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PERSPECTIVE

Perspective on mTOR-dependent Protection in Status Epilepticus

Francesca Biagioni[1,§], Roberta Celli[1,§], Filippo Sean Giorgi[2], Ferdinando Nicoletti[1,3] and Francesco Fornai[1,2,*]

[1]*I.R.C.C.S. Neuromed, Pozzilli, Italy;* [2]*Human Anatomy, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy;* [3]*Departments of Physiology and Pharmacology, University of Sapienza, Rome, Italy*

Abstract: ***Background*:** The piriform cortex, known as *area tempestas*, has a high propensity to trigger limbic epileptic seizures. Recent studies on human patients indicate that a resection containing the piriform cortex produces a marked improvement in patients suffering from intractable limbic seizures. This calls for looking back at the pharmacological and anatomical data on *area tempestas*. Within the piriform cortex, status epilepticus can be induced by impairing the desensitization of AMPA receptors. The mechanistic target of rapamycin complex1 (mTORC1) is a promising candidate.

***Objective*:** The present perspective aims to link the novel role of the piriform cortex with recent evidence on the modulation of AMPA receptors under the influence of mTORC1. This is based on recent evidence and preliminary data, leading to the formulation of interaction between mTORC1 and AMPA receptors to mitigate the onset of long-lasting, self-sustaining, neurotoxic status epilepticus.

***Methods*:** The perspective grounds its method on recent literature along with the actual experimental procedure to elicit status epilepticus from the piriform cortex and the method to administer the mTORC1 inhibitor rapamycin to mitigate seizure expression and brain damage.

***Results*:** The available and present perspectives converge to show that rapamycin may disrupt the seizure circuitry initiated in the piriform cortex to mitigate seizure duration, severity, and brain damage.

***Conclusion*:** The perspective provides a novel scenario to understand refractory epilepsy and self-sustaining status epilepticus. It is expected to provide a beneficial outcome in patients suffering from temporal lobe epilepsy.

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1. INTRODUCTION

Limbic seizures represent the most common phenotype leading to status epilepticus (SE) and seizure refractoriness. Classic drugs often fail when such a condition occurs, and seizure activity progresses into a self-sustaining, drug-resistant SE, eventually leading to death. The refractoriness of limbic SE to common antiepileptic drugs represents a life-threatening condition, which requires novel approaches to counteract severe outcomes. Recent promising data have been obtained on refractory seizures by modulating the activity of the mechanistic target of rapamycin [mTOR], [1, 2]; however, the lack of safety data and appropriate models to test such an approach for clinical purposes limits the progress of these findings.

The highly epileptogenic region within the anterior extent of the piriform cortex was described three decades ago in the seminal paper by Piredda and Gale (1985) [3]. This region, referred to as *area tempestas*, represents a small area within the mesial temporal lobe and serves as a powerful experimental tool to probe seizure circuitries as well as the effects induced by pure seizure activity in the brain. Similarly, this model was ideal for challenging potential anti-convulsant remedies within experimental settings unbiased from non-specific effects induced by systemic anticonvulsants [4-9]. This area, used so far to trigger focally experimental limbic seizures, now emerges as a crucial site in the process of the natural occurrence of refractory limbic seizures and SE in humans. In fact, very recent findings have indicated that ablation of the piriform cortex prevents seizure onset and seizure recurrence in patients affected by severe refracto-

*Address correspondence to this author at I.R.C.C.S. Neuromed, *via* dell'elettronica, 86077 Pozzilli, Italy and Human Anatomy, Department of Translational Research and New Technologies in Medicine and Surgery, The University of Pisa, *via* Roma 55, 56126 Pisa, Italy; Tel: +39 0502218667; E-mails: francesco.fornai@neuromed.it; francesco.fornai@unipi.it
§ *Co-First Authors*

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ry limbic seizures [10]. This allows sparing extensive resection of multiple regions from the mesial temporal lobe during neurosurgical approaches aimed to prevent intractable seizures and limbic SE. Thus, we may assess that a brain site, useful as an experimental tool to understand seizures, has become a real determinant of human refractory epilepsy. This is strengthened by the seizure type (limbic seizures) controlled by the piriform cortex. In this article, we provide these new vistas and novel data to foster effective mTOR-mediated therapeutic approaches in refractory limbic epilepsy. The archaic and evolutionary conserved nature of the piriform cortex from rodents to humans is likely to preserve similar circuitries and neurochemistry along with various species. Furthermore, the lack of powerful projections from the iso-thalamus may generate its unique susceptibility to develop seizure activity. In fact, limbic seizures may often become refractory to common antiepileptic drugs and may lack compensatory self-limiting mechanisms, leading to self- sustaining, long-lasting, often intractable SE. The chance to identify a small area in the human brain, which is likely to play a relevant role in shifting towards refractory seizures and SE is unique and deserves a specific focus. Within this context, experimental studies carried out in the past three decades on the role of the piriform cortex in limbic seizure generation and its specific brain circuitry may serve as a guide to improve our perspective on treating refractory epilepsy and refractory SE in humans. Previous experimental studies published by one of us (FF) in collaboration with Karen Gale [8] indicate that SE can be generated within the piriform cortex by focal micro-infusions of drugs enhancing the activity of AMPA receptors. In detail, preventing the natural desensitization of AMPA receptors either pharmacologically, with cyclothiazide (inhibitor of AMPA receptor desensitization), or *via* overexpression of the non-desensitizing GluA1 AMPA receptor subunit in the piriform cortex triggers continuous self-sustaining seizures up to SE. This can be prevented by the AMPA receptor antagonist, NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo (F) quinoxaline), thus reinforcing the crucial role of AMPA receptors in the piriform cortex to promote the transition from serial seizures to SE.

Thus, this allocortical area, which is crucial to trigger refractory limbic seizures in humans, strongly relies for its epileptogenic activity on the loss of desensitization of AMPA receptors. Conversely, AMPA receptor desensitization is expected to limit seizure duration and severity. Therefore, a failure of this mechanism, or an overabundance of naturally occurring, slowly desensitizing AMPA receptors, is likely to sustain the onset of severe and prolonged limbic seizures and SE, thereby resulting in seizure-induced brain damage [11] and seizure-induced neurodegeneration [12]. It is well known that an antiepileptic treatment with potent AMPA receptor antagonists cannot be carried out due to safety reasons and lack of selectivity. However, the connection between mTOR overactivity and AMPA receptor potentiation [13] allows us to consider the perspective of toning down the deleterious activity of AMPA receptors within the piriform cortex by suppressing mTOR. This is in line with data showing that seizures may be considered "mTORpathies," and mTOR overexpression may foster the onset of SE [14-16]. The protein complex mTOR was previously defined as the "mammalian target of rapamycin," and it is now defined as a "mechanistic target of rapamycin." This occurs in two structurally and functionally distinct complexes, mTORC1 and mTORC2. Both complexes work as a serine-threonine protein kinase, which controls several pathways, mainly the PI3K/PTEN/Akt cascade. Although both mTORC1 and mTORC2 are related to epilepsy, the role of mTORC1 is more investigated. In fact, mTORC1 is better described in the pathophysiology of epilepsy. The mTOR inhibitor, rapamycin, acts preferentially on mTORC1. It is demonstrated that mTOR upregulation increases the expression of those GluA1 AMPA receptor subunits which do not undergo natural desensitization [17]. As a consequence, one may hypothesize that inhibiting mTOR upregulation by using a classic mTORC1 inhibitor such as rapamycin leads to a loss of naturally occurring non-desensitizing AMPA receptors within the piriform cortex. This is expected to (i) reduce the transition from seizures into SE, (ii) promote self-limiting mechanisms which reduce seizure duration, (iii) mitigate seizure severity, and (iv) prevent seizure-induced brain damage.

2. RESULTS

In this article, we provide a synthetic perspective complemented by preliminary findings showing that the mTORC1 inhibitor, rapamycin, given systemically at the dose of 5 mg/Kg may occlude SE focally induced within the piriform cortex by blocking AMPA receptor desensitization. It is remarkable that the effects of rapamycin may extend at 1 hour after the onset of SE (Table **1**), which may prompt a novel drug to arrest SE once it is established. As shown in Fig. (**1**), various hippocampal subfields of the Cornu Ammonis (CA 1, 4), where SE-induced neuronal loss occurs, seem to be protected by rapamycin administered 1 hour after the onset of SE. Methods are reported in supplementary materials.

3. DISCUSSION

This hypothesis and representative data indicate that mTORC1 inhibition limits both the duration and severity of SE produced by a blocker of AMPA receptor desensitization. This effect is achieved 1 hour after the onset of SE, which strengthens the perspective of a powerful activity exerted by mTORC1 in the piriform cortex to promote seizure persistence, seizure duration, and eventually seizure propagation. It is remarkable that such an effect is not achieved by micro-infusing NMDA or AMPA receptor antagonists focally within the piriform cortex [8], which do not affect SE once it is established. In fact, the potentiation of AMPA receptors through the inhibition of its desensitizing properties is critical to trigger SE. However, when SE progresses, refractoriness to pharmacological modulation of the trigger site takes place. On the other hand, other brain areas placed downstream of the piriform cortex in the epileptic circuitry are believed to self-sustain continuous, spontaneous long-lasting seizures, characterizing the SE. The role of AMPA

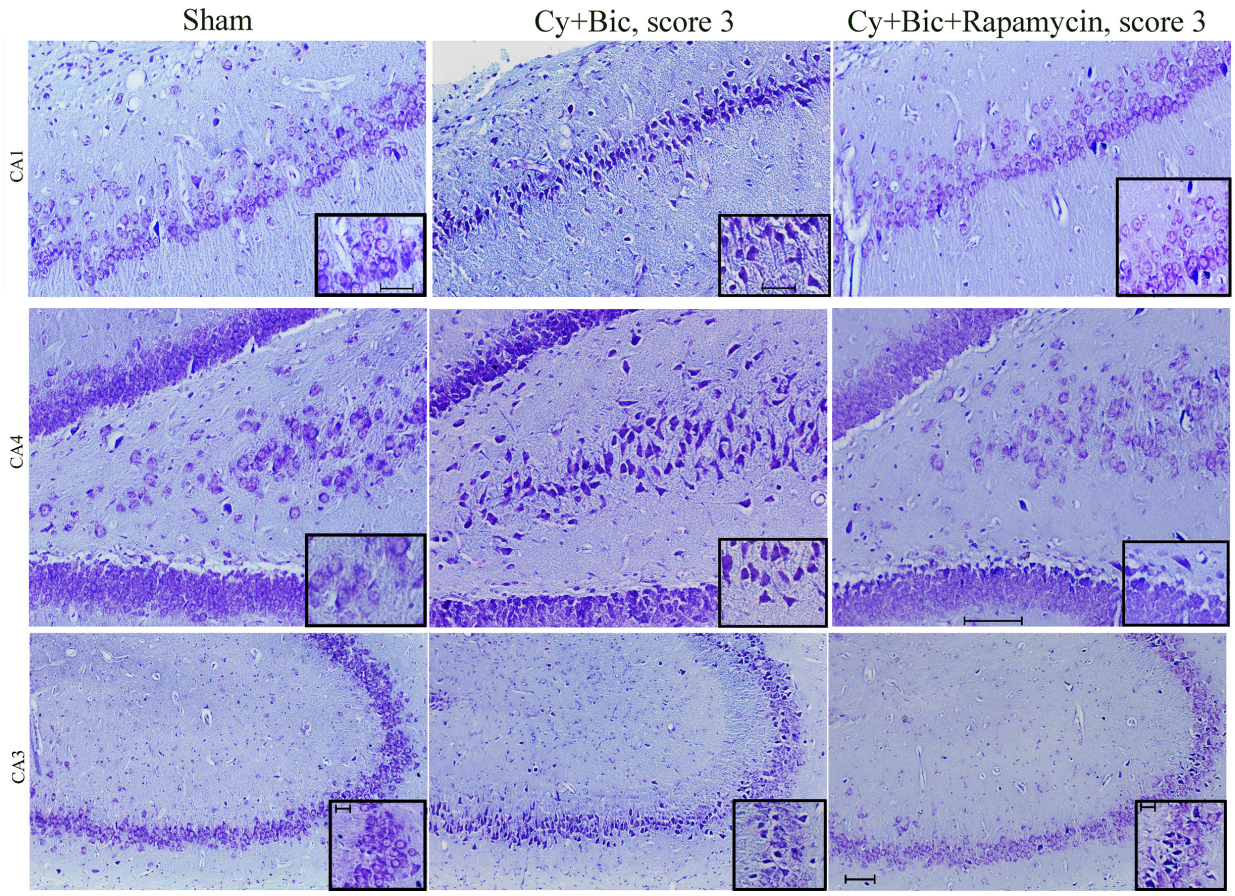

Fig. (1). Representative pictures from various hippocampal subfields (CA1; CA4, endfolium; CA3) showing a loss of pyramidal neurons and a change in morphology of spared neurons from the rat micro-infused with Cyclothiazide+Bicuculline (Cy+Bic). These changes were mostly occluded when Rapamycin (5 mg/Kg) was administered, i.p. at 1 hour following the onset of SE. In fact, the slices from the rat receiving Rapamycin were similar to the sham-operated (control rat). The slices belong to rats that were selected based on similar seizure duration and severity in order to adjust the effects of Rapamycin solely to neuroprotection (*i.e.,* ruling out the effects of seizure duration and severity). Seizures were triggered by micro-infusing Cyclothiazide (Cy) and bicuculline (Bic) within the piriform cortex of adult male Sprague Dawley rats (AP=+4 mm from the bregma, ML=+3.2 mm from the midline, and DV=−6.5 mm below the dura). Rats were sacrificed at 7 days after the seizure; brains were removed and placed in a fixing solution overnight and used for the morphological evaluation. The 10 µm thick slices were stained with Nissl staining to observe nucleic acids. Details of methods are provided along with that of Table **1** in the Supporting Materials. Scale bar 100 µm. In the insert scale bar, 60 µm. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

Table 1. Effects of rapamycin on SE evoked by cyclothiazide + bicuculline focally within the piriform cortex.

-	Seizure Duration (minutes)	Maximum Seizure score	
-	-	[1]time interval: 0-60 minutes	[2]time interval: 90-150 minutes
Cy+Bic	255.42 ± 21.38	4.14 ± 0.26	4.42 ± 0.20
Cy+Bic+ Rapamycin	138.00 ± 10.07 *	4.0 ± 0.31	1.71 ± 1.9 *

Legends: Seizures were triggered by micro-infusing Cyclothiazide (Cy) and bicuculline (Bic) within the piriform cortex of adult male Sprague Dawley rats (AP=+4 mm from the bregma, ML=+3.2 mm from the midline, and DV=−6.5 mm below the dura).
Seizure duration: time from seizure onset after micro-infusions into piriform cortex up to end seizures; time interval 0-60 minutes: a period of observation between the end of micro-infusions into piriform cortex up to 60 minutes later;
[1]time interval 0-60 minutes: a period of observation between the end of micro-infusions within piriform cortex up to 60 minutes later.
[2]time interval 90-150 minutes: a period of observation from 90 minutes after the end of micro-infusions. This corresponds to 30 minutes after the timing of systemic administration of either rapamycin ("Cy+Bic+Rapamycin" group) or saline ("Cy+Bic" group) into the piriform cortex, up to 150 minutes after the end of micro-infusion (*i.e.,* 90 minutes after either rapamycin or saline systemic administration). Rats micro-infused with Cy+Bic into the left piriform cortex experienced limbic seizures within 10 minutes after infusion, which progress to bilateral forelimb or generalized seizures; seizures become non-interrupted within 30 minutes from infusion, thus configuring a condition of SE. After administering rats with a solution of 3% DMSO in distilled water, i.p., at 60 minutes after Cy+Bic, SE persisted for up to 6 hours. Conversely, this was not the case for rats that were administered rapamycin, in which, in most cases, seizures ended within 60 minutes. Methods are reported in Supporting Materials. *$p < 0.05$ compared with "Cy+Bic.".

receptors within downstream brain areas is key in such a process. In fact, AMPA receptor antagonists, such as NBQX, are able to arrest limbic SE. Similarly, since autophagy removes AMPA receptor subunits, the effects of systemically administered rapamycin may likely inactivate AMPA receptors within the downstream areas to mitigate and arrest the propagation of SE. In summary, AMPA receptors within the piriform cortex are crucial to initiate SE and determine seizure refractoriness; however, AMPA receptors within this brain area become irrelevant to stop seizures once SE is established. AMPA receptors downstream to the piriform cortex still mediate seizure propagation.

The increase in AMPA receptor activity and the lack of desensitization are also observed in epilepsy-induced brain damage. In fact, AMPA overactivity can be significantly occluded by promoting AMPA receptor degradation, which is induced by autophagy activation. One of the major pathways inhibited by mTOR overexpression is autophagy, and enhancement of autophagy leads to a powerful degradation of AMPA receptors [18]. Therefore, it is expected that treatment with rapamycin decreases the amount of AMPA receptors, thereby preventing seizure refractoriness and SE and mitigating seizure-induced excitotoxicity [19]. The beneficial role of autophagy in seizure-induced brain damage has been recently postulated [20]. Thus, we pose the perspective that rapamycin by activating autophagy, which clears AMPA receptors in the hippocampus [18], may protect against hippocampal damage in the course of AMPA-dependent SE. This is in line with recent studies on the role of autophagy in epilepsy-induced brain damage. In this way, it is now increasingly recognized that what was once defined as mTORpathy in epileptogenesis may be mostly explained by abnormalities in the autophagy machinery. Such a scenario provides novel pharmacological perspectives to limit seizure duration and severity.

CONCLUSION

The piriform cortex is a highly conserved allocortical area in rodents and primates which is known to be highly sensitive to the epileptogenic drug. Recent data have provided a striking translational value for this brain site, which, upon removal, leads to an improvement of refractory limbic seizure and SE. Therefore, the in-depth pharmacology of the piriform cortex as deciphered in rodents and *Macaca nemestrina* represents a strong perspective that is expected to be translated into clinical practice. The current neuropharmacology of the piriform cortex indicates a crucial role for AMPA receptors desensitization. Similarly, recent evidence has emphasized the powerful role of mTOR upregulation in intractable epilepsy. This perspective article indicates the intersection between the pharmacology of SE triggered by the piriform cortex and the beneficial effect sorted by inhibiting mTOR. The present evidence sheds new light on the treatment of refractory seizures and self-sustaining SE, which extend to epilepsy-induced brain damage. Since AMPA receptor antagonists are not feasible to be used as a systemic drug to treat seizures, micro-infusions of AMPA antagonists within these regions may reveal which brain area is mostly involved. In this scenario, it is likely that the peri-amygdaloid cortex, a region placed just downstream to the piriform cortex, may have a relevant role. On the other hand, drug development of mild and safe AMPA antagonists such as perampanel may represent an effective therapeutic strategy [19].

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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