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Regulation of cell death and epileptogenesis by the mammalian target of rapamycin (mTOR)

A double-edged sword?

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Identification of cell signaling mechanisms mediating seizure-related neuronal death and epileptogenesis is important for developing more effective therapies for epilepsy. The mammalian target of rapamycin (mTOR) pathway has recently been implicated in regulating neuronal death and epileptogenesis in rodent models of epilepsy. In particular, kainate-induced status epilepticus causes abnormal activation of the mTOR pathway, and the mTOR inhibitor, rapamycin, can decrease the development of neuronal death and chronic seizures in the kainate model. Here, we discuss the significance of these findings and extend them further by identifying upstream signaling pathways through which kainate status epilepticus activates the mTOR pathway and by demonstrating limited situations where rapamycin may paradoxically increase mTOR activation and worsen neuronal death in the kainate model. Thus, the regulation of seizure-induced neuronal death and epileptogenesis by mTOR is complex and may have dual, opposing effects depending on the physiological and pathological context. Overall, these findings have important implications for designing potential neuroprotective and antiepileptogenic therapies that modulate the mTOR pathway.

Key words: kainate, apoptosis, epilepsy, seizure, rat

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Introduction

Epilepsy is one of the most common neurological disorders, affecting approximately 1% of people, and is characterized by significant morbidity and mortality. Although there are a variety of underlying causes for epilepsy, seizures themselves are often implicated in causing progressive epileptogenesis and neuronal death, contributing to medically-intractable epilepsy and co-morbid cognitive deficits. Currently available medications simply suppress seizure symptomatically, but do not appear to prevent seizure-induced brain injury or reverse the underlying mechanisms of epileptogenesis.[1] Thus, it is now widely recognized that novel therapies for epilepsy need to be developed that have neuroprotective, antiepileptogenic or disease-modifying properties.[2-4]

In order to develop disease-modifying therapies for epilepsy, a better understanding of the cellular and molecular mechanisms of epileptogenesis and seizure-induced brain injury is required. While traditionally seizure medications have targeted ion channels and neurotransmitters receptors that directly contribute to neuronal excitability, a recent trend has been to identify and target primary cell signaling pathways that initially trigger downstream mechanisms mediating neuronal injury and epileptogenesis. The mammalian target of rapamycin (mTOR) signaling pathway represents a rational candidate, because mTOR regulates numerous cellular functions and mechanisms that affect cell survival and death, neuronal excitability and epileptogenesis.[5] Furthermore, available drugs exist that specifically inhibit mTOR and could be readily tested as neuroprotective and antiepileptogenic therapies for epilepsy.

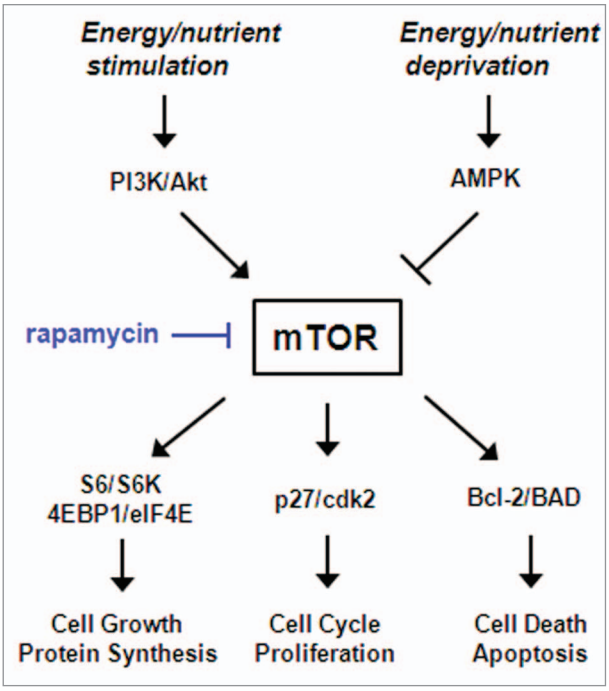

Figure 1. Regulation and effectors of the mTOR signaling pathway. In response to environmental or physiological stimuli, upstream pathways, primarily involving cascades of protein kinases, may either activate (PI3K/Akt) or inhibit (AMPK) mTOR via modulation of the tuberin-hamartin complex and Rheb GTPase (not shown). In turn, mTOR controls multiple downstream pathways/effectors that individually modulate cell growth and protein synthesis, cell cycle and proliferation, and cell death/apoptosis. Abbreviations: 4EBP1, elongation factor 4E binding protein 1; AMPK, AMP-activated protein kinase; eIF4E, elongation initiation factor 4E; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositide-3 kinase.

mTOR: A Central Regulator of Protein Synthesis, Cell Growth and Cell Death

mTOR is a serine-threonine protein kinase that is highly conserved among species and is implicated in a number of basic cellular functions, broadly related to growth, proliferation, survival, homeostasis and death.[6-9] Under physiological conditions of cellular growth and anabolism, the mTOR pathway becomes activated and promotes protein synthesis, increased cell size, and cellular proliferation, whereas during states of metabolic stress or catabolism, mTOR activity may be inhibited, thus limiting these cellular processes. mTOR has also been implicated in regulating neuronal death, in particular apoptosis, although this relationship is complicated, with mTOR having both pro-apoptotic and anti-apoptotic actions depending on the physiological or pathological conditions.

A number of upstream signaling pathways can modulate mTOR activity in response to environmental stimuli or intracellular signals, including nutrient and energy status, growth factors and stress (**Fig. 1**). Thus, during anabolic states in the presence of nutrients, growth factors or insulin, specific upstream regulators, such as the phosphatidylinositol-3 kinase (PI3K)/Akt (protein kinase B) pathway, stimulate mTOR, leading to increased protein synthesis, cellular growth and proliferation.[10,11] In catabolic states with energy or oxygen deprivation, other upstream pathways, such as AMP-kinase (AMPK), inhibit mTOR activity, thus limiting protein translation and cellular growth, proliferation and metabolism.[12,13] Activation or inhibition of mTOR by upstream pathways is generally accomplished through opposing effects on the tuberous sclerosis gene products, hamartin and tuberin, and the small GTPase protein, Rheb.

Downstream from mTOR, there are multiple signaling pathways that mediate the various functional effects of mTOR (**Fig. 1**). The anabolic actions of mTOR on cell growth are primarily accomplished by stimulation of protein synthesis. mTOR triggers activity of ribosomal S6 kinase-1 (S6K1), which phosphorylates the ribosomal protein S6, promoting ribosomal biogenesis and protein translation.[14-16] In addition, mTOR induces an inhibition of the elongation factor 4E binding protein 1 (4EBP1) and subsequent activation (release of inhibition) of the mRNA elongation initiation factor 4E (eIF4E), also triggering protein synthesis.[15,16] Parallel to regulation of the S6K/S6 and 4EBP1/eIF4E pathways in stimulating protein synthesis and cell growth, other signaling elements downstream from mTOR, such as p27/cyclin-dependent kinases, are responsible for mediating mTOR regulation of cell cycle progression and proliferation.[17-19] Furthermore, mTOR may directly modulate separate mechanisms controlling neuronal death, such as the pro-apoptotic molecules, BAD and Bcl-2.[20,21] Overall, the mTOR signaling pathway is in a central position to serve as a master regulator of multiple, interrelated functions and mechanisms relevant to cell growth, proliferation and death.

mTOR: A Central Regulator of Seizure-Related Neuronal Death and Epileptogenesis

The mTOR pathway has emerged as a leading candidate for a signaling mechanism that could be involved in seizure-related neuronal death and epileptogenesis in several types of epilepsy.[5] In mouse models of the genetic epilepsy, Tuberous Sclerosis Complex, excessive activity of the mTOR pathway due to inactivation of the upstream regulators, *TSC1* or *TSC2*, promotes epileptogenesis, neuronal hypertrophy and glial proliferation, and the mTOR inhibitor, rapamycin, prevents the development of epilepsy and the underlying cellular and histological brain abnormalities in these mice.[22,23] Rapamycin also reverses similar histological and behavioral abnormalities in related genetic models with abnormal mTOR signaling due to upstream *PTEN* gene inactivation.[24-26] By analogy, given the central role of mTOR in multiple cellular functions relevant to neuronal survival and excitability, the mTOR pathway has

also been implicated in mediating neuronal death and epileptogenesis in rodent models of acquired epilepsy due to brain injury. The mTOR pathway is activated in animal models of traumatic brain injury (TBI) and rapamycin has neuroprotective effects against neuronal death and functional deficits following TBI, although effects on posttraumatic epilepsy have not been described.[27,28] mTOR is also triggered in the pilocarpine model of acquired epilepsy and mediates axonal sprouting, a putative mechanism of epileptogenesis.[29] In a recent study, we have reported that the mTOR pathway is involved in neuronal death and epileptogenesis in the related kainate model of limbic epilepsy in rats.[30] In the kainate model, an initial episode of prolonged seizures (status epilepticus), induced by administration of the glutamate agonist, kainate, triggers neuronal death and other cellular and molecular changes that promote epileptogenesis. After recovery from the status epilepticus and following a latent period of days to weeks, these changes lead to the development of spontaneous seizures. In this model, we showed that kainate causes activation of the mTOR pathway both acutely during status epilepticus and more chronically for several weeks coinciding with the latent period of epileptogenesis.[30] The mTOR inhibitor rapamycin prevents the abnormal kainate-induced mTOR activation and, depending on the timing of the rapamycin administration, causes a variable decrease in putative cellular mechanisms of epileptogenesis, including hippocampal neuronal death, neurogenesis and axonal sprouting. Rapamycin also causes a corresponding decrease in the development of spontaneous seizures.[30] Thus, these studies suggested that mTOR plays a critical role in activating multiple downstream mechanisms of neuronal injury and epileptogenesis in the kainate model and that rapamycin has neuroprotective and antiepileptogenic actions in this model.

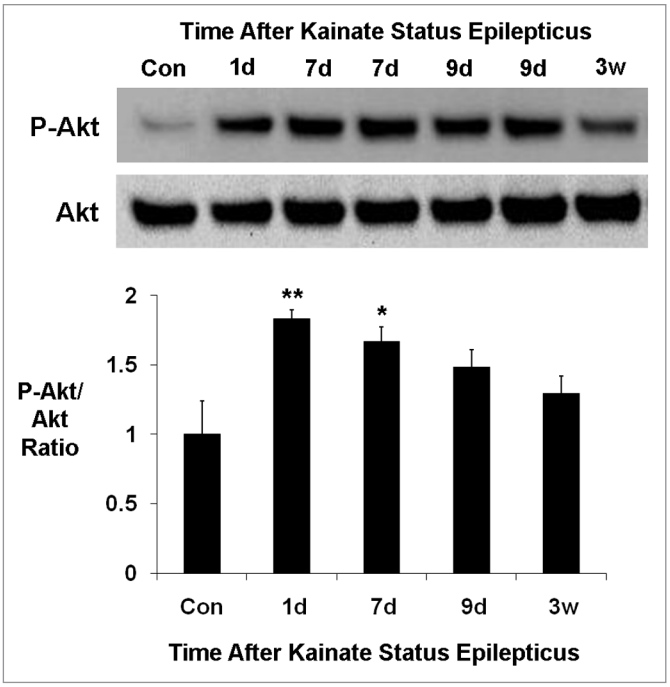

Figure 2. Kainate status epilepticus activates Akt, an upstream regulator of the mTOR pathway. Western blots of hippocampal homogenates were performed at various time points following kainate status epilepticus in adult rats. Compared to control rats, the ratio of P-Akt to total Akt was increased acutely with kainate status epilepticus and remained elevated for several weeks. *p < 0.05, **p < 0.01 by ANOVA, compared to the control group.

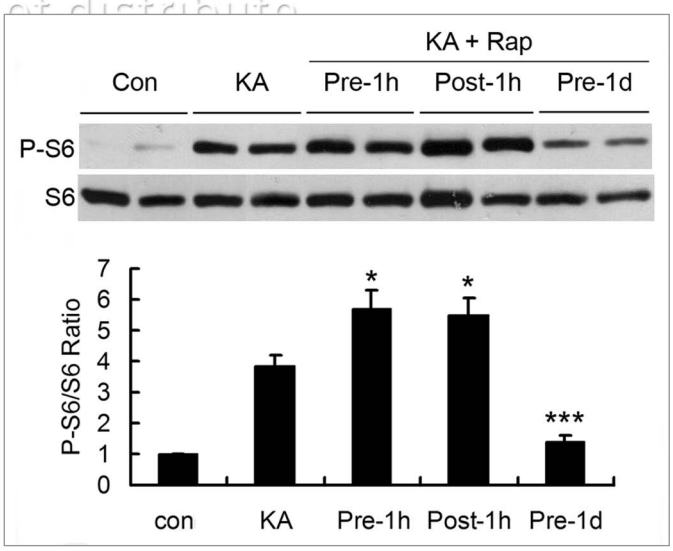

Figure 3. Rapamycin causes paradoxical exacerbation of kainate-induced mTOR activation when administered within one hour of kainate. Adult rats were injected with vehicle (Con), kainate (15 mg/kg, i.p.), or rapamycin (6 mg/kg) at different intervals before or after kainate. Kainate alone (KA) causes increased mTOR activation, as reflected by the ratio of phospho-S6 to total S6 expression measured 7 days after kainate injection, compared to vehicle (Con). Pretreatment with rapamycin one day prior to kainate inhibits the kainate-induced mTOR activation (Pre-1d). In contrast, rapamycin administered within one hour before (Pre-1 h) or after (Post-1 h) kainate causes a paradoxical increase in the kainate-induced mTOR activation. *p < 0.05, ***p < 0.001 by ANOVA, compared to the KA group.

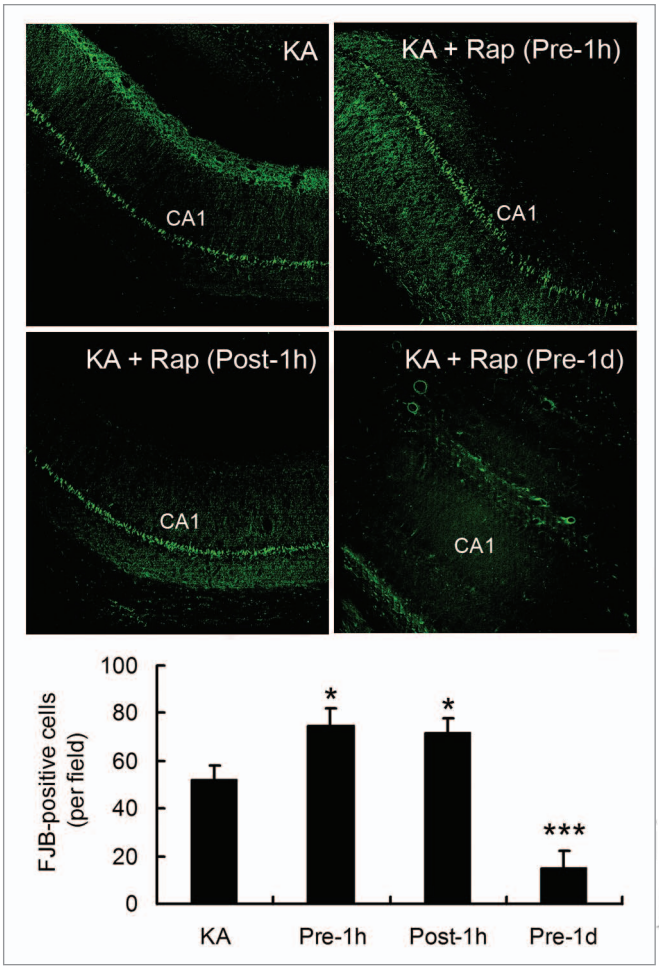

Figure 4. Rapamycin causes paradoxical exacerbation of kainate-induced cell death when administered within one hour of kainate. Kainate status epilepticus causes cell death in the CA1 region of hippocampus, as detected by Fluoro-Jade B (FJB) staining 7 days after status epilepticus. Pretreatment with rapamycin one day prior to kainate inhibits the kainate-induced neuronal death (Pre-1d). In contrast, rapamycin administered within one hour before (Pre-1 h) or after (Post-1 h) kainate causes a paradoxical increase in the kainate-induced cell death. *p < 0.05, ***p < 0.001 by ANOVA, compared to the KA group.

Upstream Activation of the mTOR Pathway by Kainate-Induced Status Epilepticus

The specific mechanisms involved in mTOR pathway regulation of seizure-related neuronal death and epileptogenesis deserve further attention. In particular, the upstream signaling pathways that trigger mTOR activation in the kainate model are not known. Given that kainate is a glutamate agonist and that large amounts of endogenous glutamate are released during status epilepticus, a rational hypothesis is that stimulation of the Akt/PI3K pathway by glutamate[31,32] and calcium influx during kainate status epilepticus causes the downstream activation of the mTOR pathway (**Fig. 1**). Other recent studies from our lab support that Akt is activated by kainate status epilepticus acutely and more chronically over a couple of weeks, correlating with mTOR pathway activation that occurs during the latent period of epileptogenesis (**Fig. 2**). In contrast, no acute alterations in activation of the AMPK signaling pathway, also upstream from mTOR, were observed with kainate (data not shown). In addition to further defining the cell signaling mechanisms involved, these findings provide potential additional strategies for preventing seizure-induced neuronal death and epileptogenesis, as the Akt/PI3K could be targeted with specific PI3K inhibitors.

Paradoxical Activation of the mTOR Pathway and Exacerbation of Neuronal Death by Rapamycin in the Kainate Model

The number and complexity of upstream and downstream signaling pathways and mechanisms that may regulate and mediate the effects of mTOR, as well as multiple positive and negative feedback steps that occur among these pathways, suggest that the mTOR pathway may have complicated, potentially dual effects on some cellular functions. For example, depending on the physiological or pathological conditions, mTOR activation has been demonstrated to have both pro-apoptotic and anti-apoptic effects.[20,21] In pilot studies to determine effective dosing regimens of rapamycin for inhibiting the kainate-induced mTOR activation,[30] we initially observed a paradoxical exacerbation of the increased mTOR pathway activity, as reflected by downstream P-S6 expression, when rapamycin and kainate were administered within a short time period of each other. Thus, in our published studies, we only injected rapamycin at least twenty-four hours before (pretreatment) or after (posttreatment) kainate in order to obtain the expected inhibition of mTOR activity.[30] Further analysis of the initial paradoxical phenomenon has confirmed that rapamycin administered within one hour of kainate injection causes a higher level of mTOR activation than kainate in the absence of rapamycin (**Fig. 3**). If rapamycin is injected at greater intervals from the kainate, the expected inhibition of mTOR activation is again observed. The paradoxical mTOR activation by rapamycin is associated with greater neuronal death several days after kainate status epilepticus (**Fig. 4**). Thus, under limited circumstances, it appears that rapamycin causes a paradoxical activation of the mTOR pathway, and depending on the situation, rapamycin has the potential to have either neuroprotective or exacerbating effects on cell death.

The specific molecular mechanisms leading to this paradoxical activation of the mTOR pathway by rapamycin in the kainate model are unknown. A speculative explanation is that during periods of especially high mTOR activity, such as during

the acute phase of kainate-induced seizure activity, mTOR inhibition by rapamycin may allow alternative feed forward pathways for stimulating downstream mTOR effectors, such as P-S6, to be activated and overcompensate for direct mTOR inhibition. Future studies are needed to define these mechanisms. Irregardless of the specific mechanisms, these findings highlight the complexity of the involvement of the mTOR pathway in responding to upstream stimuli and in turn, regulating downstream effectors. The mTOR pathway may have opposing effects on mechanisms of neuronal death and epileptogenesis, depending on the situation. This has direct clinical implications for the use of mTOR inhibitors as potential neuroprotective and antiepileptogenic agents, as there could be circumstances in which such treatment could worsen neurological status.

Conclusions

mTOR is a central signaling pathway that regulates a number of important cellular functions and mechanisms involved in seizure-related cell death and epileptogenesis. Work in animal models of both genetic and acquired epilepsies suggests that modulators of the mTOR pathway may have beneficial neuroprotective and antiepileptogenic effects. However, paradoxical effects of mTOR inhibition have also been observed and suggest that mTOR may serve as a master switch that can trigger opposing actions on neuronal death and epileptogenesis under different conditions. This dual role of mTOR needs to be considered in designing potential therapies for epilepsy that modulate the mTOR pathway.

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