The Anti-Seizure Effects of Ketone Bodies

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Overview

1. Introduction
   a. Epilepsy
   b. What is the Ketogenic diet?
   c. Ketone bodies
2. Potential mechanisms of the anti-seizure effect
3. Anti-seizure effects of acetoacetate & acetone
4. $\beta$-hydroxybutyrate too!
5. Wrap it up
What do you know about ketone bodies?
Epilepsy

- CNS disorder with episodes of abnormal brain activity that causes seizures or periods of unusual behavior, sensations, and sometimes loss of awareness
- Anyone can develop the disorder
- Symptoms vary widely; seizures can be mild (blank stares for a few seconds) or more severe (twitching of the arms and legs)
  - Temporary confusion
  - Loss of consciousness or awareness
  - Psychic symptoms such as fear, anxiety or deja vu

WHAT IS EPILEPSY?
Epilepsy is a neurological condition characterized by recurrent, unprovoked seizures.

3.4 MILLION
People in the United States have Epilepsy.

1 IN 26
People in the United States will Develop Epilepsy at Some Point in their Lifetime.

150,000
New cases of Epilepsy in the United States each Year.
Different Types of Seizures

- Focal seizures
  - Focal seizures without loss of consciousness — don’t cause a loss of consciousness, may alter emotions or change the way things look, smell, feel, taste or sound, may also result in involuntary jerking of a body part, such as an arm or leg, and spontaneous sensory symptoms such as tingling, dizziness and flashing lights.
  - Focal seizures with impaired awareness — involve a change or loss of consciousness or awareness, you may stare into space and not respond normally to your environment or perform repetitive movements, such as hand rubbing, chewing, swallowing or walking in circles.

- Generalized seizures
  - Absence seizures — often occur in children and are characterized by staring into space or subtle body movements such as eye blinking or lip smacking, may occur in clusters and cause a brief loss of awareness.
  - Tonic seizures — stiffening of your muscles, usually affect muscles in your back, arms and legs and may cause you to fall to the ground.
  - Atonic seizures — loss of muscle control, which may cause you to suddenly collapse or fall down.
  - Clonic seizures — associated with repeated or rhythmic, jerking muscle movements, usually affect the neck, face and arms.
  - Myoclonic seizures — usually appear as sudden brief jerks or twitches of your arms and legs.
  - Tonic-clonic seizures — most dramatic type of epileptic seizure and can cause an abrupt loss of consciousness, body stiffening and shaking, and sometimes loss of bladder control or biting your tongue.
Causes

- **Genetic influence** — some types of epilepsy, which are categorized by the type of seizure you experience or the part of the brain that is affected, run in families. In these cases, it's likely that there's a genetic influence.
- **Head trauma** — head trauma as a result of a car accident or other traumatic injury can cause epilepsy.
- **Brain conditions** — brain conditions that cause damage to the brain, such as brain tumors or strokes, can cause epilepsy — stroke is a leading cause of epilepsy in adults older than age 35.
- **Infectious diseases** — infectious diseases, such as meningitis, AIDS and viral encephalitis, can cause epilepsy.
- **Prenatal injury** — before birth, babies are sensitive to brain damage that could be caused by several factors, such as an infection in the mother, poor nutrition or oxygen deficiencies. This brain damage can result in epilepsy or cerebral palsy.
- **Developmental disorders** — epilepsy can sometimes be associated with developmental disorders, such as autism and neurofibromatosis.
Risk Factors

- **Age** — the onset of epilepsy is most common in children and older adults, but it can occur at any age.
- **Family history** — If you have a family history of epilepsy, you may be at an increased risk of developing a seizure disorder.
- **Head injuries** — head injuries are responsible for some cases of epilepsy.
- **Stroke and other vascular diseases** — stroke and other vascular diseases can lead to brain damage that may trigger epilepsy.
- **Dementia** — Dementia can increase the risk of epilepsy in older adults.
- **Brain infections** — infections such as meningitis, which causes inflammation in your brain or spinal cord, can increase your risk.
- **Seizures in childhood** — high fevers in childhood can sometimes be associated with seizures. Children who have seizures due to high fevers generally won’t develop epilepsy. The risk of epilepsy increases if a child has a long seizure, another nervous system condition or a family history of epilepsy.
What is the Ketogenic diet?

- High fat, low carbohydrate diet, with adequate protein
Answers!

https://answergarden.ch/884872
Ketone Bodies

- Energy-rich molecules produced by liver during fat metabolism
- Transport energy from liver to other tissues
- Small amounts are found in the blood of healthy individuals and high levels can occur in diabetics, alcoholics, and individuals with other disorders/diseases
- Fatty acid production is stimulated by glucagon and inhibited by insulin
- Two metabolisms: ketogenesis and ketolysis
- Ketogenesis is the transformation of fatty acids into Acetoacetate (AcAc) and 3-β-hydroxybutyrate (3HB) and it occurs in the mitochondria of liver cells
- Ketolysis is the conversion of ketone bodies into energy that can be used for many metabolic processes, especially important for the CNS, and it occurs in the mitochondria of many organs other than the liver
- Ketosis is a temporary state of elevated ketone bodies in the blood (ketogenic diet and ketoacidosis)
Three Ketone Bodies

- Acetoacetate (AcAc)
- 3-β-hydroxybutyrate (3HB)
- Acetone
Ketone Bodies -> Neuroprotection + Attenuation of CNS Hyperexcitability -> Anti-Seizure
Let’s Break it Down.
Mechanistic Overview

A. KBs -> ATP -> oxidative injury protection + excessive calcium release prevention
B. KBs -> inhibition of VGLUTs -> less glutamate released during synaptic transmission
C. ATP -> adenosine synthesis -> K+ exits the cell
D. KBs -> reduced inflammation
E. KBs -> hyperacetylation -> endogenous anti-oxidant increase
A. Oxidative Injury + Excessive Calcium Release Prevention

- Reconversion of KBs to Acetyl-CoA
- KBs -> inhibition of ROS production & mPTP pore
KBs -> Acetyl-CoA -> ATP

Reconversion of KBs to Acetyl-CoA

Krebs/Citric Acid Cycle: AC -> ATP

Krebs Cycle
KBs -> inhibition of ROS production

- ROS = Reactive Oxygen Species
- Unstable molecule
- Contains oxygen
- Free radicals
- Easily reacts with other molecules in a cell
- May cause:
  - Damage to DNA, RNA, and proteins
  - Cell death

Common ROSs:

- \( \cdot \cdot \cdot \) = unpaired valence electron

- Oxygen: \( O_2 \)
- Superoxide anion: \( \cdot O_2^- \)
- Hydrogen Peroxide: \( H_2O_2 \)
- Hydroxyl radical: \( \cdot OH \)
- Hydroxyl ion: \( OH^- \)
KBs -> inhibition of mPTP

- Mitochondrial permeability transition pore
- Opens in the inner mitochondrial membrane
- Enables release of Ca++ ions from the mitochondrial matrix into the intracellular space
- Prolonged mPTP opening -> collapse of mitochondrial cell potential -> cell death
B. Lower glutamate release during synaptic transmission

- Inhibition of VGLUT (vesicular glutamate transporters)
- \(\rightarrow\) decrease of glutamate loaded into synaptic vesicles
- \(\rightarrow\) decrease of glutamate quanta released during synaptic transmission
- \(\rightarrow\) decrease in hyperexcitability of CNS
C. Prevention of hyperexcitability via K+ ions

- KBs \( \rightarrow \) increase in intracellular ATP
- \( \rightarrow \) release of ATP through Pannexin channels
- \( \rightarrow \) adenosine synthesis via ectonucleotidases
- \( \rightarrow \) ADO binding to adenosine type 1 receptors
  - Coupled to indirect opening of K-ATP channels
- \( \rightarrow \) K+ ions leave the cell
- \( \rightarrow \) hyperpolarization (inhibitory)
  - Prevents hyperexcitation
D. Reduced inflammation

- KBs -> activation of HCA2 receptors on macrophage
- -> inhibition of assembly of NLRP3 inflammasomes
- -> attenuation of inflammatory mediators produced by infiltrating macrophages
E. Increase in endogenous anti-oxidants

- KB -> direct inhibition of histone deacetylases (HDACs)
- KBs -> increase in AC
  - AC = substrate for histone acetyltransferases (HATs)
- -> histone & non-histone hyperacetylation
  - Acetylation of histones -> increased gene expression
- -> increase in endogenous anti-oxidants
Mechanistic Recap

- Ketone bodies reduce hyperexcitability (as shown in figures A, B, C) through modification of the ion channels.
- Ketone bodies also increase antioxidants and decrease oxidative stress (figures A and E) through histone modification and inhibition of ROS production:
  - Oxidative stress plays a key role in pathways leading to neurodegeneration, which can lead to epilepsy
  - Antioxidants decrease oxidative stress
- Ketone bodies reduce inflammation (figure D) through modifying receptors on macrophages:
  - Steroids and other anti-inflammatory treatments were shown to decrease seizures/seizure severity
  - Febrile seizures are often caused by pro-inflammatory agents
Anti-seizure effects of Acetoacetate & acetone
Anti-seizure activity of Acetoacetate & Acetone

**Past in-vivo studies:**

Keith (1933, 1935):
- First documented testing of ketone bodies.
- Found that acetone and acetoacetate, **but not b-hydroxybutyrate**, protected rabbits against seizures induced by thujone, a constituent of the volatile oil of absinth (wormwood oil) and a known antagonist of gamma-aminobutyric acid, type A (GABAA) receptors.

(Rho et al., 2002):
- Acetone and acetoacetate, **but not b-hydroxybutyrate**, protected against sound-induced seizures in the Frings audiogenic seizure-susceptible mouse model.
- **Frings audiogenic seizure (AGS)-susceptible mice** are genetically **susceptible** to sound-induced reflex seizures. Their seizure phenotype is characterized by wild running, loss of righting reflex, tonic flexion, and tonic extension in response to high-intensity sound stimulation.
- Results: After a 10 mmol/kg i.p. injection of ACA, eight of eight mice were protected against sound-induced tonic extension. Acetone also was protective in eight of eight mice administered 10 mmol/kg acetone.
Past in-vivo studies:

(Likhodii et al., 2003):
- Acetone was found to increase the seizure threshold of rats in multiple models of seizure induction.
- Effects of acetone parallel the effects of the ketogenic diet.
- Elevation of brain acetone therefore may account for the efficacy of the KD in intractable epilepsy.

(Juge et al., 2010):
- The ability of acetoacetate to inhibit VGLUT’s & transmitter release suggests that this may account for its anticonvulsant activity.
- Researchers examined the effect of acetoacetate on 4-aminopyridine (4-AP)-evoked glutamate secretion and seizures in rats.
- 4AP is a K+ channel blocker with convulsant activity and was introduced through a micropipette directly into the rat brain.
- Administration of 4AP evoked seizures and lead to the concomitant secretion of glutamate.
- Acetoacetate reduced the intensity of 4AP evoked seizures & decreased glutamate release in a dose dependent manner. After removing AcAc from the solution, the intensity of 4AP evoked seizures increased!
Past in-vivo studies:

(Kadowaki et al., 2017):

- Investigated effects of acetoacetate on voltage dependent Ca2+ channels (VDCCs) in pyramidal cells of the hippocampus.
- The effects of acetoacetate and its analogs on VDCCs & EPSCs were evaluated using patch-clamp recording from CA1 pyramidal cells of mouse hippocampal slices.
- Results: Acetoacetate inhibited VDCCs in pyramidal cells of hippocampal slices, and reduced EPSCs in slices exhibiting epileptiform activity.

(D'Agostino et al., 2013) & (Viggiano et al., 2015, 2016):

- Recently, ketone esters have been investigated as a potential “pro-drug” capable of sustained elevation of ketone bodies.
- The R,S-1,3-butanediol acetoacetate diester (BD-AcAc2) resulted in elevated blood acetone, acetoacetate and b-hydroxybutyrate levels in rats and increased the latency to hyperbaric oxygen-induced seizures.
- Single or repeated dosing of BD-AcAc2 has been further demonstrated to increase the threshold of pentylentetrazole-induced seizures in rats.
Anticonvulsant properties of acetone, a brain ketone elevated by the ketogenic diet. (Likhodii et al., 2003)

- It is known that the KD is effective against tonic-clonic seizures, absence seizures, complex partial seizures.
- Study initiated bc it was unclear whether acetone can reproduce the broad spectrum of anticonvulsant properties like the KD.
- Study involved animal seizure models. Wistar rats, matched for age, were used to measure acetone concentrations in plasma and cerebrospinal fluid (CSF) after injections of acetone (n=30).
- Albino male rats were injected with acetone intraperitoneally.
- Different solutions of acetone in physiological saline were prepared to doses of 2, 4, 18, 16 or 32 mmol/kg.
- Dose response effects were measured in four different models:
  1) Maximal Electroshock Seizure (MES) Test - measures human tonic-clonic seizures.
  2) Subcutaneous Pentylenetetrazole Seizure (PTZ) Test - models human typical absence seizures.
  3) The Amygdala Kindling Test - models human complex partial seizures
  4) AY-9944 Seizure Test - models chronic atypical absence seizures.
Anticonvulsant Potency of Acetone

Results

The Figure, A-D, presents dose-response curves for seizure suppression in the four different animal seizure models.

Anticonvulsant potencies of acetone in the different seizure models were:

\[ \text{AY-9944} > \text{MES} > \text{PTZ} > \text{(kindling generalized)} > \text{(kindling focal)} \]

*Acetone completely suppressed generalized seizures in all four models!
Conclusions:

- Acetone suppressed acute and chronic experimental seizures of different types, indicating that it does have a broad spectrum of anticonvulsant action.

- These findings suggest that acetone would be capable clinically of suppressing tonic-clonic, typical absence, complex partial, and chronic atypical absence seizures. Perhaps most important, acetone was effective against kindled focal seizures, which, like complex partial seizures, are notoriously drug resistant.

- These results of the experiment are consistent with the hypothesis that acetone is a causal factor of the KD's anticonvulsant effects.
So Does β-hydroxybutyrate Really Have No Effect?

- While definitive evidence in this regard is not yet forthcoming recent clinical data indicate that ketone bodies (β-hydroxybutyrate) may yet be relevant to an anti-seizure effect.

- Considering all of the data and information that was presented before, there are now three recent studies that show that β-hydroxybutyrate might have a role in anti-seizure efficacy.
Minlebaev and Khazipov (2011)

- Methods Summary/Subjects: Performed a series of electrode experiments on postnatal day 5-9 non-anesthetized rat pups in vivo (took place in a live organism)
- Inhibit ketogenesis on these pups for one hour by administering insulin
  - No effect from a single flurothyl induced electrographic seizures
    - When introduced to β-hydroxybutyrate, the seizure’s effect was treated
    - How? Possibility due to a reduction of hyperexcitability and its role in raising the seizure threshold in newborn children
- Importance: Immature brain is more prone to seizure effects than the adult brain as the number of seizures occurs more often during the first months of a newborn until after a year
  - Natural ketogenesis controls brain excitability and acts as an anticonvulsant mechanism in infants
Minlebaev and Khazipov (2011)

- Graphed the onset of these electrographic seizures through the emergence of population spikes and high frequency oscillations
- Also found that GABA exerted anti-convulsive effects independently of the ketogenic state
Yum et al., (2015)

- Yum et al., investigated the effects of β-hydroxybutyrate in betamethasone NMDA model of infantile spasms
  - Did an animal model of IS (infantile spasms)
- This experiment was done to test whether β-hydroxybutyrate would affect positively or negatively towards infantile spasms since it is known to have strong antiepileptic activity effects but also have severe side effects
- Ketone Hypothesis: Mechanism where ketone bodies act as direct anticonvulsants
  - Level of blood ketone correlates with anti-seizure effect with ketogenic diet
- Methods: Pregnant rats were given betamethasone
  - Performed NMDA-triggered spasms on postnatal day P12, P13, and P15
Yum et al. (2015) Results

- When they injected the β-hydroxybutyrate once to the animal models, nothing occurred as the spams remained how it was
Yum et al. (2015) Repeated β-hydroxybutyrate

- Over three days of repeated injection of β-hydroxybutyrate delayed the onset of the seizures as well as decreasing the number of spasms occurring
  - Also, the anti-seizure effects was reinforced using repeated NMDA-triggered spasms
- They also saw a reduction in GABA, glutamate (excitatory neurotransmitter), total creatine, and macromolecule plus lipids due to randomized β-hydroxybutyrate treatment (p<0.05)
- β-hydroxybutyrate induced anxiety and memory responses in the rats with NMDA induced spasms
Significance of Yum et al. (2015)

- Saw that high concentration of ketone bodies such as β-hydroxybutyrate is significantly correlated with seizure control (for this experiment at least)
- With this data, it seems that a prolonged exposure/administration of β-hydroxybutyrate is needed to reduce the number of spasms as well as the latency of onset of seizures
- Possibly some increase fear responses and improved memory function
Kim et al., (2015)

- Persistent long term infusion of β-hydroxybutyrate over a two week period
- Model: Generic model of epilepsy in vivo of Kcna1-null mutant mouse
  - Contain essential features of human temporal lobe epilepsy
  - Method: They cut the back of the mouse’s neck and used a osmotic minipump with 10M BHB to allows this chronic infusion of BHB
- Looking closely into mitochondrial permeability transition because this complex plays an important role in regulating cell death pathways and it regulates ATP and ROS levels like ketone bodies do
Kim et al., (2015) Results

- Results:
  - $\beta$-hydroxybutyrate reduced spontaneous recurrent seizures similar to a ketogenic diet
  - Showed evidence that inhibition of mPT can result in a consistent antiseizure effect (could provide seizure control) through the modulation of mPT complex
  - Looked heavily into CypD and its implication towards the mPT complex
    - Plays a key role as a previous studies saw that a knockdown of this elevates threshold for mPT opening through modulation of intracellular calcium homeostasis

- Limitation:
  - The researchers only did this in a single animal model of epilepsy
How come these positive results are occurring now?

- This can be due to previous experiments only administering limited single dosages in vivo.

- Also, the experiments before only used certain models of acute seizure models, which is very specific in terms of severe seizure scenarios rather than looking towards models dictating spontaneous recurrent seizures which are more broad and have variety to work under.
So how does this concern us?

- The ratio of acetoacetate to β-hydroxybutyrate is approximately 1:3 in plasma of fasting or ketogenic diet treated humans and 1:2 in cerebrospinal fluid of ketogenic treated humans.
- With ketogenic diet, brain β-hydroxybutyrate dramatically increases as β-hydroxybutyrate makes up roughly 26% of dietary ketosis people’s plasma.
- Evidence of inhibitory properties of ketone bodies in vitro.
  - Seems that β-hydroxybutyrate is very situational as in the paper Chang et al., 2016 saw that high concentration of β-hydroxybutyrate had no effect towards seizure-like events.
  - However, other papers saw different results regarding seizure-like events and β-hydroxybutyrate.
- More studies → maybe use β-hydroxybutyrate as a way to elevate blood ketone levels in humans safely (off-the-counter drug).
Fun Fact

- Researchers at Georgia State University found that \( \beta \)-hydroxybutyrate is found to have anti-aging effects on vascular systems (2018)

Article Link:
Main Takeaway

- Ketone bodies have an anti-seizure effect; however, more research needs to be done in a clinical setting.
- While it was originally found that 3HB had no effect, upon further examination, it was determined that it does in fact have an anti-seizure effect along with the other two ketone bodies.
What do you know about ketone bodies pt. 2
More Answers!

https://answergarden.ch/884873
Resources for Introduction

https://onlinelibrary.wiley.com/doi/full/10.1002/%28SICI%291520-7560%28199911/12%2915%3A6%3C412%3AID-DMRR72%3E3.0.CO%3B2-8

https://www.mayoclinic.org/diseases-conditions/epilepsy/symptoms-causes/syc-20350093

Mechanistic recap:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4428026/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3378051/
Resources for Mechanism Section

https://www.sciencedirect.com/topics/neuroscience/glycolysis

http://academic.brooklyn.cuny.edu/biology/bio4fv/page/coup_ox.htm


http://www.srmuniv.ac.in/sites/default/files/files/KETONEBODYSMETABOLISM.pdf

http://umich.edu/~chemh215/W02PRESENT/SSG3/ssg3/


Resources for Acetoacetate and Acetone Section

https://jamanetwork.com/journals/archneurpsyc/article-abstract/645623

https://jamanetwork.com/journals/archneurpsyc/article-abstract/646623


Resources for Methods


Study 1 Link
https://vpn.ucsd.edu/+CSCO+0075676763663A2F2F6E702E7279662D7071612E70627A++/S092012111100714/Main.pdf?_tid=695fa4ff-dfc0-44c6-a186-7cdfe3b9b61f&acdnat=1551236241_d2e5acc3edbc891f9636ae6f8bf0bbb
More Resources for Methods

Study 2:
https://vpn.ucsd.edu/+CSCO+0075676763663A2F2F6E702E7279662D7071612E70627A++/S0920121115300358/1-s2.0-S0920121115300358-main.pdf?_tid=9328255f-3dcd-42ba-b01b-c4c8951a48de&acdnat=1551236283_0521ecbdca0a4e26020d934b1514f991
Resources for β-hydroxybutyrate