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SYSTEMATIC REVIEW

Branched-Chain Amino Acids and Seizures: A Systematic Review of the Literature

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Abstract

Background Up to 40% of patients with epilepsy experience seizures despite treatment with antiepileptic drugs; however, branched-chain amino acid (BCAA) supplementation has shown promise in treating refractory epilepsy.

Objectives The purpose of this systematic review was to evaluate all published studies that investigated the effects of BCAAs on seizures, emphasizing therapeutic efficacy and possible underlying mechanisms.

Methods On 31 January, 2017, the following databases were searched for relevant studies: MEDLINE (OvidSP), EMBASE (OvidSP), Scopus (Elsevier), the Cochrane Library, and the unindexed material in PubMed (National Library of Medicine/National Institutes of Health). The searches were repeated in all databases on 18 February, 2019. We only included full-length preclinical and clinical studies that were published in the English language that examined the effects of BCAA administration on seizures.

Results Eleven of 2045 studies met our inclusion criteria: ten studies were conducted in animal models and one study in human subjects. Seven seizure models were investigated: the strychnine (one study), pentylenetetrazole (two studies), flurothyl (one study), picrotoxin (two studies), genetic absence epilepsy in rats (one study), kainic acid (two studies), and methionine sulfoximine (one study) paradigms. Three studies investigated the effect of a BCAA mixture whereas the other studies explored the effects of individual BCAAs on seizures. In most animal models and in humans, BCAAs had potent anti-seizure effects. However, in the methionine sulfoximine model, long-term BCAA supplementation worsened seizure propagation and caused neuron loss, and in the genetic absence epilepsy in rats model, BCAAs exhibited pro-seizure effects.

Conclusions The contradictory effects of BCAAs on seizure activity likely reflect differences in the complex mechanisms that underlie seizure disorders. Some of these mechanisms are likely mediated by BCAA's effects on glucose, glutamate, glutamine, and ammonia metabolism, activation of the mechanistic target of rapamycin signaling pathway, and their effects on aromatic amino acid transport and neurotransmitter synthesis. We propose that a better understanding of mechanisms by which BCAAs affect seizures and neuronal viability is needed to advance the field of BCAA supplementation in epilepsy.

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Key Points

Recent studies have shown that the branched-chain amino acids valine, leucine, and isoleucine can modulate seizure activity in both human epilepsy and animal models of epilepsy.

The majority of studies in humans and animal models of epilepsy demonstrated potent anticonvulsant effects of branched-chain amino acids; two animal studies however suggested possible pro-convulsant effects.

The complex effects of branched-chain amino acids on seizures are likely mediated by their modulation of glucose, glutamate, glutamine, and ammonia metabolism, activation of the mechanistic target of rapamycin signaling pathway, and their effects on aromatic amino acid transport and neurotransmitter synthesis.

1 Introduction

Epilepsy is a heterogeneous group of chronic neurological disorders characterized by recurrent spontaneous seizures [1]. Seizures are typically unpredictable and occur without warning, and are often associated with significant morbidity including memory and cognitive impairment. Epilepsy affects approximately 1% of the population [2] and carries a significant societal burden, with annual costs in the USA as high as US$20 billion [3]. Current epileptic therapies fail to control seizures in up to 40% of patients; therefore, there is a great need for the discovery of new methods to treat seizures [4, 5]. In most cases, the cause of epilepsy is unknown, and the pathophysiology of how epilepsy develops after a brain insult (i.e., epileptogenesis) is poorly understood.

Although the precise mechanisms that underlie seizures have not yet been fully elucidated, it is generally thought that seizures reflect either a global or focal perturbation in the balance of excitatory (e.g., glutamatergic, acetyl cholinergic) and inhibitory (e.g., GABAergic) neurotransmission [1, 6–8]. As the primary excitatory neurotransmitter in the brain, glutamate plays an important role in neuronal signaling. Sustained high levels of extracellular glutamate in the brain lead to excessive stimulation of neuronal glutamate receptors and subsequent abnormal neurotransmission, increased excitability, seizures, and neuronal loss [9]. It has been postulated that blood-derived branched-chain amino acids (BCAAs) valine, leucine, and isoleucine account for 20% of the de novo synthesis of brain glutamate [10]. The synthesis occurs via transfer of the BCAAs' amino group to α-ketoglutarate in the mitochondria of astrocytes, in a reaction catalyzed by branched-chain aminotransferase. Branched-chain amino acids are abundant in the blood, particularly after a meal, and readily enter the brain [11] via large neutral amino acid transporter 1 (LAT1) [12] and the solute carrier family 6 member 15 (SLC6A5) [13], thus providing an important mechanistic link between peripheral amino acid metabolism and brain glutamate homeostasis.

Moreover, studies have suggested that BCAAs can indirectly modulate cerebral metabolism by perturbing the synthesis of the biogenic amines dopamine (DA), serotonin (5-HT), and norepinephrine (NE) [11]. These neurotransmitters have numerous important effects in the brain, and their dysregulation has been implicated in several disease states such as depression, Parkinson's disease, and epilepsy [14–16]. The biogenic amine neurotransmitters are synthesized from aromatic amino acids (ArAAs) [17], which are known to compete with BCAAs for transport across the blood–brain barrier (BBB) [18, 19]. Thus, changes in peripheral BCAA concentrations can alter the synthesis of DA, 5-HT, and NE in the brain by affecting the transport kinetics of ArAAs across the BBB [20].

In recent years, increasing evidence suggests that the BCAAs serve critical functions in the brain (Fig. 1), including the facilitation of cognition and behavior, neurotransmitter synthesis, intracellular signaling, immune modulation, and mitochondrial health [21–25]. However, there are conflicting data on the role of BCAAs in the pathophysiology of brain disorders such as amyotrophic lateral sclerosis, maple syrup urine disease, traumatic brain injury, malignant brain tumors, and epilepsy [26–31].

Concomitant measurements of electroencephalography (EEG) and intracerebral microdialysis samples in humans with medically refractory epilepsy have demonstrated that during epileptic seizures, both BCAAs and glutamate are elevated in the extracellular compartment in areas of the brain involved by the seizures, suggesting that BCAAs might play an important physiological role in seizures [32]. Moreover, animal studies have observed that a deficiency in BCAAs results in increased susceptibility to seizures [33]. To date, few studies have examined the effects of BCAA administration on seizures in several epilepsy models and in humans, with varying results. The purpose of this systematic review was to evaluate all preclinical and clinical studies that investigated the effects of BCAA administration on seizures. In exploring the effects of BCAAs on seizures in various types of epilepsies, some themes have emerged that provide insights into the mechanisms by which BCAAs might impact seizures. A better understanding of these mechanisms might facilitate the development of novel treatments for epilepsy.

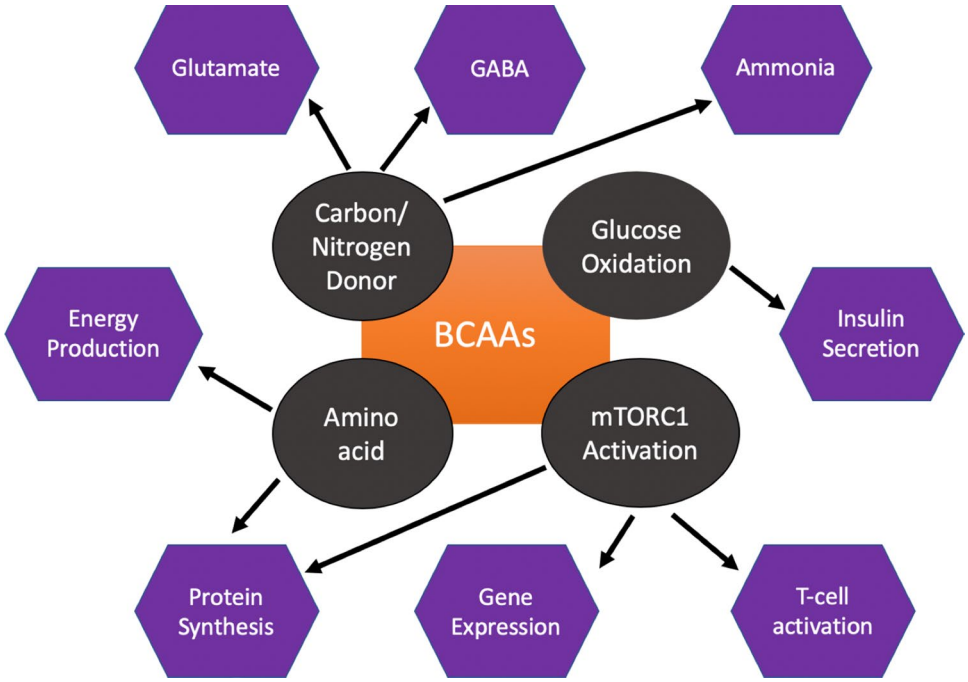

Fig. 1 Roles of branched-chain amino acids (BCAAs) in the body. Branched-chain amino acids are important for several biological processes such as the metabolism glutamate, gamma-aminobutyric acid (GABA), and ammonia; insulin secretion; mammalian target of rapamycin complex 1 (mTORC1) activation, protein synthesis, and energy production

2 Methods

2.1 Search Methodology

On 31 January, 2017, a senior medical librarian performed a comprehensive search of multiple databases: Ovid MEDLINE (1946-January week 4 2017), National Library of Medicine PubMed for unindexed material, Ovid EMBASE (1974–2017 January 31), Scopus, and Cochrane Library. The searches were repeated in all databases on 18 February, 2019 to update the results.

To formulate the search, we began with the Yale MeSH Analyzer (http://mesh.med.yale.edu/) using key articles to produce relevant controlled vocabulary and keyword terms. We also read abstracts and the full text of key articles to generate concepts and synonyms. The key articles were also used for validating the success of the searches. Results in each database were limited to articles written in English. The search strategy is detailed in the Electronic Supplementary Material.

The original search retrieved a total of 3091 references, which were pooled in EndNote and de-duplicated to 1810. The references were uploaded to Covidence for screening. Two separate screeners (SEG and EC) evaluated the titles, abstracts, and full text of the eligible articles. References of the articles meeting the inclusion criteria were reviewed to ensure comprehensiveness. The updated search retrieved 503 references. Duplicate citations were removed, which resulted in an additional 273 new articles for review. Of this set, 38 citations had already been present in the original set of references from the 2017 search and were removed. In total, 2045 unique articles were reviewed.

2.2 Inclusion Criteria

This systematic review only included full-length preclinical and clinical studies that were published in the English language and that examined the effects of BCAA administration on seizures.

2.3 Study Selection

Two reviewers (SEG and EC) independently evaluated the titles and abstracts of the retrieved articles. Abstracts that did not include sufficient information to determine eligibility for inclusion were retrieved for full-text evaluation. The two reviewers independently evaluated all full-text articles and determined eligibility, and disagreements were resolved by consensus-based discussion between two reviewers.

2.4 Data Extraction and Analysis

The same two reviewers independently conducted study evaluation and data extraction. The data extracted included the species, sample size (total and in each group), seizure type and model (for animal studies), results, and conclusions. A qualitative summary composed of these descriptive properties was created for each included study.

3 Results

A total of 3594 articles were retrieved from the systematic literature search. After removing the duplicates, 2045 articles were then screened (Fig. 2). Of these articles, 2034 were excluded. Of the 11 included articles, the effects of seizures were tested in one clinical study in children with refractory epilepsy, as well as in seven animal models of epilepsy: strychnine (one study), pentylenetetrazole (PTZ; two studies), fluorothyl (one study), picrotoxin (two studies), Genetic Absence in Epilepsy in Rats from Strasbourg (GAERS; one study), kainic acid (KA; two studies) and methionine sulfoximine (MSO; one study) (Table 1). In three of the aforementioned studies, a BCAA mixture was administered; in the other studies, one or more BCAAs were individually administered (Fig. 3). Branched-chain amino acids were found to have anticonvulsant effects in the above-mentioned clinical study, and in five animal models of epilepsy. The GAERS model exhibited minor pro-seizure effects of BCAAs. The MSO model demonstrated reduced seizure propagation with short-term BCAA supplementation, whereas long-term supplementation worsened seizure propagation and caused neuron loss.

3.1 Effects of Branched-Chain Amino Acids (BCAAs) [Administered as a Mixture] on Seizures in Laboratory Animals

3.1.1 Methionine Sulfoximine Model

Methionine sulfoximine has been shown to suppress the activity of glutamine synthetase (GS) locally in the brain [34]. In the MSO model of mesial temporal lobe epilepsy (MTLE), GS is inhibited in the right hippocampal formation in rats via a long-term intra-hippocampal infusion of MSO [34]. We recently studied the effects of BCAA supplementation on seizures in 16 MSO-treated rats [35]. The

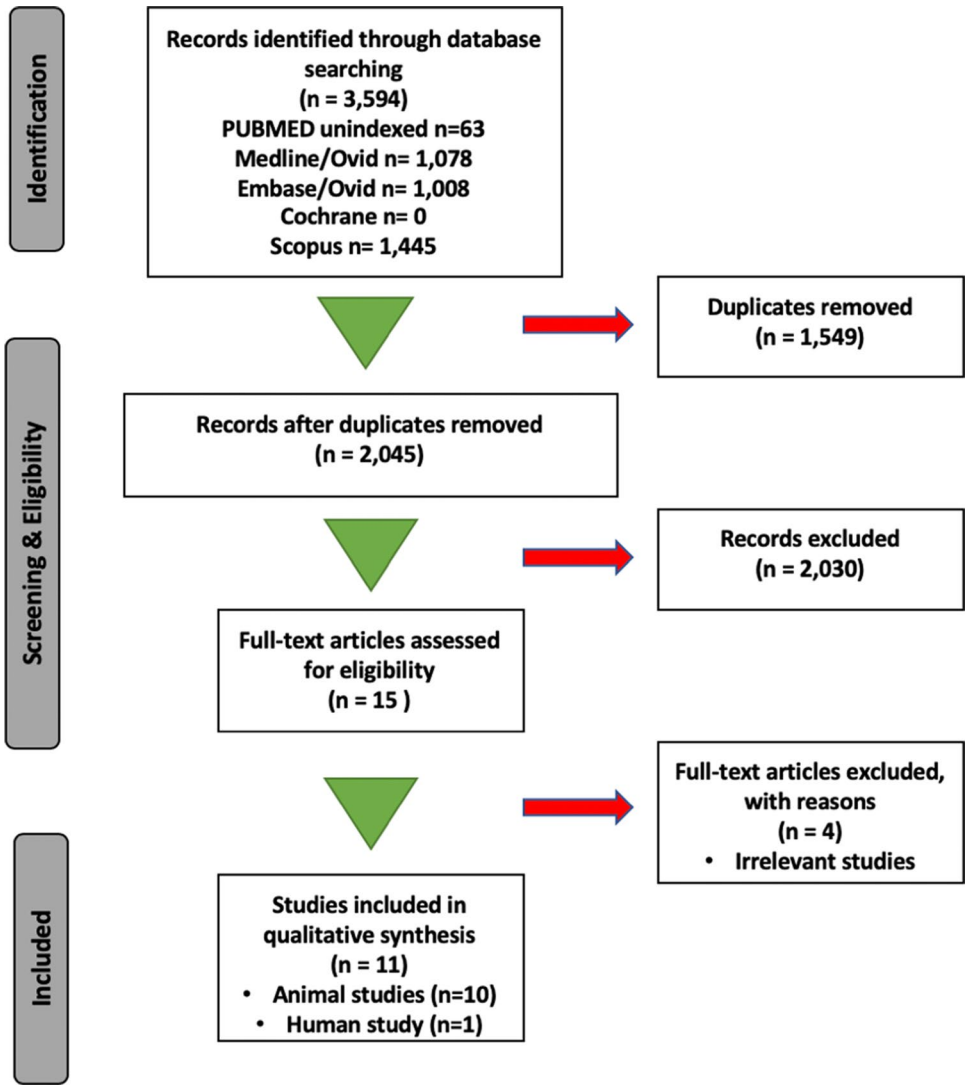

Fig. 2 Study flow chart. We identified 2045 studies by a systematic review, of which 11 met our inclusion criteria. In total, one human study and seven animal models of epilepsy were extracted in which the effects of branched-chain amino acid administration on seizures were investigated

Table 1 Characteristics of studies included in this systematic review

Author, year (epilepsy model)	Species	Sample size	BCAA	Experimental paradigm	Results	Conclusions
Preclinical studies						
Jeske, 1974 [44] (strychnine)	Mouse	5–10 per group	Leucine	Leucine was administered either 10 min (25 µg/kg) or 60 min (100 mg/kg) prior to SC strychnine administration (25 µg/kg)	All seizures were abolished when leucine was administered before strychnine injection	Leucine had anticonvulsant effects in a strychnine-induced model of epilepsy
Pinto, 1988 [47] (PTZ and fluorothyl)	Mouse	Average 14 per group	Valine	1000 mg/kg aspartame ± valine (1000 mg/kg) was administered orally 1 h prior to PTZ (50–75 mg/kg) or fluorothyl (10%)	Valine alone was ineffective in both models, but blocked the pro-convulsant effects of aspartame	Pro-convulsant action of aspartame was blocked by valine when given concomitantly
Skeie, 1992 [38] (picrotoxin)	Rat	10 in the experimental and control groups, respectively	4% combination of leucine, isoleucine, and valine	4% BCAAs were administered IP 2 h prior to seizure induction with picrotoxin IP (10 mg/kg)	There was an increased latency time to seizure onset in the experimental group	Injection of a 4% BCAA solution increased the seizure threshold to picrotoxin
Skeie, 1994 [39] (picrotoxin)	Rat	10 per group	Leucine, isoleucine, valine, or a mixed AA solution	Valine, leucine, or isoleucine (300 mg/kg IP) was administered 120 min prior to picrotoxin injection	The latency time to seizure onset was increased in animals pretreated with BCAAs	Valine, leucine, and isoleucine increased the seizure threshold to picrotoxin, whereas a balanced AA solution had no effect
Dufour, 1999 [46] (PTZ)	Rat	10 in the experimental and control groups, respectively	Leucine, isoleucine, valine, or a mixed AA solution	Leucine, isoleucine, valine, a mixture of AAs, or saline (control) was administered in rats 2 h prior to the administration of PTZ	The latency to SWD and to tonic-clonic seizures was increased in the leucine and isoleucine groups	Leucine and isoleucine, but not valine, increased the latency to absence-like and tonic-clonic seizures, but had no effect on the duration of tonic-clonic seizures
Dufour, 2001 [53, 54] (GAERS)	Rat	10 per treatment group	Leucine, isoleucine, or valine	Leucine, isoleucine, valine, α-KIC, a balanced AA solution (300 mg/kg IP), or saline was administered in GAERS rats	Leucine, isoleucine, valine, and α-KIC increased the number of SWD per 20 min. The duration of SWD was increased only in the valine and isoleucine group	BCAAs and α-KIC, when injected IP, increased the number of SWD in GAERS and a minor effect on their duration
Hartman, 2015 [57] (KA)	Mouse	8–36 per treatment group	L-Leucine or D-leucine	A single IP injection of L- or D-leucine (3 mg/kg or 300 mg/kg) was administered 3 h before or 15–20 min after KA injection	D- or L-leucine suppressed seizure activity when administered before seizure induction. However, only D-leucine terminated seizures when administered after seizure onset	L- and D-leucine pretreatment had potent anticonvulsant effects, but only D-leucine was effective in terminating seizures when administered after seizure onset

Table 1 (continued)

Author, year (epilepsy model)	Species	Sample size	BCAA	Experimental paradigm	Results	Conclusions
Holden, 2017 [58] (KA)	Mouse	13	D-Leucine	D-Leucine (1.5% w/v) was administered in the drinking water in animals with KA-induced seizures	D-Leucine treatment decreased the number of days with seizures in the dark cycle and the seizure frequency in the light cycle	D-Leucine is protective against seizures in a light-dependent manner
Gruenbaum, 2019 [35] (MSO)	Rat	8 in each (experimental and control) group	4% mixture of leucine, isoleucine, and valine	A 4% BCAA mixture in water was administered in drinking water ad libitum to MSO infused animals for 21 days	In BCAA-treated rats, there was an increase and decrease in relative percent of convulsive seizures observed in the first and third week, respectively	Long-term BCAA supplementation facilitated the spread of seizures from the hippocampus to motor cortex, which was associated with an increase in seizure-associated neuron loss
Human studies						
Evangeliou, 2009 [60]	Human	17	A mixture of leucine, isoleucine, and valine	Children (aged 2–7 years) with refractory epilepsy were administered an oral mixture of BCAAs daily for 6–12 months along with a KD (started 6–24 months prior)	The addition of BCAAs to the ketogenic diet significantly reduced seizure activity compared with the KD alone	BCAAs may increase the anticonvulsant effectiveness of the KD

AA amino acid, *α-KIC* α-ketoisocaproate, *BCAA* branched-chain amino acid, *GAERS* genetic absence epilepsy rat from Strasbourg, *h* hours, *IP* intra-peritoneally, *KA* kainic acid, *KD* ketogenic diet, *min* minutes, *MSO* methionine sulfoximine, *PTZ* pentylenetetrazole, *SC* subcutaneous, *SWD* spike-wave discharge

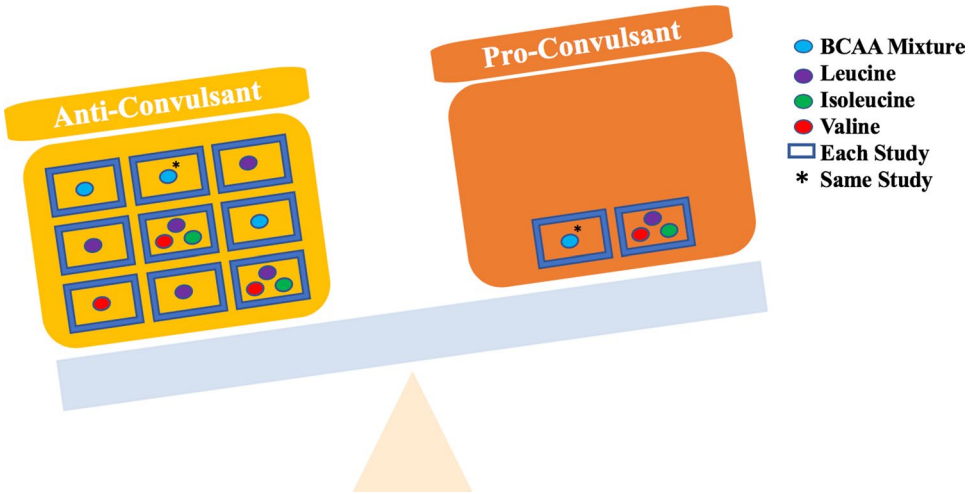

Fig. 3 Overview of studies that investigated the effects of branched-chain amino acids (BCAAs) on seizure activity. From a total of 11 studies, two studies demonstrated that BCAAs exhibited pro-convulsant effects, whereas the other nine studies demonstrated anticonvulsant effects. Three studies used a BCAA mixture in the experimental group, and the remaining studies investigated the effects of leucine, valine, and isoleucine when administered individually

MSO-treated rats were randomized to receive either unrestricted access to water or a 4% BCAA solution in water, and seizure activity was monitored for a period of 21 days. Although BCAA ingestion was ineffective in reducing the frequency of convulsive and non-convulsive seizures, short-term BCAA ingestion reduced seizure propagation (reflected by a reduction in the relative percent of convulsive seizures), while long-term oral supplementation with BCAAs worsens seizure propagation (reflected by an increase in the relative percent of convulsive seizures) and seizure-associated neuron loss. The study suggests that the effect of BCAAs on seizure propagation in MTLE might be dependent on the duration of exposure.

3.1.2 Picrotoxin Model

The chemo-convulsant picrotoxin antagonizes the GABA-benzodiazepine receptor complex and is a noncompetitive channel blocker for $GABA_A$ Cl^- channels [36]. The resulting reduction in Cl^- permeability inhibits the target neuron. Picrotoxin also antagonizes $GABA_C$ receptors allosterically [37]. After an intraperitoneal (IP) injection of picrotoxin, mice exhibit tonic seizures that follow a brief latent period, which facilitates the study of novel treatments on the seizure threshold [38].

We identified two studies that investigated the effect of BCAA administration prior to picrotoxin-induced seizures. In the first study [38], 20 male Wistar rats were randomized to receive either an IP injection with a 4% BCAA solution or placebo (8 mL/kg), and receive an IP injection of picrotoxin (10 mg/kg) 120 min later. Ten rats were injected with the 4% BCAA solution, which contained 1.24 g valine, 1.38 g leucine, and 1.38 g isoleucine for every 100 mL. The authors reported a significant increase in the latency time between the picrotoxin injection and seizure onset in the rats pretreated with BCAAs. The mean latency time was 11.2 min for the BCAA-treated group and 8.3 min for the placebo-treated group ($p < 0.03$). Because the seizure threshold to picrotoxin was increased by BCAAs, the authors proposed that effect of BCAAs might be due to its interactions with the GABA-benzodiazepine receptor complex. A second study further examined this relationship [39], and investigated the effect of individual BCAAs on the seizure threshold to picrotoxin (described below).

3.2 Effects of BCAAs (Administered Individually) on Seizures in Laboratory Animals

3.2.1 Strychnine Model

Intraperitoneal or subcutaneous injection of strychnine in rodents results in acute generalized tonic-clonic seizures [40, 41]. Strychnine acts as a selective competitive antagonist of chloride channel-associated postsynaptic glycine receptors, thereby blocking the inhibitory action of glycine [42]. The convulsions generated with this model are refractory to most standard antiepileptic drugs [43] and exhibit tonic or tonic-clonic seizure patterns. The model provides a reliable method to assess the effects of novel antiepileptic drugs by quantifying the number of spontaneous seizures before and after treatment.

We identified one study in which the anticonvulsive effects of amino acid derivatives of 4-aminoantipyrine on strychnine-induced convulsions were investigated [44]. Male Swiss mice (weight 18–20 g, $n = 5$–10) received subcutaneous injections of strychnine (25 µg/20 g) to induce seizures. Various amino acid derivatives were injected subcutaneously 10, 30, and 60 min prior to seizure induction to observe for potential anticonvulsive effects. The authors demonstrated that subcutaneous injections of L-leucine (100 mg/kg) and the α-methyl ester of 4-gamma-glutamyl-antipyrine (100 mg/kg) 10 and 60 min prior to seizure induction

completely abolished convulsions, whereas DL-valine and DL-leucine failed to demonstrate anticonvulsive effects.

3.2.2 Pentylenetetrazole Model

Pentylenetetrazole is a $GABA_A$ receptor antagonist that is used to induce both acute seizures and chemical kindling in rodents. Pentylenetetrazole administered via the IP or subcutaneous route at doses of 60–100 mg/kg results in myoclonic jerks, clonus and tonic extensions after 5–9 min. Repeated administrations at sub-threshold doses of 20–40 mg/kg can induce the kindling phenomenon, and can thereby serve as a chronic epilepsy model [45].

We identified two studies that examined the effect of BCAAs on PTZ-induced seizures. The first study [46] investigated whether the administration of BCAAs or the ketoacid of leucine (α-ketoisocaproic acid; α-KIC) 2 h prior to seizure induction would modulate the effect of absence-like and tonic-clonic seizures. A total of 20 non-epileptic, 5-month-old male Wistar rats were subcutaneously injected with PTZ weekly (40 mg/kg). This regimen resulted in absence-like and spike-and-wave discharges (SWD) followed by paroxysmal EEG activity and tonic-clonic seizures. The ten rats in the experimental group received pretreatment with injections of leucine, isoleucine, valine, or α-KIC (each 300 mg/kg) 2 h before administration of PTZ. The ten rats in the control group received saline injections along with a balanced mixture of amino acids, Vamin® (Pharmacia, Canada Ltd., Montreal, QC, Canada; 300 mg/kg). The primary outcome measures in this experiment were latency to the first SWD or tonic-clonic seizure, the number of SWD episodes per minute of recording before the onset of the tonic-clonic phase, the total duration of the period of SWD preceding the occurrence of the tonic-clonic seizure, and the total duration of the convulsive seizure. Pretreatment with leucine or isoleucine significantly increased the latency to the first SWD (+101–104%), the latency to the tonic-clonic seizure (+53–62%), and the duration of the SWD period preceding the convulsive seizure (+39–62%). However, while leucine and isoleucine delayed the occurrence of tonic-clonic seizures, they had no effect on seizure duration. Valine, α-KIC, and the commercial mixture of amino acids had no effect on seizures. The authors noted that the difference in efficacy between valine and leucine/isoleucine may be due to the relatively low affinity of valine to the transport system of neutral amino acids across the BBB [18].

A second study [47] demonstrated that valine was effective in blocking the pro-convulsive effects of aspartame on PTZ-induced seizures in male CD-I mice (average $n = 24$/group). One hour before inducing seizures with PTZ (50–75 mg/kg), the mice were administered valine (1000 mg/kg) and/or aspartame (1000 mg/kg) orally. In mice receiving treatment with only aspartame (1000 and 2000 mg/kg), the authors demonstrated a significant increase in the percentage of animals that experienced PTZ-induced seizures ($p < 0.05$). Interestingly, the pro-convulsant effects of aspartame were blocked in mice that received concomitant administration of valine (1000 mg/kg). However, when administered alone, valine had no effect on seizure frequency. The authors found that treatment with aspartame elevated levels of phenylalanine in the brain. In this case, valine effectively antagonized the transport of phenylalanine into the brain.

3.2.3 Flurothyl Model

With repeated exposure to flurothyl, a $GABA_A$ receptor antagonist, mice demonstrate spontaneous seizures and generalized clonic seizures that remit within 1 month [48]. Animals are placed in an airtight chamber, where the volatile flurothyl vapors are inhaled. Absence-like seizures and partial seizures typically occur after short exposure to flurothyl, while clonic and tonic-clonic seizures occur after longer exposures [49]. Findings suggest that the mechanism for flurothyl-induced seizures is in part mediated by the benzodiazepine-binding site of the GABA-benzodiazepine receptor complex [50].

We identified a study that examined the pro-convulsive effects of aspartame on flurothyl-induced seizures in male CD-I mice (average $n = 14$/group) [47]. Valine (1000 mg/kg) and/or aspartame (1000 mg/kg) was administered orally 1 h prior to seizure induction with flurothyl inhalation (10% solution in 95% ethanol, 0.05 mL/min). The authors found that doses of 1000, 1500, or 2000 mg/kg of aspartame significantly reduced the time to clonic seizure (395, 381, and 339 s; $p < 0.05$). As with the PTZ model above, concurrent administration of valine (1000 mg/kg) blocked the pro-convulsant effects of aspartame and prevented a reduction in the time to clonic seizures.

3.2.4 Picrotoxin Model

Following the previously described study with the picrotoxin model of epilepsy [38], a second study [39] was performed to investigate the effects of individual BCAAs on picrotoxin-induced seizures. Fifty male Wistar rats were divided into five groups of ten rats, and each group received a different pretreatment 120 min before the injection of picrotoxin. Rats were randomized to receive either an IP injection with valine (300 mg/kg), leucine (300 mg/kg), isoleucine (300 mg/kg), a balanced amino acid solution (300 mg/kg), or placebo (saline, 3 mL). All four BCAA pretreatments resulted in a significant increase in the latency time to seizure onset compared with the placebo and balanced amino acid solution.

3.2.5 Genetic Absence Epilepsy in a Rat Model

The GAERS model utilizes a strain of rats that exhibits recurrent non-convulsive seizures, accompanied by bilateral and synchronous SWD by electroencephalography (EEG) [51]. Drugs that are effective against absence seizures in humans have been shown to reduce SWD in GAERS in a dose-dependent manner. However, drugs that are used for convulsive and focal seizures have no effect on seizures in GAERS. Findings suggest that the absence seizures from GAERS result from disturbances in the thalamocortical circuitry [52].

We identified a study (published in two independent papers) that investigated the effects of BCAAs on epileptic seizures in GAERS [53, 54]. Groups of ten GAERS rats were administered saline or 300 mg/kg of leucine (22 mg/kg), isoleucine (16.7 mg/kg), valine (18.2 mg/kg), α-KIC, or a balanced amino acid solution via IP injections. Electroencephalography was recorded for 40 min during each hour for 5 consecutive hours, and the cumulative duration of SWD was measured during 20-min periods of EEG recording. Injections of leucine, isoleucine, valine, and α-KIC resulted in an increased number of SWD per 20 min. The duration of SWD was not affected by a single injection of leucine or α-KIC. Valine increased the duration of SWD at 260 min post-injection, and isoleucine increased the duration of SWD at 220 min and 260 min post-injection. The balanced amino acid solution had no effect on the number of durations of SWD. The experiment demonstrated that BCAAs and α-KIC, when injected intraperitoneally, increased the number of SWD in GAERS and had a minor effect on their duration.

3.2.6 Kainic Acid Model

Kainic acid is a cyclic analog of L-glutamate that acts as a potent agonist to the AMPA/kainate class of glutamate receptors [55]. Infusion with KA can result in neurodegeneration and seizures. Kainic acid can be used to create a rodent model that closely mimics temporal lobe epilepsy in humans, with a latent period and subsequent spontaneous seizures, which are refractory to many antiepileptic drugs [56]. The KA model generates focal-onset (localization-related) seizures, which contribute to many cases of epilepsy in adults [57].

Two recent studies examined the anti-seizure effects of L-leucine in the KA model of epilepsy. In the first study [57], 5-week-old C57BL/6 mice were administered an IP injection of KA. The mice were administered a single IP injection of L- or D-leucine (3 mg/kg or 300 mg/kg) 3 h before or 15–20 min after KA injection. The study demonstrated that a single low dose of L-leucine injected 3 h prior to KA-induced seizures potently suppressed seizure activity, whereas the high dose only had intermediate anticonvulsant effects. D-Leucine also suppressed seizure activity in a manner at least as effective as L-leucine. While both forms of leucine provided potent anticonvulsant effects as pretreatments, only D-Leucine effectively terminated seizures, even when administered after the seizure onset. The authors noted that the mechanism of action of D-leucine is unclear because there is no known function for endogenous D-leucine in eukaryotic biology, and that D-leucine is not incorporated into mammalian proteins.

Another recent study investigated the effects of D-leucine on KA-induced spontaneous seizures [58]. Mice were administered an IP injection of KA (25–100 mg/kg body weight until the mouse went into status epilepticus), and EEG was monitored for 4 weeks before, after, and during administration of ad libitum D-leucine (1.5% w/v) in the drinking water. While no differences were observed in the overall number of seizure days or frequency of seizures, D-leucine protected against seizures during the dark cycle. The seizures recurred during the dark cycle for the 4 weeks after the D-leucine treatment was withdrawn.

3.3 Effect of BCAA Mixture on Seizures in Humans

The ketogenic diet can be a useful adjunct in the treatment of medically refractory epilepsy, especially in children [59]. In one study [60], 17 children aged 2–7 years and diagnosed with intractable epilepsy were administered an oral mixture of BCAAs daily for 6–12 months in addition to the ketogenic diet, which was initiated 6–24 months prior. One hundred grams of the supplementary BCAA mixture comprised 45.5 g leucine, 30 g of isoleucine, and 24.5 g of valine. Patients were started with a daily dose of 5 g mixed in food or beverages, with weekly increments of 5 g up until a maximum dosage of 20 g/day. Over the course of the experiment, the addition of BCAAs to the ketogenic diet resulted in a significant reduction in seizure frequency. Three patients experienced a complete cessation of seizures, and two other patients experienced an additional reduction in seizures from 70% on the ketogenic diet to 90%. Two patients on the ketogenic diet experienced a marked improvement from 50% and 60% reductions before BCAAs supplementation to 80% and 90% reductions following BCAA administration. Side effects were reported to be minimal, with three patients reporting a slight but transient increase in heart rate at the beginning of treatment.

4 Discussion

4.1 Possible Mechanism of BCAAs on Seizures

In this systematic review, we identified 11 studies that examined the effects of BCAA administration on seizures. One

of these studies was conducted in children with medically refractory epilepsy, and the remaining ten studies were conducted in seven animal models of epilepsy, including the strychnine, PTZ, fluorothyl, picrotoxin, GAERS, KA, and MSO models. With the exception of the GAERS model of absence seizures and long-term BCAA supplementation in the MSO model, all other studies demonstrated anticonvulsant properties of BCAAs. Although the mechanisms by which BCAAs impact seizures are complex and poorly understood, several potential mechanisms have been suggested and are reviewed here.

4.2 Proposed Anticonvulsant Effects of BCAAs

It has been suggested that BCAAs exert their anticonvulsant effects by affecting neuronal excitation and inhibition, specifically by enhancing the metabolism and clearance of brain glutamate, as well as facilitating GABA synthesis and GABAergic neurotransmission (summarized in Fig. 4). Chronic elevation of glutamate in the extracellular compartment of the brain has been implicated as a key pathophysiological cause of epileptic brain damage and seizures [6, 9, 61]. Branched-chain amino acids are thought to have several effects on glutamate metabolism and neurotransmission. First, there is evidence that BCAAs might reduce seizure activity by decreasing the neuronal glutamate levels available for synaptic release [53]. Blood-derived BCAAs are rapidly taken up astrocytes, where they transaminate α-ketoglutarate, thereby producing glutamate and branched-chain ketoacids (BCKAs). Because BCKAs are poorly oxidized in astrocytes, they are transported out of the astrocyte to the extracellular fluid, where they are taken up by neurons. In the neuron, BCKAs are transaminated back to BCAAs in a reaction that consumes glutamate [54, 60, 62, 63]. In this manner, BCAAs decrease the neuronal glutamate available for subsequent synaptic release (Fig. 4, reaction 1).

Second, it is thought that BCAAs facilitate the clearance of glutamate from the brain. The astrocytic glutamate that is produced from the transamination of α-ketoglutarate by BCAAs is rapidly converted to glutamine by GS [64]. Depending on the energy needs of the neuron, the newly formed glutamine is either taken up by neighboring neurons, where it is packaged into vesicles for excitatory neurotransmission, or is rapidly cleared from the brain via transport to the blood [65, 66]. Moreover, BCAAs may facilitate the clearance of glutamine from the brain via obligate exchange transporters, by which BCAA influx from the blood to the brain is equal and opposite to glutamine efflux from the brain to the blood [67]. In summary, the net effect of BCAA uptake into the brain is that it facilitates both the degradation of neuronal glutamate and the clearance of astrocytic glutamate in the form of glutamine (Fig. 4, reaction 2).

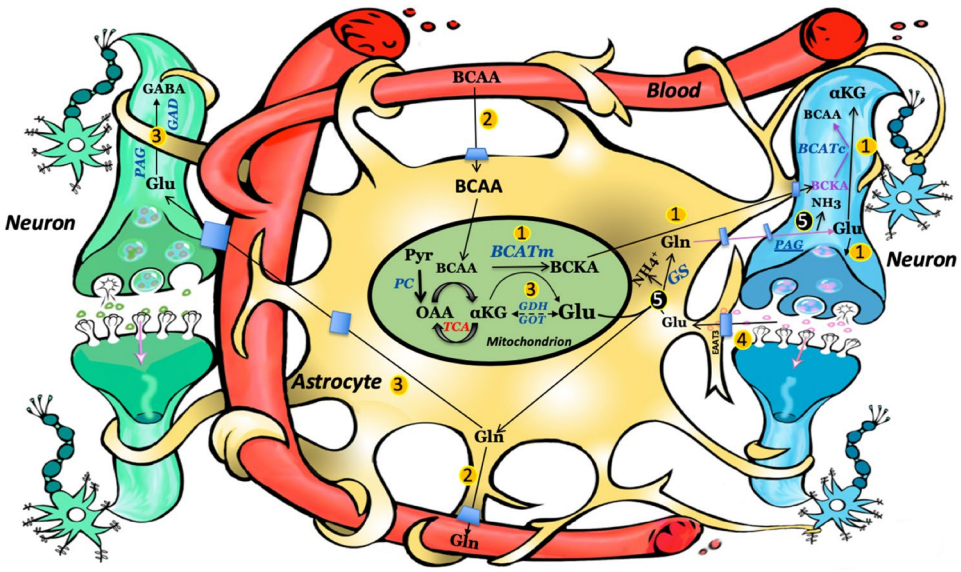

Fig. 4 Branched-chain amino acid (BCAA)-glutamate-glutamine shuttle, and the proposed mechanisms underlying the effects of BCAAs on seizures. Anticonvulsant effects: (1) decrease in neuronal glutamate concentration; (2) increased glutamate clearance via glutamine, BCAA obligate transport; (3) increased gamma-aminobutyric acid (GABA) production in GABAergic neuron; and (4) increased uptake of extracellular glutamate via excitatory amino acid transporter 3 (EAAT3). Pro-convulsant effects: (5) increased ammonia production. *aKG* alpha-ketoglutaric acid, *BCATc* cytoplasmic branched-chain amino acid transaminase, *BCATm* mitochondrial branched-chain amino acid transaminase, *BCKA* branched-chain alpha-ketoacid, *GAD* glutamic acid decarboxylase, *GDH* glutamate dehydrogenase, *Gln* glutamine, *Glu* glutamate, *GOT* glutamic oxaloacetic transaminase, *GS* glutamine synthetase, NH_3 ammonia, *OAA* oxaloacetate, *PAG* phosphate-activated glutaminase, *PC* pyruvate carboxylase, *Pyr* pyruvate, *TCA* tricarboxylic acid cycle

There is further evidence that the anticonvulsant effects of BCAAs may be GABA mediated, either by direct stimulation of GABA receptors or by increasing GABA production. The administration of BCAAs are thought to favor the production of GABA by inhibiting both glutamate dehydrogenase activity [68, 69] and glutamate oxaloacetate transaminase [59], two enzymes that play a key role in the degradation of astrocytic glutamate. In this way, glutamine production from glutamate is favored over α-ketoglutarate production, and the newly formed glutamine can then be shuttled to GABAergic neurons where it can be used for GABA synthesis (Fig. 4, reaction 3). Moreover, studies have demonstrated that 5-aminovaleric acid, a valine analog with anticonvulsant effects [70], facilitates GABA-mediated neurotransmission by direct interaction with $GABA_B$ receptors [71, 72], by blocking glial uptake of extracellular fluid GABA [73] or by inhibiting GABA degradation [72].

The ketogenic diet effectively reduces the frequency and severity of seizures, and is used as an adjunct therapy in treating patients (especially children) with epilepsy [74, 75]. The BCAAs, in combination with the ketogenic diet, was shown to be more effective in reducing the frequency of epileptic seizures than the ketogenic diet alone [60]. It is unclear from this study whether the anticonvulsant effects of BCAAs resulted from synergism with the ketogenic diet, or whether their actions were mediated by an entirely different mechanism. Leucine is a known ketogenic amino acid, and its degradation produces the anticonvulsant ketones acetoacetate and β-hydroxybutyrate [76]. Moreover, during ketosis, plasma and brain concentrations of BCAAs markedly increase, causing some experts to speculate that BCAAs and the ketogenic diet share key anticonvulsant mechanisms [59, 77–79]. It is important to note, however, that several studies have observed that the BCAA-induced reduction in seizures occurs regardless of the level of ketosis [57], suggesting that BCAAs may have anticonvulsant properties that are independent of their ketogenic effects.

Another mechanism by which BCAA is thought to exert its anticonvulsant effects is by promoting glucose homeostasis. Studies have shown that BCAAs are involved in critical nutrient signaling pathways in the hypothalamus, and play an important role in maintaining a state of metabolic balance [80]. Leucine produces a robust synergetic effect with insulin to regulate glucose levels, and isoleucine and valine suppress endogenous glucose production [81]. Importantly, both hyperglycemia and hypoglycemia can induce seizure activity or increase seizure susceptibility in animals and humans [82–84]. Clinical studies have shown that the people with hyperglycemia have an increased predisposition to seizures [85, 86], possibly by attenuating potassium (adenosine triphosphate) channels [87]. Moreover, both in vivo and in vitro studies have demonstrated that a threshold blood glucose concentration is necessary for adequate synaptic transmission [83]. In the presence of seizures, an increase in BCAA concentrations might result in an improved metabolic balance, followed by a subsequent reduction in seizure activity.

Last, BCAAs might modulate seizure activity by interacting with mammalian target of rapamycin (mTOR) receptors. Mammalian target of rapamycin is an atypical serine/threonine kinase, which is present in two different complexes: mammalian target of rapamycin complex 1, which is inhibited by rapamycin, and mammalian target of rapamycin complex 2, which is insensitive to rapamycin. It is well known that BCAAs are potent activators of the mTOR receptor, thereby initiating a cascade of cell signaling pathways [88–90]. Although the activation of the mTOR signaling pathway is thought to play an important role in several cellular and molecular processes that affect neuronal excitability and seizure activity, the exact mechanism by which mTOR activation affects seizures is poorly understood [91]. Studies have shown that activation of the mTOR signaling cascade stimulates the neuronal uptake of extracellular glutamate [92] via excitatory amino acid transporter 3 [93], thereby facilitating the clearance of extracellular glutamate (Fig. 4, reaction 4). The mechanisms by which BCAA-mediated mTOR activation modulates seizure activity warrants further study.

4.3 Pro-Convulsant Effects in the Genetic Absence in Epilepsy in Rats from Strasbourg Model and the Methionine Sulfoximine Model

The study conducted in the GAERS model of absence seizures demonstrated a slight pro-seizure effect after BCAA administration. The injection of leucine and α-KIC in the GAERS model resulted in a decrease in intracerebral glutamate levels. The authors postulated that a decrease in glutamatergic neurotransmission would thereby promote GABAergic neurotransmission, which is known to cause seizures in the GAERS model [52]. Moreover, the study demonstrated that leucine administration activated the urea cycle in the thalamus, which reflects an increase in ammonia production in the brain [54, 94]. Ammonia is inherently neurotoxic, and further induces cellular apoptosis by increasing the extracellular fluid (ECF) levels of potassium in the brain [95], which is also thought to play an important role in absence seizures in the GAERS model.

In the MSO model of MTLE, long-term BCAA supplementation worsened the spread of seizures from the hippocampus to the motor cortex, as evidenced by an increase in the relative percent of convulsive seizures. In the presence of the GS deficiency that characterizes this model, high levels of BCAAs in the brain are thought to result in increased extracellular glutamate and slowed glutamate-glutamine cycling [9, 96, 97], increased ammonia production

(Fig 4, reaction 5) [54, 98], decreased ammonia clearance [98], and perturbed neurotransmitter concentrations [99]. These effects might also account for the neuron loss associated with long-term supplementation of BCAAs in this model. Seizure-induced neuron loss, along with the elevated ammonia and elevated glutamate levels typically present in MTLE [100], might explain the increased seizure propagation observed with long-term BCAA exposure.

The pro-convulsant effects of BCAAs can also be explained by a decreased synthesis of biogenic amines DA, 5-HT, and NE in the setting of high peripheral BCAA levels. Monoamines (e.g., 5-HT and catecholamines such as DA and NE) are known to be protective against seizure activity, while 5-HT and DA dysregulation has been implicated in epilepsy. Many studies have suggested that serotonergic and dopaminergic neurotransmission are essential in controlling seizure susceptibility [101]. Moreover, some commonly used antiepileptic drugs are thought to work by increasing the levels of 5-HT and DA [15, 102, 103]. Selective serotonin reuptake inhibitors, which increase the level of serotonin available at the synapse, have also shown promise in treating refractory epilepsy [104, 105]. Similarly, the use of vagal nerve stimulation to treat refractory epilepsy exploits the anticonvulsant properties of NE [106]. The decreased synthesis of these neurotransmitters in the setting of high BCAA concentrations can be accounted for by a decrease in ArAAs transport into the brain [11]. Dopamine, 5-HT, and NE are synthesized from the ArAAs tryptophan, tyrosine, and phenylalanine [17, 107, 108], which together with BCAAs and other neutral amino acids, compete for transport across the BBB by the same transport system. Although these transporters are typically saturated at physiological plasma concentrations [18, 19, 109], a sudden increase in peripheral BCAA concentrations that follows a short-term BCAA administration can increase the uptake of BCAAs into the brain, thereby decreasing the uptake of ArAAs [20]. Consequently, a reduction in the concentration of precursor amino acids (tryptophan, tyrosine, and phenylalanine) results in a reduction in the synthesis of 5-HT, DA, and NE [110–112], which has been associated with neuronal loss [113, 114]. Therefore, in the aforementioned MSO study [35], the pro-convulsant effects and neuronal loss observed with long-term BCAA supplementation might be explained by a reduction in biogenic amine neurotransmitter synthesis.

4.4 Implications for BCAA Treatment in Human Epilepsy

Although some animal models of epilepsy and one human study have demonstrated potent anti-seizure effects of BCAAs, many questions remain unanswered regarding optimal dosing and duration of BCAA treatment, and which epilepsy conditions might benefit most. Notably, the use of BCAAs in treating epilepsy should be cautioned by recent studies that have shown neurotoxic effects when high concentrations of BCAAs are present in the brain, particularly in conditions where BCAA metabolism or glutamate clearance is perturbed [27, 115, 116]. Based on these observations, taken together with the effects of BCAAs on seizures, we can speculate that the anti-seizure effects of BCAAs in the brain must be met by three key physiological conditions.

First, the anticonvulsant effects (or pro-convulsant effects in the case of the GAERS model) of BCAAs are likely dependent on the efficient uptake from the blood to the brain. For example, studies have shown that compared with leucine and isoleucine, valine has a five to ten-fold lower affinity for the large neutral amino acid transporters (LNATs) through which BCAAs are transported into the brain [18]. As such, valine has less anticonvulsant effects than leucine or isoleucine in animal models of epilepsy [46, 47]. Moreover, although PTZ administration results in seizures, PTZ also facilitates the uptake of leucine from the blood to the brain [117], where it can inhibit seizure activity. This may in part explain the potent anticonvulsive effects of leucine in the context of PTZ-induced seizures.

Second, the anticonvulsant effects of BCAAs are likely dependent on effective BCAA metabolism, thereby preventing the build-up of high concentrations of BCAAs in the brain. This is especially evident in maple syrup urine disease, in which decreased BCAA metabolism results in increased BCAAs and seizures [31]. When BCAAs are present in high concentrations owing to inefficient metabolism, they have deleterious effects on brain energy metabolism [118] and brain development [119] Branched-chain amino acids have been shown to stimulate ammonia production in the brain [54, 94], which is neurotoxic as discussed above. Moreover, high concentrations of BCAAs result in the accumulation of toxic metabolites that induce oxidant cortical injury [120].

Third, the anticonvulsant effects are likely dependent on efficient glutamate metabolism and clearance in the ECF. Interestingly, although BCAAs contribute to glutamate production in astrocytes, the peripheral injection of BCAAs does not normally increase ECF glutamate levels in the brain [53]. Rather, the newly synthesized astrocytic glutamate is rapidly converted to the biologically inert amino acid glutamine, which either enters the glutamate-glutamine cycle or is transported back to the blood. In conditions where glutamate production from BCAAs are increased (i.e., in glioma) or glutamate clearance is perturbed (i.e., amyotrophic lateral sclerosis), BCAA administration has indeed resulted in neurotoxic effects [121]. Importantly, some forms of medically refractory epilepsy such as MTLE are characterized by a deficiency in GS and slowed glutamate-glutamine cycling [9]. This might explain why long-term

BCAA supplementation worsened seizure propagation and neuron loss in the MSO model of MTLE.

Fourth, the anticonvulsant properties of BCAAs can be attributed to their role in metabolic regulation via glucose homeostasis. A reduction in seizure threshold has been associated with perturbed glucose levels [122], and case studies have described non-ketotic hyperglycemic seizures in humans [123, 124]. Increased BCAAs, via hypothalamic signaling [125], can help promote glucose homeostasis and improve energy balance. Moreover, BCAAs, together with insulin, can stimulate mammalian target of rapamycin complex 1 signaling pathways to modify messenger RNA translation [126]. This process likely functions as an anabolic signal to alter tissue growth in areas of high metabolic activity (e.g., within a seizure focus) [125].

5 Conclusions

Epilepsy often results in significant individual disability and has a high negative economic impact on public health. In up to 40% of patients, seizures recur despite medical therapy, highlighting the need for the development of better methods of treating seizures. In recent years, BCAAs have been shown to have several effects in the brain, and can either stimulate or inhibit seizures. The mechanistic link between BCAAs and seizure activity has not been fully elucidated. Here, we systematically reviewed ten animal studies and one human study that investigated the effects of BCAAs on seizure activity. In most epilepsy models and in humans, BCAAs were shown to have potent anticonvulsant effects while in the GAERS epilepsy model and long-term BCAA treatment in the MSO model, BCAAs had pro-convulsant effects. The effects of BCAAs on seizure activity likely reflects the numerous and complex cellular pathways by which BCAAs are thought to act, including biogenic amines, glucose, glutamate, GABA, and ammonia homeostasis in the brain. Moreover, the different effects of BCAAs on seizures might reflect differences in the complex pathophysiological mechanisms of the underlying seizure disorder. In conditions in which BCAAs have inhibited seizure activity, the anticonvulsant effects of BCAAs are likely dependent on effective uptake of BCAAs from the blood to the brain, effective BCAA metabolism in the brain, effective metabolism and clearance of extracellular glutamate in the brain, and tighter glucose homeostasis. Future studies should further elucidate the mechanisms by which BCAAs affect seizure activity. Ultimately, BCAA-associated metabolic pathways might be useful therapeutic targets in the treatment of some medically refractory epilepsy conditions.

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Compliance with Ethical Standards

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