

## RESEARCH HIGHLIGHT

## Isovaline: A unique amino acid with antiepileptic drug properties

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**Current anti-epileptic drugs (AEDs) primarily act by decreasing excitation or increasing inhibition of the neuronal network. This is achieved by inactivating Na<sup>+</sup> or Ca<sup>2+</sup> ion channels and decreasing glutamate release or by enhancing GABAergic influence. Despite using these AEDs, approximately 30% of epileptic patients remain intractable. As a consequence, there is a clear need to develop new AEDs that may work via novel mechanisms to provide greater efficacy. With this in mind, we investigated whether isovaline, a unique amino acid with a similar chemical structure to glycine and GABA, could fill this role. Previously, we showed that isovaline attenuated seizure-like events (SLEs) *in vitro* via a novel mechanism. In this research highlight, we discuss our latest published findings which demonstrate the efficacy of isovaline in an *in vivo* rat model of epilepsy.**

**Keywords:** Rats; Seizures; Epilepsy; Electrophysiology; Behavior

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Epilepsy is one of the most common neurological disorders in the world, affecting sixty-five million people worldwide<sup>[1]</sup>. Anti-epileptic drugs (AEDs) are the preferred treatment option for managing epilepsy disorders since they are more pragmatic than other proposed treatments such as surgical resection or electrical stimulation. Older AEDs such as phenytoin, valproate and phenobarbital primarily act on voltage-gated ion channels<sup>[2, 3]</sup> or GABA<sub>A</sub> receptors<sup>[4]</sup>; however, these drugs do not provide adequate seizure control in 30% of epileptic patients and poignantly underscores the considerable need for new and improved AEDs<sup>[5]</sup>. In achieving these goals, 2<sup>nd</sup> generation AEDs were developed which expanded upon the mechanism(s) thought to underlie the older generation AEDs, but possessed slightly different toxicity and drug-interaction profiles. Now, more recent AEDs are being developed with novel chemical structures and mechanisms (see review<sup>[6]</sup>). Here, we discuss our research of isovaline in

*in vitro* and *in vivo* models of epilepsy<sup>[7, 8]</sup> to raise awareness that this amino acid provides antiepileptic efficacy through a novel mechanism. As a consequence, isovaline could represent a new class of drugs for the treatment of epilepsy.

Isovaline is a non-proteinogenic amino acid initially identified in the Murchison carbonaceous meteorite. This amino acid is present as a racemic mixture, which is unlike most proteinogenic amino acids which are predominantly S-enantiomers<sup>[9, 10]</sup>. Notably, the chirality of amino acids is known to have significant pathophysiological effects<sup>[11]</sup> although it's unclear what importance this has for isovaline in AED efficacy since our research only focused on its natural form. Prior to our investigation of isovaline for AED utility, this amino acid was investigated for its effects on nociception<sup>[12-14]</sup>. More specifically, Puil and colleagues at the University of British Columbia injected mice with strychnine into the lumbar intrathecal space, or administered formalin,

glutamate, or prostaglandin E2 into the hindpaw to elicit acute and chronic pain<sup>[14, 15]</sup>. After, isovaline was given intravenously (IV) or intrathecally to these animals and either approach induced significant antinociceptive effects. Since the current strategy for developing AEDs is to have these drugs provide efficacy to a variety of disease states and disorders, it is particularly noteworthy that isovaline can serve as an AED and as an analgesic drug<sup>[6]</sup>.

In our first paper, given the similarity of isovaline to other inhibitory neurotransmitters, we investigated whether isovaline could attenuate seizure-like events (SLEs) or primary afterdischarges (PADs) in hippocampal slices using extracellular field recordings in the CA1 region<sup>[7]</sup>. After evoking PADs with tetanic stimulation of the Schaffer collaterals or inducing recurrent SLEs with either low (0.25 mM) Mg<sup>2+</sup> and high (5 mM) K<sup>+</sup> or 100  $\mu$ M 4-aminopyridine (4-AP), we found that bath perfusion of 250  $\mu$ M isovaline abolished these SLEs and PADs in 3 of 8 brain slices and significantly attenuated the amplitude and duration in the remaining 5 slices. We next attempted to unmask isovaline's mechanism of action by recording from both pyramidal cells and interneurons in the hippocampus using the whole-cell patch clamp technique. Interestingly, we found that isovaline had a greater inhibitory effect on interneuronal activity if these neurons were more active. Therefore, isovaline appeared to attenuate hippocampal interneurons in an activity-dependent manner. Furthermore, this effect was still present in the presence of AMPA, NMDA and GABA<sub>A</sub> receptor blockers, which suggested that this effect was due to direct modulation of inherent interneuronal ion channels. In contrast, isovaline had no direct effect on pyramidal neuron firing at baseline levels during aCSF perfusion. However, we noted that isovaline attenuated pyramidal neuronal hyperactivity during low Mg<sup>2+</sup>/high K<sup>+</sup> or 4-AP treatment, presumably from increased inhibitory drive from interneurons.

Our recent paper expanded on our *in vitro* study by examining whether isovaline effects on SLEs and PADs translated to behavioral and electrophysiological outcomes *in vivo*<sup>[8]</sup>. To be consistent with our *in vitro* study, we injected a single intraperitoneal (IP) dose of 4-AP to elicit generalized convulsions and epileptiform activity in rats<sup>[16-18]</sup>. In this study, we employed two strategies to assess the efficacy of isovaline as an AED in these seizing animals. In one approach, we recorded local field potentials (LFPs) in the hippocampus of anesthetized rats before and after 4-AP treatment. We observed recurrent epileptiform activity after 4-AP injection, which were abolished 15-20 minutes after IV tail vein injection of 150 or 500 mg/kg isovaline.

Conversely, IV tail vein injection of saline did not alter the duration, amplitude or frequency of epileptiform activity in the hippocampus. In another group of animals, we examined whether isovaline could affect seizing behavior. We found that 4-AP readily induced generalized convulsive seizures characterized by wild-running and jumping behavior within 30 minutes after injection. Saline injection did not alter these behaviors, but after 150 or 500 mg/kg isovaline IV tail vein injection, only occasional head nodding and staring episodes characteristic of mild partial seizures<sup>[19]</sup> were present. In the last part of our study, we examined whether the use of isovaline would coincide with significant adverse side effects such as sedation on animal behavior. To accomplish this, we utilized the open field test (OFT) and monitored the distance travelled and the time spent immobile before isovaline injection and 30 minutes and 2 hours post-isovaline treatment at both dosages. We found that isovaline at low or high dose had no effect on any of these measures.

In summary, our studies reveal a strong potential for isovaline to serve as a novel AED that is interneuron-specific and activity-dependent, which does not induce sedation. We speculate that these properties would have minimal impact on cognitive function in non-seizing brains, which strengthens our position that isovaline has utility and pragmatism as an AED. To further validate the efficacy of isovaline as an AED, we are continuing our research using other animal models of epilepsy to reveal more specific uses of this unique amino acid in managing specific epilepsies. However, before isovaline can be seriously considered for clinical application, it is critical that future studies are undertaken to examine the pharmacology and toxicology of isovaline and its metabolites, possible drug-interactions with other medications and the identification of the ion channel(s) modulated by isovaline in hippocampal interneurons.

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### Conflict of Interest

The author declares that they have no conflict of interest.

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