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Review

New experimental therapies for status epilepticus in preclinical development

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ABSTRACT

Starting with the established antiepileptic drug, valproic acid, we have taken a novel approach to develop new antiseizure drugs that may be effective in status epilepticus. We first identified that valproic acid has a potent effect on a biochemical pathway, the phosphoinositide pathway, in *Dictyostelium discoideum*, and we demonstrated that this may relate to its mechanism of action against seizures in mammalian systems. Through screening in this pathway, we have identified a large array of fatty acids and fatty acid derivatives with antiseizure potential. These were then evaluated in an in vitro mammalian system. One compound that we identified through this process is a major constituent of the ketogenic diet, strongly arguing that it may be the fatty acids that are mediating the antiseizure effect of this diet. We further tested two of the more potent compounds in an in vivo model of status epilepticus and demonstrated that they were more effective than valproic acid in treating the status epilepticus.

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1. Introduction

Valproic acid is a branch-chain fatty acid, 2-propylpentanoic acid. Its antiseizure effects were discovered while using it as a solvent to dissolve a series of potential new antiepileptic drugs [1]. Subsequently, valproic acid and its salt, sodium valproate, have become widely used as broad-spectrum antiepileptic drugs.

Valproic acid is available as an intravenous formulation and has been used in the treatment of status epilepticus in humans. In a small randomized, prospective study, valproic acid was at least as effective as intravenous phenytoin and levetiracetam when used as second-line therapy but with a superior cardiovascular safety profile compared with phenytoin [2]. In a pseudorandomized study, valproic acid was shown to be superior to levetiracetam (although at a lower dose than is often used) in the treatment of status epilepticus [3]. More recently, glycine, amide, and carbamate derivatives of valproic acid have also been shown to have antiseizure potential and to be effective in status epilepticus, stopping the status epilepticus and providing varying degrees of neuroprotection [4].

There are, however, potential downsides to the treatment of status epilepticus with valproic acid. High-dose valproic acid can precipitate hyperammonemia and associated encephalopathy [5] and also fulminant liver failure in people with mitochondrial cytopathies [6].

Surprisingly, despite years of research, valproic acid's mechanism of action has remained unclear. Here, we have provided insight into valproic acid's putative mechanism of action. Further, we used a simple model system, *Dictyostelium*, to screen a range of medium-chain fatty acids and their derivatives for efficacy in seizures and status epilepticus. Lastly, we showed that these therapies provide both neuroprotection and antiseizure effects in in vitro and in vivo models of seizures and status epilepticus.

2. Screening for antiepileptic drugs

The modern era of antiepileptic drug discovery began in 1937 when a cat model of seizures (the maximal electroshock model) devised by Merritt and Putnam was used to screen over 700 compounds resulting in the discovery of the antiseizure effect of phenytoin [7]. Later, the National Institute of Neurological Diseases (USA) started an antiepileptic drug screening program [8], which has screened over 25,000 compounds through animal seizure models; this program has been

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remarkably effective and has identified many of the new antiepileptic drugs currently in use. The problem with this approach, however, is that efficacy in acute seizure models is usually demonstrated prior to any understanding of mechanism of action. Moreover, the screening takes place in acute seizure models rather than in spontaneous seizures of chronic epilepsy models, and there is rarely testing of efficacy in models of status epilepticus.

The advantage of nonmammalian model systems is that it can be much more straightforward, through mutagenesis, to determine putative mechanisms of action [9]. The use of *ex vivo* mammalian models (e.g., neuronal cultures and acute slice preparations) can also facilitate a mechanistic approach. We, therefore, set out to determine the mechanisms of action of valproate in these model systems and, through that, to identify potential new antiepileptic drugs.

3. Valproic acid and *Dictyostelium*

Dictyostelium discoideum is a social amoeba that shares many of its enzymes and biochemical pathways with mammalian systems; thus, it has proven to be an excellent model for understanding specific aspects of cellular signaling in human disease [10,11]. *Dictyostelium* permits rapid and easy genetic manipulation and screening of mutant libraries as pharmacogenetic approaches to understanding drug action.

During times of starvation, *Dictyostelium* cells move towards one another and coalesce to form fruiting bodies (Fig. 1). The mechanisms underlying this process are well understood. Cell surface receptors detect extracellular cyclic AMP and then activate an intracellular signaling cascade involving phosphoinositides, resulting in chemotaxis. We first found that valproic acid at clinically relevant concentrations blocks this chemotaxic cell movement [12] through rapidly reducing phosphoinositide production in moving *Dictyostelium* cells [12,13]. Importantly, the phosphoinositide pathway is a phylogenetically conserved pathway that is present in mammalian cells. We next set out to determine where valproate acted in the phosphoinositide pathway. This is challenging in mammalian systems in which enzyme inhibitors are often nonspecific and rarely completely effective. However, it is substantially easier in *Dictyostelium* because of the ability to rapidly delete any gene of choice provided that the protein product is not vital. We have used this method to examine the effect of valproic acid on PI3Ks (phosphatidylinositol 3-kinases), a family of enzymes that are most commonly associated with the production of phosphoinositide [14]. Here, we employed a single *Dictyostelium* cell line with all six genes encoding these proteins deleted. Valproic acid blocked phosphoinositide production in these cells in the absence of the PI3K enzymes, indicating that valproic acid did not work through changing the activity of these enzymes.

We have extended this work to mammalian neurons and in vivo animal seizure models [17] and have shown that that acute seizures in a rat seizure model or in an in vitro model of seizure-like activity decrease hippocampal phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) levels and reduce protein kinase B (PKB/AKT) phosphorylation. These changes were prevented with valproic acid treatment. Moreover, valproic acid's effect on seizure-like activity was blocked by drugs that target phosphoinositide signaling.

4. *Dictyostelium* and drug discovery

Another significant advantage of *Dictyostelium* over other model systems is that it can be used as a rapid throughput screen of drugs/chemicals because of the abrupt change in the behavior of cells following drug exposure [16]. The observations that valproic acid reduces phosphoinositide production and that this likely plays a role in its antiseizure effect provide an approach to develop a rapid throughput assay to identify compounds with a similar mode of action. Restricting this assay to compounds that are chemically similar to valproic acid (a branch-chain fatty acid) enabled us to explore the correlation of the structure of branch- and straight-chain fatty acids in this assay to establish the structure–activity relationship of these compounds [14]. The observation that only small changes in the structure (e.g., the addition of an extra carbon) have a profound effect on the compound's activity indicated that these compounds were not working through some nonspecific effect of fatty acids on this pathway but that a more precise interaction was taking place.

We, thus, identified a group of compounds that are effective in a nonmammalian assay, which relates to a mechanism of action of valproic acid, and had also established a structure–function relationship for efficacy in this assay [14]. We next determined whether these compounds are effective in a mammalian system using epileptiform activity induced in ex vivo hippocampal–entorhinal cortex slices by removing magnesium from the perfusion solution or by adding a convulsant [15]. We used these in vitro screens of epileptiform activity for four main reasons: (1) these models avoid the confounders of drug metabolism and the blood–brain barrier; (2) such systems are simple and permit high-throughput screening; (3) such methods reduce animal use; and (4) these models have seizures that are resistant to many antiepileptic drugs; valproate only shows a partial effect. These in vitro mammalian systems refined our chemical space and identified a host of potential antiepileptic drugs [14,18,19].

5. Mechanisms of the ketogenic diet and efficacy in status epilepticus

One of the compounds that we identified in our *Dictyostelium* and in vitro seizure assays that had a particularly marked effect was

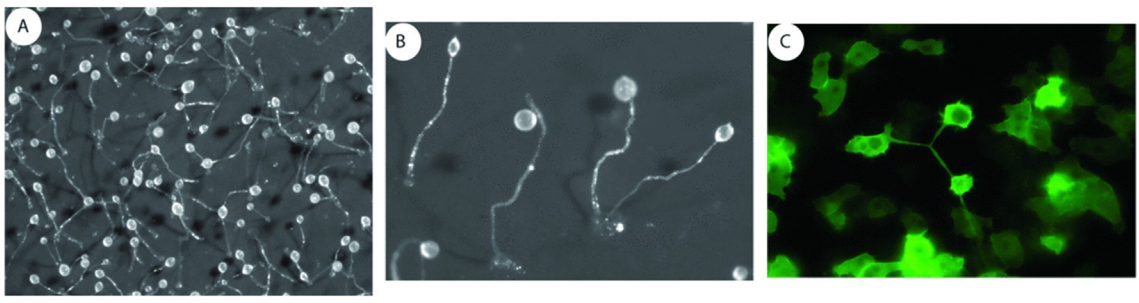

Fig. 1. *Dictyostelium* has been used as an animal replacement model to identify new compounds for seizure control. (A) Bird's-eye view of a field of *Dictyostelium* fruiting bodies, with each comprising a round spore head and a stalk. (B) Side angle view of four mature *Dictyostelium* fruiting bodies, each approximately 1 mm in height. (C) A field of *Dictyostelium* cells expressing a fluorescently tagged protein, zizB [15]. Each cell is approximately 10 μm in diameter, and overexpression of this protein causes a defect in cell division (seen as three joined cells undergoing cytokinesis).
From Ref. [16].

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