneration

Neuronal degeneration was one of the first recognized hallmarks of MTLE. Resected hippocampi from MTLE patients have decreased size and a rubber-like stiffness. Slices of these hippocampus observed on the microscope have a reduction of the number of neurons preferentially in CA1, CA3, and the hilus (CA4), whereas CA2 and the dentate nucleus seemed to be spared (de Lanerolle et al., 2003). Extrahippocampal neuron loss has also been observed in MTLE in the entorhinal cortex, pyriform cortex, and amygdala (Ben-Ari and Dudek., 2010). The role and mechanism of neuronal degeneration in MTLE remain unclear.

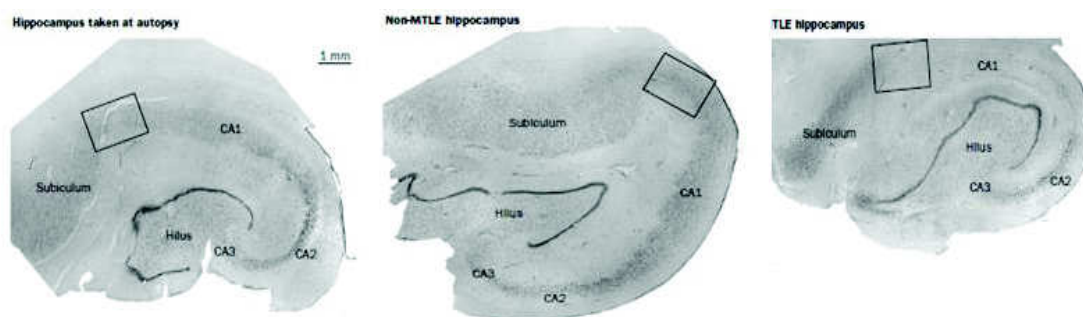


Figure 1: **Nissl-stained coronal sections of hippocampi from representative cases**

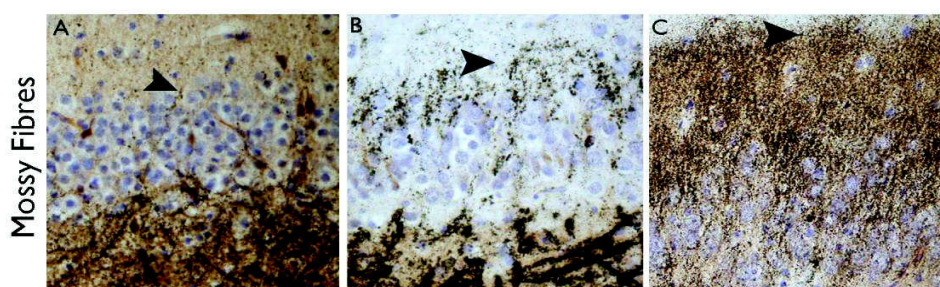
Density and distribution of neurons are similar in autopsy and non-MTLE hippocampi. By contrast, the MTLE hippocampus shows severe loss of neurons, particularly in areas CA1 and CA3 and the dentate hilus. Magnification is the same for all sections (adapted from Eid et al., 2004)

### Gliosis

Gliosis is the common denominator of various neurological diseases including human and animal epileptic tissue (Pitkanen and Sutula, 2002; Sharma et al., 2007; Thom, 2014). Chronic astroglial activation increases inflammatory cytokine production, potentiates excessive synaptic activity and induces a migration to the epileptic focus, resulting in sclerosis. Whether these effects are responsive to MTLE or inducers of MTLE has yet to be answered.

## Mossy Fiber Sprouting

Mossy fiber sprouting is characterized by dentate granule cell axons forming synapses with cells in the granule cell layer and inner molecular layer rather than in the CA3 region of Ammon's horn (Sharma et al., 2007). Which changes deriving from seizures conduct to sprouting are currently unknown. Gliosis and the release of growth factors, cytokines, and adhesion molecules from activated astrocytes and microglia (Yang et al., 2010) probably are in the basis of the sprouting phenomenon. Sprouting is progressive and is brought on by recurrent seizures (Pitkanen and Sutula, 2002). Hyperexcitation hypothesis purport that dentate granule cells become hyperexcitable as a result of recurrent sprouting since mossy fibers are glutamatergic axons and establish excitatory circuits within the inner molecular layer (Sharma et al., 2007). Conversely, other investigators believe that mossy fiber sprouting serves to reform inhibitory circuits that are lost during the initial damage of neurons, supported by the fact that mossy fibers synapse primarily on inhibitory interneurons in control animals (Gorter et al., 2001). Additionally, it has been noted that mossy fiber sprouting occurs secondary to neuron loss (Jankowsky and Patterson, 2001). Observations of reinnervation of dormant basket cells by mossy fibers and of collateral sprouts from interneurons forming inhibitory feedback to granule cells also reinforces the antiepileptogenic hypothesis (Ben-Ari and Dudek, 2010). Recently, Sharma and colleagues (2007) noted that aberrant sprouting precedes seizure onset in rats exposed to kainic acid (KA). This observation does not clarify the question of the role of mossy fibers, unfortunately, because whether the sprouting itself leads to seizure production or the inability to sprout further leads to seizure production is unclear. Clarifying the role that mossy fibers play in epileptogenesis is an important step in better understanding epilepsy and epileptogenesis and may lead to new treatment options.



**Figure 2. Axonal sprouting in Hippocampal sclerosis (HS)/temporal lobe epilepsy (TLE).**

Mossy fibre sprouting identified with Timm staining in human hippocampal sclerosis (HS) (A-C). In (A) the black silver granules are mainly confined to the subgranular zone and significant sprouting into the molecular layer (arrow) is not observed. In (B) there is focal sprouting in the molecular layer (arrow) and in (C) marked sprouting is shown with a dense band of zinc positive granules in the molecular layer (adapted from Thom M 2014).

## Potencial Targets for Seizure Control

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The need for new AEDs is a widely-recognized goal for the improved treatment of epilepsy ([Baulac and Pitkanen, 2008](#)). Innovative treatments may either be targeted to epileptogenesis, the morphological and functional changes leading to epilepsy after an initial brain insult, or to ictogenesis, the processes involved in initiation, propagation and amplification of seizures in the epileptic brain. Possible features of new AED targets include proteins with more subtle influence on excitatory neurotransmission to avoid the common side effects of many AEDs. Anti-neuroinflammatory ([Venazzi et al., 2011](#)) or neuroprotective properties ([Acharya et al., 2008](#)) could also yield disease-modifying effects that would mitigate the underlying pathology.

### Adenosine – A Retaliatory Metabolite

The ribonucleoside adenosine (ADO) has early evolutionary origins and likely played a role in prebiotic evolution ([Oro and Kimball, 1961](#)). Importantly, ADO is not only part of the energy molecule Adenosine Triphosphate (ATP) but also of Ribonucleic Acid (RNA), the nucleic acid thought to be at the origin of life ([Lahav, 1993](#); [Dworkin et al., 2003](#); [Robertson and Joyce, 2012](#)). While ATP reflects the energy pool in the environment, RNA reflects the metabolic activities of a cell. Thus, ADO assumes a central place between energy availability and metabolic demands and has therefore been termed a retaliatory metabolite ([Newby et al., 1985](#)). The early evolutionary principle to conserve energy was likely a rise in ADO as a consequence to ATP depletion and to use the increase in ADO as a negative feedback regulator to attenuate all cellular activities that consume energy. This early evolutionary principle is omnipresent in all living systems and in every human organ. In the brain, epileptic seizures cause a rapid drop in energy, which results in the generation of ADO levels that can exceed the basal level more than 40 times ([During and Spencer, 1992](#)); it is this rise of ADO that acts as endogenous terminator of seizures and which is responsible for the postictal refractoriness that normally follows a seizure ([Lado and Moshe, 2008](#)). Seizure suppression by ADO depends on the activation of G protein coupled adenosine A1 receptors ([Fredholm et al., 2005a](#)); however, new evidence suggests that ADO retains important ADO receptor-independent regulatory functions, which are based on interactions with mitochondrial bioenergetics, interference with enzyme reactions, and epigenetic functions. Thereby ADO assumes a unique role as homeostatic network regulator.

## Adenosine Receptor-Dependent Pathways

A number of ADO's actions are mediated by a group of specific receptors, G protein-linked transmembrane proteins of the P1 family, distinguished from the P2 ATP receptor family. Four members of the P1 family have been cloned in mammals: adenosine subtype 1 (A1R), 2A (A2AR), 2B (A2BR) and 3 (A3R) receptors ([Fredholm et al, 2011](#)). Homologous genes have been found in numerous other animal groups ([Sazanov et al, 2000](#); [Petersen et al., 2003](#); [Dolezelova et al., 2007](#); [Boehmler et al., 2009](#); [Malik and Buck, 2010](#)). These receptors have biochemical specificity as each act through a particular set of G proteins to influence second messengers: A1R and A3R activation inhibit the second messenger cAMP production, whereas A2AR and A2BR activation increase cAMP ([Fredholm et al., 2011](#)). Other second messengers such as diacylglycerol, inositol triphosphate, and  $\text{Ca}^{2+}$  are also modulated. Each receptor presents a distinct pharmacology, and each has a particular distribution in tissues and cell types. A1Rs are expressed most highly in brain, whereas A2BRs and A3Rs have their highest expression in the periphery ([Dixon et al., 1996](#)). Within the brain, A1Rs are widespread with particularly high levels in the limbic system, whereas A2ARs are expressed mostly in the basal ganglia ([Dixon et al., 1996](#)). ADO can have powerful receptor-mediated effects on synaptic transmission in the brain ([Fredholm et al., 2011](#)). Presynaptic A1Rs inhibit synaptic release of most, if not all, neurotransmitters, with an apparently greater effect on excitatory transmission. Thus, if ADO levels are raised sufficiently, synaptic transmission can be blocked altogether. On the postsynaptic side, A1Rs hyperpolarize membranes by opening inwardly rectifying  $\text{K}^+$  channels. These combined A1R effects play a major role in the efficacious anticonvulsant effect of ADO and A1R agonists ([Boison, 2007](#)). The effect of A2ARs on network excitability is less clear, and more anatomically restricted, but if seizures reflect brain network imbalance, then one seizure model suggests that A1Rs and A2ARs may cooperate to promote homeostasis ([De Sarro et al., 1999](#)).

## Mitochondrial Bioenergetics

Mitochondria generate ATP via oxidative phosphorylation, and this is the main process of energy generation. Regarding the relationship between ADO and ATP, intracellular ADO is dephosphorylated from Adenosine 5'-monophosphate (AMP) and is converted back to AMP via adenosine kinase (ADK). The ADO-AMP cycle is linked to ADP and ATP through ADK. Thus, ADO is linked tightly to energy metabolism. Whereas mitochondrial uncouplers decrease ATP and increase ADO, mitochondrial enhancers which boost ATP levels also appear to increase ADO. Therefore, improving

mitochondrial bioenergetics has the potential to offer dual benefits of improving metabolic dysfunction and restoring ADO homeostasis (Boison et al., 2013).

The intracellular concentration of ATP is nearly 50 times higher than that of AMP (Arch and Newsholme, 1978) and about 10,000 times higher than that of ADO (Pazzagli et al., 1995; Delaney and Geiger, 1996) and minor decreases in intracellular ATP leads to a large rise of intracellular ADO level. Thus, various excitatory stimuli cause decreased brain energy and a subsequent increase in ADO (Shepel et al., 2005). As a retaliatory metabolite ADO is thought to be one of the keylinks between neuronal network homeostasis and mitochondrial bioenergetics with both adenosine receptor-dependent and independent pathways.

### Ketogenic Diet

In the early 1920s, the ketogenic diet was first introduced to treat patients, mainly children, with refractory epilepsy. However, with the introduction of diphenylhydantoin in the following decade the diet faded from the clinical arsenal. In the 1990s its clinical interest was renewed and since then more palatable diet variants were developed.

The diet is high in fat and low in carbohydrate and protein, providing sufficient protein for growth but insufficient amounts of carbohydrates for all the metabolic needs (Freeman et al., 2006). During high rates of fatty acid oxidation, in mitochondria, large amounts of acetyl-CoA are generated, leading to the synthesis of three ketone bodies - hydroxybutyrate, acetoacetate, and acetone. Ketone bodies pass into circulation, causing serum levels to rise severalfold, and then are utilized as an energy source in extrahepatic tissues, including the brain. The ketone bodies are converted to acetyl-CoA by D-hydroxybutyrate dehydrogenase, acetoacetate-succinyl-CoA transferase, and acetoacetyl-CoA thiolase and then enter the Krebs cycle within brain mitochondria, leading to ATP production (Masino and Geiger, 2008).

Ketone bodies have an important role on seizure protection and the proposed mechanisms for this occurrence are:

- a) glutamate transport into synaptic vesicles (vesicular glutamate transporter VGLUT2), is inhibited by the ketone body acetoacetate (Juge et al, 2010), decreasing glutamate release;
- b) increased production of the inhibitory neurotransmitter  $\gamma$ -Aminobutyric acid (GABA) by glutamate recycling via glutamine; reduction of brain derived neurotrophic factor (BDNF) and its receptor TrkB expression, both being

implicated in epileptogenesis, by a decline in the cytosolic reduced nicotinamide adenine dinucleotide (NADH) ([Garriga-Canut et al., 2006](#));

- c) ATP activation of nearby ATP-sensitive potassium ( $K_{ATP}$ ) channels ([Haller et al., 2001](#); [Tanner et al., 2011](#)), generating hyperpolarizing  $K^+$  currents ([Ashcroft and Gribble, 1998](#));
- d) Increased extracellular ADO levels by mitochondrial ATP and consequent reduction of neuronal excitability by activating pre and postsynaptic A1Rs.

The increase on extracellular ADO levels is critical to the therapeutic success of the ketogenic diet ([Masino and Geiger, 2008](#)).

### DNA Methylation Hypothesis

Epigenome modifications which include changes in DNA methylation, histone tail modifications, and incorporation of histone variants are mechanisms by which network homeostasis can be dramatically altered and consequently alter the entire gene expression profile of a tissue. There are a number of epilepsy-associated neurological diseases that are directly attributed to primary genetic mutations and result in secondary deregulation of the epigenome ([Kobow and Blumcke, 2011](#)). Gene promoters from MTLE patients have altered DNA methylation patterns and decreased DNA methyltransferase gene expression ([Kobow et al., 2009](#); [Zhu et al., 2012](#)). This hypermethylated state forms the basis of the methylation hypothesis of epileptogenesis, suggesting that seizures by themselves can induce epigenetic chromatin modifications and thereby aggravate the epileptogenic condition ([Kobow and Blumcke, 2011](#)). Hypermethylation of DNA can be triggered by a variety of mechanisms, however, the mechanisms underlying the gradual increase in DNA methylation during the course of epileptogenesis are not well characterized and subject of speculation ([Kobow and Blumcke, 2012](#)). The epigenetic drift hypothesis suggests that a gradual shift in the ratio of active DNA demethylation and *de novo* methylation, triggered by an injury and modified by environmental and intrinsic factors leads to increased DNA methylation, altered gene expression, and an altered (e.g., seizure) phenotype ([Feil and Fraga, 2011](#)).

Peripheral changes in the transmethylation pathway are conserved within the brain. It was recently found that hippocampal ADO levels regulate the global state of DNA methylation by shifting the equilibrium constant of the transmethylation pathway either increasing (high ADK and low ADO) or decreasing (low ADK and high ADO) methylation. These ADO dependent changes in DNA methylation are receptor-independent and can be evoked by either a single pharmacological bolus of ADO (icv

administration) or in response to endogenous changes in ADK expression or activity ([Williams-Karnesky et al., 2013](#)).

As an obligatory endproduct of transmethylation, ADO's tone non-specifically drives the transmethylation pathway by regulating substrate availability. Consequently, ADO's tone does not regulate site specific DNA methylation, but instead the homeostasis of the DNA-methylome.

Through this mechanism, astrogliosis and associated overexpression of ADK could contribute to continued epileptogenesis through maintenance of a hypermethylated state of hippocampal DNA ([Williams-Karnesky et al., 2013](#)).

### **Unbalance of Adenosine Kinase in the Brain**

Astrocytes have the main regulative function on maintenance of extracellular ADO levels. The astrocyte-based enzyme ADK phosphorylates adenosine to AMP driving the influx of ADO into the astrocyte through equilibrative transporters. Injuries to the brain such as trauma, stroke or SE trigger an acute surge in ADO, which is accompanied by transient downregulation of ADK ([Clark et al, 1997](#); [Pignataro et al, 2008](#)). The initial injury and the associated surge in ADO can trigger several mechanisms possibly implicated in epileptogenesis, among which the induction of A2AR expression in glial cells appears to play a prominent role. The proliferation of astrocytes and thereby the development of astrogliosis is in part regulated by the ratio of the different ADO receptors expressed on the astrocyte membrane. The increased activation of A2ARs by an injury- associated surge in ADO can increase astrocyte proliferation and activation, whereas the blockade of excitatory A2ARs prevents the induction of astrogliosis by BDNF, which is a known transactivator of the A2AR ([Hindley et al., 1994](#); [Brambilla et al., 2003](#); [Rajagopal et al., 2004](#)). ADO deficiency in human epilepsy has directly been identified via the analysis of microdialysis samples. A 25% reduction of ADO in the epileptogenic versus the contralateral control hippocampus was found ([During and Spencer, 1992](#)).

## Focal Adenosine Augmentation

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Systemic augmentation of ADO signaling by either A1 receptor agonists or by ADK-inhibitors effectively suppress seizures in a wide range of epilepsy models including those resistant to conventional AEDs ([Gouder et al., 2003](#); [McGaraughty et al., 2005](#); [Benarroch, 2008](#)). Unfortunately, systemic augmentation of ADO signaling is not a therapeutic option due to widespread, mainly cardiovascular, side effects, and due to the liver toxicity of ADK disruption ([Boison et al., 2002](#); [Fredholm et al., 2005a](#)). Therefore, focal ADO augmentation approaches, with the aim to reconstruct ADO homeostasis within an epileptogenic brain area, become a therapeutic necessity. Focal ADO augmentation to suppress epileptic seizures was first successfully performed in rat kindling models of epilepsy using intraventricular implants engineered to release ADO ([Huber et al., 2001](#)). Importantly, focal ADO augmentation, in contrast to systemic ADO augmentation, was not associated with any sedative side effects ([Guttinger et al., 2005](#)). Unfortunately, duration of seizure control (12 days) was limited by a reduced life expectancy of the encapsulated cells ([Huber et al., 2001](#)). Currently, new approaches are being developed to make therapeutic use of ADO augmentation:

- Silk is a Food and Drug Administration (FDA) approved biocompatible biopolymer that can be engineered for controlled focal drug release. Infrapocampal implants of silk which were engineered to release defined daily doses of up to 1 mg of ADO provided complete control of seizures in the kindled rat for the duration of the ADO release (1 mg ADO per day for 10 days) ([Szybala et al., 2009](#)). Mouse embryonic stem cells and human mesenchymal stem cells were also engineered to release ADO from silk implants ([Shetty and Hattiangady, 2007](#))
- ADO releasing stem cells based on genetic disruption of the endogenous adenosine kinase (adk) gene or by expressing a micro RNA (miRNA) directed against adk gene via a lentiviral vector ([Fedele et al., 2004](#); [Ren et al., 2007](#)) potently suppressed kindling epileptogenesis after transplantation into the infrapocampal fissure with upregulation of ADK ([Li et al., 2007b](#)). Marked astrogliosis attenuation, normalization of ADK expression levels and total absence of seizures were registered three weeks later, supporting a novel and persistent antiepileptogenic effect of focal ADO delivery.

## Discussion

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Epilepsy is a neuronal disorder characterized by abnormal and excessive neuronal firing where each seizure represents a rapid loss of homeostatic equilibrium, with altered energy and molecular gradients, and a corresponding interruption of normal behavior and consciousness. Because having a seizure can increase the likelihood of future seizures, seizures themselves contribute to epileptogenesis ([Boison et al., 2013](#)).

Over the past few decades, many studies have sought to identify and treat key changes associated with epilepsy and spontaneous seizures. Many morphological and biochemical changes identified aggravate epilepsy and may steer to a refractory state, where no AED therapy is able to control seizures. MTLE is the most frequent of those drug resistant epilepsies.

The majority of MTLE patients have HS lesions, including astrogliosis, neuronal death and aberrant mossy fiber sprouting (e.g. [de Lanerolle et al., 2003](#); [Bae et al., 2010](#); [Yang et al., 2010](#)). The aberrant sprouting of mossy fibers occurs secondary to neuron loss and so, a comprehensive knowledge of this phenomenon will have a key role for the development of new treatment options.

At the present, antiepileptic drugs focus on neuronal targets, mainly glutamatergic and GABAergic mechanisms or ion channels. These therapies are largely symptomatic and do not affect the underlying disease processes. These drugs often result in deleterious side effects including cognitive impairment. Moreover, a large subpopulation of patients does not respond to current AEDs, suggesting that there is a great need for new therapeutic directions ([Wiebe and Jette, 2012](#)).

Endogenous mechanisms for the termination of seizure activity include the release of high enough amounts of ADO, contributing to seizure arrest and postictal refractoriness. Because ADO modulates neuronal activity it is considered an endogenous anticonvulsant. The activation of ADO inhibitory A1 receptors decreases excitatory neurotransmission, and for this reason several ADO-based therapies for epilepsy are currently under development.

Ketogenic diet has been confined to a last resort therapy for refractory epilepsy, nevertheless this diet often succeeds in control of seizures when drugs fail. This indicates that the metabolic changes produced by the diet tap into anticonvulsant mechanisms that are not targeted by existing medication. The diet is unlikely to

produce treatment resistance and significant untoward side effects (Boison et al., 2011): One of these mechanisms is the enhancement of ADO levels, leading to a net inhibition of excitatory transmission; other proposed mechanisms include disruption of glutamatergic synaptic transmission, inhibition of glycolysis, and activation of ATP-sensitive potassium channels. Follow up studies will show how potent it is the antiseizure action of this diet by the integration of individual findings on metabolic modification of neuronal activity.

ADO-dependent changes in DNA methylation were pinpointed as an underlying mechanism for the antiepileptogenic properties of ADO therapy. Several targets that function by either interacting with DNA or playing a role in gene transcription and translation responded to ADO therapy and exhibited a decrease in the promoters' DNA methylation. This fact constitutes a benefit since the use of DNA methyltransferase (DNMT) inhibitors is associated with complications or side effects (Williams-Karnesky et al., 2013).

Pathologically increased expression of ADK is linked to astrogliosis in many central nervous system (CNS) pathologies, leading to reductions in ADO tone and leaving neurons more susceptible to damage (Boison, 2008). It has been observed that the upregulation of ADK occurs before gross morphological changes in the hippocampus, offering a time window for therapeutic intervention (Gouder et al., 2004). Consequently, the use of ADK inhibitors may possibly slow down hippocampus' deterioration.

Local administration of ADO in the hippocampus in animal kindling models through icv injection markedly attenuated seizures and contributed to postictal refractoriness. Systemic administration of selective agonist analogues of the A1 inhibitory ADO receptor or selective antagonist analogues of the A2A excitatory ADO receptor mimicked ADO icv injection, but the occurrence of intense cardiovascular side effects were also observed. Being ADO an ubiquitary molecule, its administration *per se* (and its analogues) leads to numerous adverse effects. Nowadays we find a new era in which focal approaches of ADO augmentation are the leading strategies for seizure control: Cell- and polymer- based focal ADO augmentation therapies were found to provide effective seizure control (Boison, 2009a; Boison & Stewart, 2009); The transplantation of ADO releasing stem cells (modified by viral-mediated insertion of antisense sequences) potently suppressed kindling epileptogenesis (Li et al., 2007b). This viral ADK antisense approach apparently causes permanent reversal of ADK-based ADO dysregulation in the epileptogenic focus in which ADK levels are

pathologically high (Boison, 2010a). Yet it is necessary to establish the efficacy of the virus in post SE chronic models of epilepsy.

Polymer-, and in particular silk-based ADO delivery (Wilz et al., 2008; Szybala et al., 2009), are ideal to start clinical safety and feasibility tests of focal ADO-augmentation. Notwithstanding, these systems need to be further improved in order to provide long-term delivery options for ADO. Furthermore, the translational potential of such studies in humans is uncertain.

Rapamycin has recently received broad attention as a potential broad-spectrum effector in seizure suppression and irregular structural modification of the epileptic hippocampus (Chong et al., 2010). This drug affects the mammalian target of rapamycin (mTOR) protein, which is involved with growth factor production, neuronal plasticity and remodeling, glial activation, and cellular motility. As such, it would seem that rapamycin has the potential to be a catch-all therapy for MTLE and for epilepsy as a whole. Because rapamycin was originally approved as a cancer chemotherapeutic (powerfull inhibitor of mitosis and motility of cells) it may not be safe as a long-term MTLE treatment.

New therapeutic targets, which do not include the increase of ADO in the hippocampus, have been emerging. Therapies based on the mechanisms now evidenced from the study of rapamycin in the hippocampus of rats, in induced metabolic changes in neurons by ketogenic diet, and in the mechanisms involved in neuro-inflammation, are rapidly developing. However, in the near future, the ADO-based therapies are the ones that most likely will jump to clinical trial's assays.

## Conclusions

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The number of AEDs available for treatment of MTLE are greater than ever, but the effectiveness of any AED remains arguably unchanged. Contraindications are better managed today but effective seizure-eliminating therapy is not ([Löscher and Schmidt, 2011](#)).

Much of the mystery of epilepsy is now a “chicken and egg” phenomenon. Novel experiments will allow further insights on the interplay among ion channels, cellular signaling, neurotransmitter release and reuptake, and inflammatory factors in pre-epileptic and epileptic tissue. Basic understanding of morphological changes such as mossy fiber sprouting, gliosis, and neuron death may lead to identification of specific molecules released either as a result of cellular changes or as a prequel to the changes. These molecules may constitute good targets for future therapeutic approaches.

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## Annex A

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### **“Epilepsia do lobo mesial temporal: novas abordagens terapêuticas emergentes” - Resumo**

#### **Introdução**

A epilepsia é uma doença neurológica crónica, sendo a mais comum em todo o mundo, com cerca de 50 milhões de pessoas afectadas por esta patologia. A epilepsia do lobo mesial temporal (MTLE) é a forma de epilepsia parcial mais comum em adultos e uma das formas de epilepsia farmacorresistente mais frequentes, sendo necessário o recurso à intervenção cirúrgica. A adenosina (ADO) é uma molécula sinalizadora extracelular, capaz de coordenar a homeostasia nas células, com efeitos de protecção e reparação tecidual. No sistema nervoso tem um papel neuromodulador bastante específico, controlando a transmissão e plasticidade sináptica e coordenando as redes neuronais. Esta dupla acção, aliada à evidência de que a ADO actua no cérebro como um anticonvulsivante endógeno e que a sua disfunção pode estar envolvida no processo epileptogénico, faz da ADO uma molécula cuja compreensão dos seus mecanismos de síntese e inactivação permitirá desenvolver estratégias terapêuticas que visem a cura desta patologia mais do que o tratamento sintomático.

#### **Objectivos**

Esta revisão pretende destacar a importância dos mecanismos fisiológicos mediados pela ADO na epilepsia, em particular na MTLE. Destina-se a resumir as evidências atuais das diversas estratégias terapêuticas emergentes nos últimos anos, que num futuro próximo pode dar origem a medicamentos inovadores para o tratamento da epilepsia.

#### **Epilepsia**

A epilepsia é uma desordem cerebral caracterizada pela predisposição para gerar crises epiléticas, pelas consequências neurobiológicas, cognitivas, psicológicas e sociais desta condição. O diagnóstico de epilepsia é assumido pelos clínicos quando decorrem pelo menos duas crises epiléticas não provocadas, embora outros considerem apenas uma convulsão, desde que haja uma causa predisponente ou uma descarga interictal generalizada sugestiva de predisposição genética. As convulsões resultam de descargas excitatórias anormais de neurónios corticais e a sua manifestação sintomática depende da localização destas no córtex e da sua medida e

padrão de propagação no cérebro, sendo altamente variável, mas estereotipada na maioria dos doentes. Embora a probabilidade de diagnosticar epilepsia seja cerca de 3%, a sua prevalência real é próxima de 0,8%. Na maior parte dos casos a causa da epilepsia não é conhecida. As causas identificadas variam com a idade do doente, sendo os síndromes hereditários mais comuns nas crianças, o traumatismo e o AVC em adultos. Os fármacos anti-epilépticos (AED) (ex.: Fenitoína, valproato de sódio, carbamazepina) constituem o tratamento de primeira linha para a epilepsia. Os AEDs de nova geração (ex.: levetiracetam e lacosamida) têm menores efeitos laterais, melhor adaptação mas não alteraram a percentagem de pacientes com epilepsia farmacorresistente (~ 30%). Os alvos destes fármacos visam o aumento da transmissão inibitória (GABAérgica), a redução da transmissão excitatória (glutamatérgica), a modulação da liberação de neurotransmissores e de canais iónicos dependentes de voltagem.

## MTLE

A epilepsia do lobo mesial temporal (MTLE), a forma mais comum de epilepsia farmacorresistente, é frequentemente associada a lesões prévias, incluindo traumas, convulsões febris, e infecção, levando ao estado epiléptico. Após a lesão inicial, decorre um período de latência de 5 a 10 anos sem sintomas ou complicações. Quando a atividade convulsiva recomeça a se manifestar, é normalmente controlável com medicação (período silencioso), mas no decurso da doença, os sintomas tornam-se incontroláveis com AEDs comuns. Pensa-se que o período latente envolve mudanças estruturais e bioquímicas iniciadas pela lesão primária que conduzem ao aparecimento das convulsões. Lesões criadas pela astrogliose (cicatriz astrogliar), morte neural e arborização aberrante das fibras musgosas têm sido observados em tecido epiléptico humano e animal. As alterações bioquímicas subjacentes permanecem obscuras, mas muitos estudos indicam que a convulsão inicial causa alterações estruturais primárias que continuam a desenvolver-se no decurso da doença. Além das alterações macro-estruturais, muitas alterações bioquímicas e de sinalização têm sido descritas, como a sobre-regulação de indicadores inflamatórios por aumento de citocinas pro-inflamatórias e fatores de crescimento. A dificuldade da hipótese inflamatória é que, apesar da inflamação do cérebro na infância poder preceder a epilepsia no adulto, doentes epilépticos que iniciaram a sua patologia já na idade adulta também apresentam indicadores inflamatórios no foco epiléptico, não relacionados com um episódio inflamatório anterior.

## Alterações morfológicas

### Degeneração Neuronal

A degeneração neuronal foi uma das primeiras características associadas à MTLE reconhecidas, com alterações no hipocampo incluindo diminuição de tamanho, endurecimento e perda de neurónios, especialmente nas áreas CA1, CA3 e no hilo (CA4) e perda de neurónios extra hipocampais no córtex entorrínico, piriforme e amígdala. A importância bem como os mecanismos de degeneração neuronal na MTLE permanecem pouco claros.

### Gliose

A gliose pode revelar-se num aumento de cerca de dez vezes da micróglia ativada. A hipótese da cicatriz glial epileptogénica propõe que os astrócitos reativos libertam fatores neurotróficos que conduzem à arborização axonal, à formação de sinapses e à hiperexcitabilidade. A ativação crónica dos astrócitos aumenta a produção de citocinas inflamatórias, potencializa a atividade sináptica excessiva (direta ou indiretamente) e induz a migração para o foco epiléptico, resultando em esclerose. Se estes efeitos são a consequência da MTLE ou a causa desta, continua ainda sem resposta.

### Arborização das fibras nervosas

A arborização é caracterizada por formação de sinapses das células axoniais granulosas do dentado com células da camada granular e da camada molecular interna sobretudo na região CA3 do corno de Ammon. A ocorrência de gliose, a libertação de fatores de crescimento, citocinas, adesões moleculares oriundas da ativação de astrócitos e micróglia são alguns dos mecanismos que se assumem estar na base deste fenómeno. A hipótese da hiperexcitabilidade defende que as células granulosas do dentado se tornam hiperexcitáveis devido à arborização recorrente. Outros investigadores afirmam que o aparecimento de células musgosas serve para reformular circuitos inibitórios que se perderam durante os danos iniciais nos neurónios. O esclarecimento do papel que as fibras musgosas desempenham na epileptogénese é importante para a compreensão desta patologia, podendo conduzir a novas opções de tratamento.

### Alvos potenciais para o controlo de convulsões

Pesquisas intensivas estão em curso na tentativa de obter novos AEDs que visem proteínas modulatórias diferentes na transmissão excitatória. Sabendo que a neuro-

inflamação com libertação de inúmeras citocinas e interleucinas estão na base da epileptogénese, o desenvolvimento de anti-inflamatórios neuronais com propriedades neuroprotetoras poderá atenuar as alterações decorrentes das convulsões, reduzindo assim a doença subjacente.

### **Adenosina – um metabolito retaliatório**

A ADO não é apenas um metabolito do ATP, que reflete o reservatório de energia, mas também do RNA, que reflete as atividades metabólicas da célula. Assim, esta assume um papel central entre a disponibilidade energética e as exigências metabólicas, sendo apelidada de “metabolito retaliatório”. O princípio para conservar energia assenta no aumento da ADO resultante do consumo de ATP, que vai atenuar todas as atividades da célula que consomem energia para preservar as reservas de ATP. No cérebro, as convulsões provocam uma queda rápida de energia, resultando no aumento dos níveis de ADO até cerca de 40 vezes. A ADO vai atuar como um terminador endógeno das convulsões, assim como induzir um ambiente de depressão pós-convulsiva que impede a propagação dos estímulos a partir do foco epiléptico. A supressão das convulsões pela ADO depende da ativação dos recetores inibitórios de adenosina A1 (A1R), acoplados à proteína G. Evidências recentes sugerem que a ADO intervém numa série de funções importantes de regulação independentes dos seus receptores, nomeadamente interações com a bioenergética mitocondrial, interferência com reações enzimáticas e nas funções epigenéticas. Assim sendo, a ADO, assume um papel único enquanto regulador da homeostasia.

### **Vias dependentes do receptor de adenosina**

Um vasto conjunto de mecanismos produzidos pela ADO é mediado pela interacção com recetores purinérgicos específicos, da família P1, que se distinguem da família de recetores purinérgicos sensíveis ao ATP, da família P2. Existem 4 membros da classe P1: A1R, A2AR, A2BR e A3R. Cada recetor apresenta uma farmacologia distinta e tem uma distribuição particular nos tecidos e sub-tipos celulares: Ao nível do cérebro os A1R são expressos principalmente, no sistema límbico, os A2AR nos núcleos da base e os A2BR e A3R principalmente na periferia. Os A1R localizados pré-sinápticamente inibem a libertação sináptica da maioria dos neurotransmissores, com um efeito aparentemente maior na transmissão excitatória. No componente pós-sináptico, os A1R hiperpolarizam as membranas através da abertura dos canais retificadores de potássio, sensíveis ao ATP. Estes dois efeitos combinados da ADO e dos seus agonistas selectivos para os A1R, contribuem para a inibição da excitabilidade da rede sináptica, exercendo um efeito anti-convulsivante marcado. O efeito que resulta da

activação dos A2AR na excitabilidade neuronal é menos evidente, e anatomicamente restrito. Assim, os A1R e A2AR podem cooperar para promover a homeostasia.

### Bioenergética mitocondrial

O mecanismo da ADO na manutenção da homeostasia do neurónio deve-se à sua interação direta com a bioenergética mitocondrial e com o metabolismo energético, através do ciclo Adenosina-AMP. Assim sendo, a reposição da bioenergética mitocondrial pela ADO tem o potencial de oferecer o duplo benefício de melhorar a disfunção metabólica e restaurar a homeostasia neuronal.

### Dieta Cetogénica

Desde 1920, a dieta cetogénica é utilizada para tratar doentes (principalmente crianças) com epilepsia refractária aos AEDs. A sua composição é maioritariamente à base de lípidos com pequenas quantidades de hidratos de carbono e de proteínas. A elevada oxidação dos ácidos gordos gera grandes concentrações de Acetil-CoA e consequente síntese de corpos cetónicos, que passam a ser utilizados como principal fonte de energia para os tecidos. Os corpos cetónicos têm um papel anti-convulsivante importante: reduzem a excitabilidade neuronal ao diminuir a secreção de glutamato; aumentam a transmissão inibitória ao aumentar a produção de GABA; reduzem a expressão do factor neurotrófico BDNF e do seu receptor TrkB (ambos relacionados com a epileptogénese) ao reduzir a glicólise; levam à mobilização de ATP mitocondrial que vai por sua vez activar canais de potássio sensíveis ao ATP ( $K_{ATP}$ ) hiperpolarizando os neurónios pós-sinápticos. A metabolização deste ATP mitocondrial aumenta também os níveis de ADO extracelulares, que por sua vez vai reduzir ainda mais a excitabilidade neuronal ao activar os A1R pré e pós-sinápticos. A ADO vai inibir pré-sinápticamente o influxo de cálcio e vai activar pós-sinápticamente os canais rectificadores de  $K^+$ , potenciando a hiperpolarização induzida pelo ATP nos neurónios pós-sinápticos. Este aumento nos níveis de adenosina extracelular desempenha um papel fundamental no sucesso terapêutico da dieta cetogénica.

### Hipótese da metilação do DNA

Há uma série de doenças neurológicas relacionadas com a epilepsia que são diretamente atribuídas a mutações genéticas primárias, resultando na desregulamentação secundária do epigenoma. O que se observa nos tecidos excisados de pacientes com MTLE são promotores de genes com padrões de hipermetilação do DNA e com expressão diminuída do gene da DNA

metiltransferase. Os mecanismos subjacentes ao aumento da metilação do DNA na epileptogénese não estão bem caracterizados e são alvo de grande especulação. A hipótese epigenética sugere que a mudança gradual no rácio de desmetilação e metilação de novo do DNA, desencadeada por uma lesão precipitante e modificada por fatores intrínsecos e ambientais, conduz ao aumento da metilação, expressão alterada de genes e um fenótipo alterado (ex: convulsão). Enquanto produto obrigatório da transmetilação, a ADO exerce um efeito tónico sobre a via de transmetilação regulando a disponibilidade do substrato e consequentemente a homeostasia do metiloma do DNA. A astrogliose e a sobre-expressão da ADK no hipocampo, ao reduzirem os níveis tónicos de ADO, podem contribuir para a epileptogénese através da manutenção de um estado hipermetilado do DNA do hipocampo.

### **Desequilíbrio da Adenosina cinase no cérebro**

Os astrócitos constituem a via principal de recaptção da ADO, controlando a sua disponibilidade extracelular através do balanceamento da expressão da enzima astrocitária ADK. Esta enzima ao converter a ADO em AMP, promove o seu influxo para o astrócito, através de transportadores equilibrativos. Lesões no cérebro desencadeiam um aumento agudo de ADO e a regulação negativa transitória de ADK. A lesão inicial e o aumento associado de ADO podem induzir a expressão de A2AR nas células da glia e que estão implicados na proliferação e ativação de astrócitos. A astrogliose é um achado típico patológico que tem sido consistentemente associado à sobre-expressão da ADK, responsável pelos níveis reduzidos de ADO.

### **Aumento focal de adenosina**

O aumento sistémico dos níveis de ADO ou dos seus agonistas selectivos não é uma opção terapêutica devido aos seus efeitos secundários generalizados, principalmente cardiovasculares. Do mesmo modo o recurso a inibidores da ADK não é opção devido à toxicidade hepática destes compostos. O aumento focal no hipocampo de ADO pode ultrapassar todos os efeitos secundários decorrentes da sua administração sistémica, repondo a sua homeostasia no foco epiléptico. As pesquisas que visam a obtenção de dispositivos de libertação local de ADO no hipocampo epiléptico estão em franco desenvolvimento.

Os primeiros implantes testados eram feitos com membranas semipermeáveis que libertavam doses controladas de ADO no hipocampo. Posteriormente foram feitos implantes com membrana de seda (menos imunogénica) contendo células-tronco embrionárias, manipuladas para libertar ADO. Estes implantes controlaram por

completo as convulsões nos ratos epiléticos, efeito esse de curta duração, devido às quantidades limitadas de ADO ou ao tempo de vida curto das células nos dispositivos. Novos dispositivos contendo células-tronco, com sequências anti-sense contra o gene *adk*, foram testados. Três semanas depois da sua implantação registou-se uma atenuação marcada da astrogliose, a normalização dos níveis de ADK e a ausência total de crises nos hipocampos dos ratos epiléticos, sugerindo uma regulação persistente dos níveis de ADO. Estes dados são promissores uma vez que só o restabelecimento definitivo dos níveis de ADO no hipocampo por estes dispositivos poderá ser viável terapeuticamente.

## Discussão

Ao longo das últimas décadas, muitos estudos têm tentado identificar as alterações morfológicas e bioquímicas que perpetuam a progressão da epilepsia até um estado refratário, resistente ao controlo por AEDs. A MTLE é a epilepsia farmacorresistente mais frequente, e a maioria dos doentes com MTLE apresenta lesões escleróticas hipocámpais, incluindo astrogliose, morte neuronal e arborização aberrante das fibras musgosas. O esclarecimento destes achados histológicos é um passo importante na melhor compreensão da epileptogénese, e de novas opções de tratamento.

Os AEDs disponíveis visam o tratamento sintomático, não se registando quaisquer modificações nos processos subjacentes da epilepsia. Apesar dos AEDs de terceira geração causarem menos efeitos adversos, não conseguem tratar ainda a grande subpopulação de doentes com epilepsia farmacorresistente, o que implica desenvolver opções terapêuticas inovadoras. A ADO modula a atividade neuronal, sendo considerada um anti-convulsivo endógeno por diminuir a transmissão excitatória. Tendo em conta essa evidência, novas terapias baseadas na ADO estão atualmente a ser desenvolvidas.

O uso da dieta cetogénica tem sido sucesso no controlo de crises epiléticas (sobretudo em crianças) quando os AEDs falham. As alterações metabólicas por ela produzidas ativam mecanismos anticonvulsivos diferentes dos alvos visados pela medicação existente. O aumento dos níveis de ADO é um dos principais mecanismos anticonvulsivos ativados, contribuindo para uma inibição global da transmissão excitatória. Outras alterações metabólicas identificadas, contribuem para o potente efeito anti-convulsivo produzido por esta dieta.

Mudanças na metilação do DNA no hipocampo induzidas pela ADO foram apontadas como um dos mecanismos subjacentes às propriedades anti-epileptogénicas desta

molécula. A redução da metilação do DNA hipermetilado em hipocampus de ratos epiléticos pela ADO constitui um benefício, uma vez que o uso de inibidores da DNA metiltransferase (DNMT) na redução das crises convulsivas, está associado a vários efeitos adversos.

O aumento patológico de ADK, decorrente da astrogliose, reduz os níveis tónicos de ADO, deixando os neurónios mais susceptíveis a danos. Sabendo que a sobre-regulação da ADK ocorre antes da ocorrência de modificações morfológicas significativas no hipocampo, há um período de tempo em que é possível modificar esta sobre-expressão, recorrendo ao uso de inibidores da ADK como agentes terapêuticos.

A administração sistémica de ADO ou dos seus agonistas estáveis para o controlo das crises epiléticas é impraticável devido aos numerosos efeitos adversos, maioritariamente no sistema cardiovascular. Uma nova era de pesquisa de técnicas que aumentem focalmente a ADO está em franca expansão. Dispositivos que libertam ADO, contendo polímeros de libertação controlada ou células histaminais manipuladas geneticamente, controlaram as crises em modelos de rato epilético. Células histaminais contendo sequências anti-sense contra o gene *adk*, incorporadas em implantes de seda, reverteram de forma definitiva a desregulação da ADK, reduziram a astrogliose e cessaram completamente as crises nos ratos epiléticos.

Apesar dos dispositivos de libertação de ADO serem neste momento os mais plausíveis para testes clínicos de segurança e viabilidade, estes sistemas necessitam de ser melhorados para fazerem libertação de ADO durante períodos prolongados. Além disso, ainda é incerto a translação dos estudos feitos em modelos animais para o homem.

## Conclusões

Tem-se verificado um vasto aumento do número de AEDs disponíveis para o tratamento da MTLE, mas a sua eficácia no tratamento e possível cura mantêm-se inalterada. Novas experiências irão permitir esclarecer melhor a forma como interagem os canais iónicos com a sinalização celular, a libertação de neurotransmissores e os fatores inflamatórios em tecidos epiléticos e pré-epiléticos. A compreensão das alterações morfológicas como a arborização das fibras musgosas, gliose e morte dos neurónios pode levar à identificação de moléculas específicas libertadas como resultado de alterações celulares ou como precursoras das mudanças e que poderão ser bons alvos para futuras abordagens terapêuticas.