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The mTOR Signaling Pathway in the Brain: Focus on Epilepsy and Epileptogenesis

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Abstract Recent evidence suggests that an altered mammalian (mechanistic) target of rapamycin (mTOR) signaling pathway and its pharmacological modulation might be implicated in several neurological diseases including epileptogenesis. mTOR is a molecular sensor, which regulates protein synthesis, enhancing mRNA translation of genes involved in the regulation of cell proliferation and survival, working as part of two distinct multimeric complexes known as mTORC1 and mTORC2. mTOR is an evolutionarily highly conserved serine/threonine kinase belonging to the phosphoinositide 3-kinase-related kinase family and represents one of the most recently studied pathways in relation to epilepsy and epileptogenesis, due to its suggested pivotal role in many aspects of cellular proliferation and growth also including neurodegeneration, neurogenesis, and synaptic plasticity. In this review, we report the cellular and molecular features of mTOR and related pathways, analyze their function in the brain including all current related evidence of their role, and finally, discuss the possible involvement of mTOR signaling in epileptogenesis and epilepsy, giving further consideration to future developments in this area.

Keywords Rapamycin · Antiepileptogenic · Tuberous sclerosis complex (TSC) · Seizure · Temporal lobe epilepsy (TLE) · Inflammation

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Introduction

The mammalian target of rapamycin (mTOR), also known as mechanistic target of rapamycin or FK506 binding protein 12-rapamycin associated protein 1, is a 289-kDa serine/threonine protein kinase that is involved in a wide spectrum of fundamental cellular biochemical and physiological processes ranging from regulation of cell growth, development, and proliferation to adaptive immune function [1, 2]. Rapamycin itself (also known as sirolimus) is a naturally occurring macrolide antibiotic molecule derived from the Easter Island soil-dwelling bacterium *Streptomyces hygroscopicus* that is widely used as an antifungal, immunosuppressant, and anticancer agent, through its specific ability to inhibit some mTOR functions [3]. Not surprisingly, the molecular factors and downstream target signaling molecules associated with the mTOR pathway are numerous and complex [4]. In the central nervous system (CNS), mTOR has been associated with the development of synaptic plasticity and memory function, as well as neuronal repair mechanisms after injury [5]. Recent evidence also suggests that an altered mTOR signaling pathway might be implicated in several neurological diseases such as Alzheimer's, Parkinson's, and Huntington's diseases as well as tuberous sclerosis, epilepsy, and epileptogenesis [5, 6]. The term epileptogenesis refers to a cascade of events, with or without a previously identified insult (e.g., traumatic brain injury, infection, or genetic predisposition), which culminates in the occurrence of repetitive spontaneous seizures [7]. Currently, epileptogenesis or the "silent (latent) phase" is defined operationally as the period intervening between a previous brain insult and the appearance of the first spontaneous seizure [7–9]. Many molecular and cellular alterations have already been identified in both humans and experimental animal models (for a review, see [7]) as being associated with epileptogenesis, with involvement of several inflammatory and

immune mediators [10] and changes in gene expression [11] with the aim of identifying new targets for novel antiepileptogenic therapies; however, this goal has not been completely achieved, and some concerns about possible adverse effects linked with therapeutic treatment target intervention have been raised [7, 12]. During epileptogenesis, many changes including neurodegeneration, neurogenesis, gliosis, and recruitment of inflammatory cells into the brain have been characterized. However, the whole story is complicated by the progressive nature of epileptogenesis, where such changes are differently involved during the various phases of the process [13]; this has led to many controversies in the literature, particularly when considering gene expression, where the majority of observed changes seem to be laboratory specific and are rarely in common with findings reported in other publications [12]. In our view, more studies are warranted in order to clarify the exact sequence of mechanisms involved in the epileptogenic process.

An antiepileptogenic drug can also be defined as a treatment able to prevent the development of spontaneous seizures. In this light, several clinical trials have been conducted in human patients, looking for possible antiepileptogenic effects of some established currently marketed antiepileptic drugs (AEDs) following brain trauma, but none of the tested compounds were shown to prevent seizure onset [14]. A number of experimental studies using different agents and animal models have also attempted in the past to demonstrate or prove antiepileptogenic efficacy of AEDs; however, translation of findings to humans obviously remains difficult, and a better understanding of the epileptogenic process seems to be mandatory in order to improve the chances of success [7, 14]. At least some of the changes taking part in the epileptogenic process are also in common with other neurological disorders and might be responsible for the development of comorbid diseases in epilepsy (e.g., depressive disorders, cognitive and emotional impairment). Therefore, this aspect should also be taken into account as a further stimulus for intensifying our efforts to understand epileptogenic mechanisms [15]. Most current "first generation" AEDs (e.g., phenobarbital, phenytoin, carbamazepine, valproate) and more newer compounds (e.g., lamotrigine, gabapentin, topiramate, tiagabine, levetiracetam) as far as we know act primarily to counteract cellular mechanisms involved in generating the hyperexcitability symptoms of epilepsy [16–18]. Accordingly, several alternate molecular targets have been proposed over the years, both as antiepileptic and antiepileptogenic modulatory sites [7, 19, 20]. Within these, mTOR and its associated signaling pathways have been some of the most recently studied, due to their suggested pivotal role in many aspects of cellular proliferation and growth, also including neurogenesis, age-related neurodegeneration, synaptic plasticity, and memory formation [21]. In this review, we will summarize the main functions of mTOR and related pathways in the brain, including the most recent experimental evidence of their physiological roles, and discuss the possible involvement of mTOR in epilepsy and epileptogenesis, with a particular focus on likely future developments in this area, including a better understanding of roles, new drug development, and disease progression.

The mTOR Signaling Pathway: an Overview

mTOR is a molecular sensor, which under the influence of various growth factors, mitogens, and hormones, regulates protein synthesis, enhancing mRNA translation of genes involved in the regulation of cell proliferation and survival and promoting cell cycle progression from G1 to S phase. The mTOR kinase works as part of two distinct multimeric complexes, known as mTORC1 and mTORC2 [5, 22–24]. mTOR is an evolutionarily highly conserved serine/threonine kinase belonging to the phosphoinositide 3-kinase (PI3K)-related kinase family. It lies at the nexus of the regulatory network and acts as a sensor that integrates extracellular and intracellular events [25] (Fig. 1).

Many important oncogenic signaling molecules such as PI3K, AKT (PKB), epidermal growth factor receptor (EGFR), human epidermal receptor growth factor 2 (HER2/neu), and BCR-ABL stimulate cell proliferation, growth, and survival, by working as upstream modulators of mTOR kinase [26] (see Table 1 for a list of signaling components). Most of these modulators have been studied using various experimental protocols and their mechanisms have been clarified. Similarly, the downstream signaling molecules regulated by the mTORC complexes have been identified and their regulation and role has been studied [27]. Although interest in mTOR has dramatically increased in the past few years, it is evident that little is still known about the number and identity of mTOR activity effectors [5, 28, 29]. mTOR is activated by phosphorylation and exerts its function mainly through two different, but related complexes, namely, mTORC1 and mTORC2 [4, 30–32] (Fig. 2). The two different complexes are constituted by mTOR and several partners with two common proteins shared by both mTORC1/2: mLST8 (mammalian lethal with Sec13 protein 8, also known as GβL), which represents a protein homologue of the heterotrimeric G protein β subunit, being a positive regulator of both complexes [33], and DEPTOR (DEP-domain containing mTOR-interacting protein), which is a recently identified physiological negative regulator of both mTORC1/2 complexes [34]. mTORC1 is completed by further two specific proteins, RAPTOR (regulatory-associated protein of mTOR), which is a positive regulator also involved in substrate recruitment, and PRAS40 (proline-rich AKT substrate of 40 kDa), which

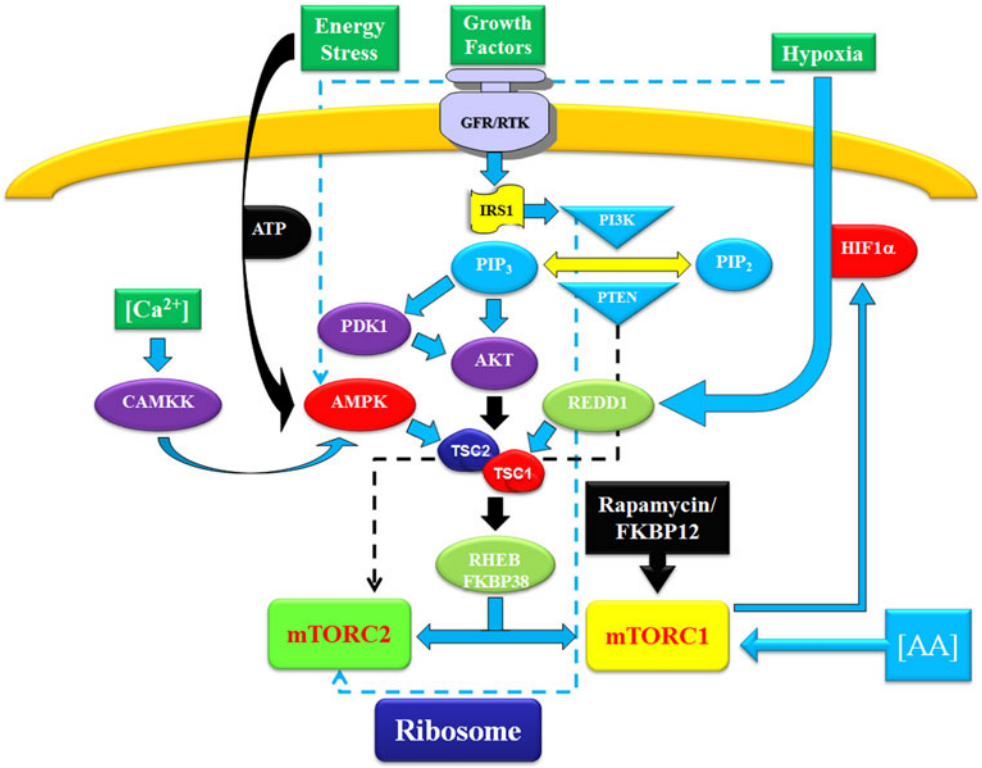

Fig. 1 Overview of the mTOR signaling pathway. Mammalian target of rapamycin (mTOR) is a 289-kDa serine/threonine protein kinase and a member of the phosphatidylinositol 3-kinase-related kinase (PIKK) family. The mTOR pathway is regulated by a wide variety of cellular signals, including mitogenic growth factors, hormones such as insulin, nutrients (amino acids, glucose), cellular energy levels, and stress conditions. A principal pathway that signals through mTOR is the PI3K/AKT signal transduction pathway. Signaling through this pathway is initiated by growth factor receptors. These include insulin-like growth factor receptor (IGFR), platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), and the Her family. The signal from the activated receptors is transferred directly to the PI3K/AKT pathway. PI3K then catalyzes the conversion of membrane-bound phosphatidylinositol (4,5)-bisphosphate (PIP_2) to phosphatidylinositol (3,4,5)-triphosphate (PIP_3). PIP_3 then activates AKT, which can also be activated by phospholipid-dependent kinase-1 (PDK-1). AKT works indirectly on mTOR through the actions of the TSC1/TSC2 complex (tuberous sclerosis complex). The association of the two proteins TSC1 (hamartin) and TSC2 (tuberin) produces a complex that through TSC2 inactivation of a Ras family small GTPase known as Ras homolog enriched in brain (RHEB) inhibits mTOR. Adenosine 5′-monophosphate-activated protein kinase (AMPK) can also modulate mTOR. Increases in the cellular adenosine 5′-monophosphate (AMP)/adenosine triphosphate (ATP) ratio promote AMPK phosphorylation, which in turn phosphorylates TSC2, apparently promoting its activation. This inhibits the action of mTOR activity. The protein REDD1 potently inhibits signaling through mTOR, working downstream of AKT and upstream of TSC2 to inhibit mTOR functions. Another well-known drug inhibitor of mTOR is rapamycin. When complexed with its cellular receptor, FK506 binding protein-12 (FKBP12), rapamycin binds directly to mTOR to inhibit downstream signaling. *Blue arrows* indicate activation. *Black arrows* indicate inhibition

has a suppressive action, disinhibited by phosphorylation by the serine/threonine kinase AKT. PRAS40 is also known as AKTS1 (AKT1 substrate [35]). mTORC2 has six components, three in common with mTORC1 (mTOR, DEPTOR, and mLST8) and three specific proteins—RICTOR (rapamycin-insensitive companion of mTOR), which plays a role in the activating interaction between mTORC2, and tuberous sclerosis complex 2 (TSC2), which is a direct activator of this complex [36]; mSIN-1 (mammalian stress-activated protein kinase interacting protein), which is necessary for the assembly of the complex and for its capacity to phosphorylate AKT [37]; and PROTOR-1 (protein observed with RICTOR-1), which has been shown to bind to RICTOR [38] and seems to play a role in enabling mTORC2 to efficiently activate serum- and glucocorticoid-induced kinase 1 (SGK1) [39]. The precise function of the mTORCs is still under debate. Whereas the role of mTORC1 in response to growth factors, mitogens, nutrients, and stress has been well established for regulating cell growth and proliferation, the role of mTORC2 has been less studied and it seems to be more involved in cell survival and cycle progression, being insensitive to nutrients or cellular energy conditions. However, mTORC2 phosphorylates AKT in response to hormones or growth factors and regulates the actin cytoskeleton and cell survival. These findings not only reveal the crucial role of mTOR signaling in physiology and pathology, but also reflect the extraordinary complexity of the mTOR signaling network [27] (Fig. 2).

Table 1 Summary table of mTOR signaling components and relative molecular, physiological, and pathological functions

Abbreviation	Component	Role and function	References
AKT kinase (also known as PKB)	v-akt murine thymoma viral oncogene homolog; protein kinase B	One of the most important serine–threonine kinases for cell survival. After phosphorylation, AKT regulates different cellular processes including cell growth, proliferation, apoptosis, and glucose metabolism	[73]
AMPK	AMP (adenosine 5′-monophosphate)-activated protein kinase	AMPK is a sensor of energy status that maintains cellular energy homeostasis. Furthermore, it regulates mitochondrial biogenesis and disposal, autophagy, cell polarity, and cell growth and proliferation	[198]
FKBP12	FK506 binding protein-12	FKBP12 binding with rapamycin inhibits mTORC1. Furthermore, it regulates intracellular calcium release, cellular trafficking, and gene expression	[199]
FKBP38	FK506 binding protein-38	FKBP38 has a role in apoptosis and is further involved in the regulation of mTOR, regulation of neural tube formation, regulation of cellular hypoxia response, and hepatitis C virus replication	[199, 200]
HIF-1α	Hypoxia-inducible factor 1α	Transcription factor regulating a wide spectrum of biological processes, such as angiogenesis, inflammation, bioenergetics, proliferation, motility, and apoptosis	[201]
IRS1	Insulin receptor substrates	Carries out various functions downstream of insulin (and IGF) receptors by providing a juxtamembrane localization signal for PIP_3 generation, amplifying the signal engendered by receptor autophosphorylation and engaging an array of substrates that account for the diverse actions of insulin	[202, 203]
PDK1	Phospholipid-dependent kinase-1	PDK1 has an essential role in regulating cell migration especially in the context of PTEN deficiency. It can also directly activate AKT	[204]
PI3Ks	Phosphoinositide 3-kinases	PI3K then catalyzes the conversion of membrane-bound PIP_2 to PIP_3. They control key signaling pathways in cancer cells, leading to cell proliferation, survival, motility, and angiogenesis	[205]
PIP3	Phosphatidylinositol (3,4,5)-triphosphate	The downstream effects of increased PIP_3 levels are diverse and cell type specific. Increased proliferation, survival, and motility are some of the main cellular effects associated with the increased PIP_3 levels that could contribute to its tumorigenic effects	[206]
PTEN	Phosphatase and tensin homolog	PTEN dephosphorylates the 3-phosphoinositide products of PI3K, therefore, negatively regulates the PI3K–AKT–mTOR pathway, which is an important regulator of cell growth and survival. Relevant role in tumorigenesis	[207]
REDD1	Regulated in development and DNA damage responses 1	REDD1 is activated under stress conditions such as hypoxia and negatively modulates mTOR signaling	[208]
RHEB	Ras homolog enriched in brain	RHEB is a Ras family small GTPase which activates mTORC1. Its expression is increased after seizures and by NMDA receptors	[209, 210]
TSC1 and 2	Tuberous sclerosis complex; TSC1=hamartin; TSC2=tuberin	The TSC1/2 complex has been found to play a crucial role in an evolutionarily conserved signaling pathway that regulates cell growth: the mTORC1 pathway. In the CNS, the TSC1/2 complex not only regulates cell growth/proliferation, but also orchestrates an intricate and finely tuned system that has distinctive roles under different conditions, depending on cell type, stage of development, and subcellular localization	[211]

For details on the role of all these components, see also Fig. 1 and Table 3

mTORC complexes also differ in their sensitivity to rapamycin that inhibits mTOR by complexing with the binding protein FKBP12 (FK 506-binding protein of 12 kDa) and subsequently inhibiting mTOR phosphorylation. mTORC2 was originally thought to be rapamycin insensitive [40]; however, further studies demonstrated that after prolonged treatment, rapamycin inhibits the assembly and function of mTORC2 as well, at least in some cell lines [41]. In an attempt to develop better drugs to define the role of the mTOR signaling pathway, several new molecules have been developed that can inhibit the function of both mTORC1 and mTORC2 and also demonstrate efficacy in preclinical cancer models such as in vitro and in vivo models of breast, ovarian, lung, hematological, and prostate malignancies [25, 42–45] (Table 2).

As mentioned above, several upstream regulators and downstream targets have been identified for both mTORC complexes (Fig. 1; Table 3). In the following subsections, an overview of the general functions of both mTORC1/2 is given, whereas the roles of mTOR in the brain and particularly in epileptogenesis are summarized in the later sections of the review.

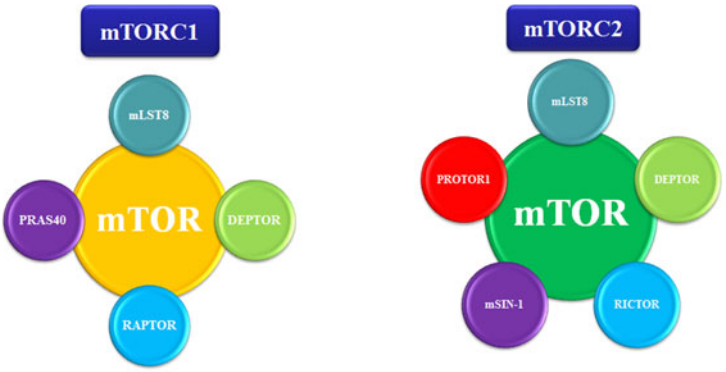

Fig. 2 mTOR complexes and their known substrates. mTOR can form two distinct complexes, mTORC1 and mTORC2, depending on its associated protein binding partners

Known Substrates for mTORC1		Known Substrates for mTORC2
S6Ks	PKCs	AKT
4E-BPs	PP2A	PKCs
CLIP-170	p21Cip1	RHO
eEF2	p27Kip1	SGKs
ODC	Rb	FAK
HIF-1a	STAT3	GTPases
Lipin	ULK1	

Table 2 Overview of mTOR inhibitors and modulators

mTORC1-selective inhibitors	Mixed/indirect inhibitors
Deforolimus (AP23573)	3,3-Diindolylmethane (DIM)
Everolimus (RAD001)	Curcumin
Rapamycin	Genistein
Temsirolimus (CCI-779)	Resveratrol epigallocatechin gallate (EGCG)
mTORC1/mTORC2 dual inhibitors	mTOR/PI3K dual inhibitors
AZD2014	BGT226
AZD8055	GDC0980
CC223	GSK2126458
INK128	LY294002
Ku0063794	NVP-BBD130
OSI-027	NVP-BEZ235
OXA01	**PF04691502**
Palomid 529	PI-103
PP242	PI-540
PP30	PI-620
Torin1	PKI402
TORKi	**PKI587**
WAY001	SF1126
WAY600	WJD008
WYE132	XL765
WYE354	
WYE687	

mTORC1 Upstream Regulators and Downstream Targets

The mTORC1 rapamycin-sensitive complex exerts several important cellular functions such as regulation of cell growth and proliferation and survival, by sensing mitogen, growth factor, and nutrient (amino acids and energy) signals [46]. As a consequence, mTORC1 is activated indirectly by growth factors through several steps starting with the activation of type I insulin-like growth factor receptors (IGFR) which stimulates PI3K to catalyze intracellular phosphatidylinositol-3,4,5-triphosphate (PIP_3) synthesis. Increased PIP_3 results in full activation of AKT which then positively regulates mTOR [47].

A major discovery in the last decade demonstrated that the tumor suppressor proteins tuberous sclerosis 1 and 2 (TSC1 and 2, also named hamartin and tuberin) encoded by the TSC1 and TSC2 genes, respectively, form a heterodimer which acts as a modulator between AKT and mTOR [48–50]. Very recently, Zeng et al. [51] showed that induced Tsc2 mutations in mice intrinsically caused a more severe neurological phenotype than Tsc1 mutations, characterized by epilepsy, severe neuronal disorganization, and premature death, which was related to an abnormal (higher) activation of mTOR. These heterodimers bind to the GTPase Rheb (the RAS homolog enriched in the brain) which in turn activates mTOR; therefore, the hamartin–tuberin complex normally acts as an inhibitor of mTOR function. The way by which Rheb activates mTOR is still not clear, even if it has been suggested that Rheb might antagonize FKBP38 (FK506-binding protein 38) an endogenous inhibitor of mTOR [52, 53].

Table 3 Overview of downstream substrates for mTOR complexes and relative molecular, physiological, and pathological functions

Downstream	Role and function	References
mTORC1		
S6Ks (P70 ribosomal protein S6 kinase 1/2)	Ubiquitously expressed and involved in the control of cell growth	[65, 66]
4E-BPs (eukaryotic initiation factor 4 (eIF4) binding proteins	Phosphorylation of 4E-BPs leads to disinhibition of eIF4 and gives rise to the initiation of translation	[64]
CLIP-170 (cytoplasmic linker protein-170)	Regulates microtubule arrangements	[212]
Eukaryotic elongation factor 2 (eEF2) kinase	Promotes the GTP-dependent translocation of the nascent protein chain from the A-site to the P-site of the ribosome	[213]
Ornithine decarboxylase (ODC)	Key enzymes in the polyamine biosynthetic pathway	[214]
Hypoxia-inducible factor 1α (HIF-1α)	Transcription factor regulating a wide spectrum of biological processes, such as angiogenesis, inflammation, bioenergetics, proliferation, motility, and apoptosis	[201]
Lipin	Plays a role in lipid biosynthesis and might intervene in DAG production in the brain	[67]
PKCδ and PKCε	Play important roles in signaling for various growth factors, cytokines, and hormones	[215]
Protein phosphatase 2A (PP2A)	Represents a family of highly and sophistically regulated phosphatases also involved in Alzheimer's disease	[216]
p21Cip1 and p27Kip1 cyclin-dependent kinase inhibitors	Involved in tumor cell proliferation and survival	[217, 218]
Retinoblastoma protein (Rb)	A nuclear phosphoprotein that regulates growth by inhibiting the cell cycle at G1 phase	[219]
Signal transducer and activator of transcription 3 (STAT3)	Transduces signals from some cytokines (e.g., IL-6 and IL-10) to regulate expression of genes involved in cellular processes	[68]
mTORC2		
AKT kinase	One of the most important kinases for cell survival. After phosphorylation, AKT regulates different cellular processes including cell growth, proliferation, apoptosis, and glucose metabolism	[73]
PKCs (protein kinase Cs)	Modulation of protein translation and other intracellular targets to modulate actin organization and cell motility and possibly other functions	[74–76]
RHO GTPases	Involved in the actin cytoskeleton	[40]
SGK1 (serum- and glucocorticoid-induced kinase 1)	Regulates, by phosphorylation, processes such ion transport and growth	[77]

Energy and stress represent further regulators of mTORC1, which responds as a sensor to cellular energy status through the AMP-activated protein kinase (AMPK), which in turn phosphorylates TSC2 and enhances its activity [54]. Therefore, under low energy conditions, AMPK becomes activated, leading to an inhibition of mTOR through TSC2 phosphorylation and the cascade described above [50]. It is worth noting that in the brain, AMPK can be directly activated by a calmodulin-dependent protein kinase kinase independently from AMP levels, but dependent on Ca^{2+} concentration [55]. In this system, AKT can phosphorylate TSC2 directly, causing inhibition and therefore activation of mTOR, but under high ATP levels, AKT inhibits phosphorylation of TSC2 via AMPK, also leading to mTOR activation [56]. Hypoxia (low oxygen tension), also negatively regulates mTORC1, inducing a hypophosphorylation. The protein REDD1 (encoded by the REDD1 gene: regulated in development and DNA damage responses), which is induced in response to stresses such as hypoxia or DNA damage, seems to act upstream of TSC1/2 [57] even though some studies also implicate AMPK-dependent mechanisms [58]. The dysregulation of mTORC1 under hypoxic stress was recently reviewed by Vadysirisack and Ellisen [59]. It is worth noting that since stress can regulate mTORC1, experimental stresses associated with animal handling, drug administration, or other manipulations might confound laboratory results leading to unreliable data. Therefore, particular attention should be paid to experimental environment and protocols during such experiments.

The last upstream positive mTOR regulators are nutrient amino acids (particularly leucine). The mechanism again seems to act upstream of TSC1/2 [48]; however, this has been debated [60, 61] and further studies are necessary to clarify the exact mechanism by which amino acids regulate mTOR functions. Indirectly, many other molecules might also be able to regulate the activation/inhibition of mTORC1/2 such as phosphatase and tensin homolog (PTEN) which acts at odds with PI3K [62] or all PI3K enhancers which interact and stimulate both PI3K and AKT [63]. For convenience, these other factors will not be

discussed in this review, unless they are also involved in the epileptogenic process (for a review, see [27]).

The downstream targets of mTORC1 in mammals are various: the best characterized are S6Ks (p70 ribosomal protein S6 kinase 1/2) and 4E-BPs (eukaryotic initiation factor 4 (eIF4) binding proteins [64]). S6Ks are ubiquitously expressed and involved in the control of cell growth [65, 66]. 4E-BPs are repressors of the translation initiation factor eIF4. Phosphorylation of 4E-BPs leads to disinhibition of eIF4 and gives rise to the initiation of protein translation. Furthermore, mTOR has also been involved in the regulation of some other proteins such as cytoplasmic linker protein-170 (CLIP-170), eukaryotic elongation factor 2 kinase (eEF2K), ornithine decarboxylase (ODC), glycogen synthase, hypoxia-inducible factor 1α (HIF-1α), lipin, protein kinase C (PKC)δ and PKCε, protein phosphatase 2A, p21Cip1 and p27Kip1 cyclin-dependent kinase inhibitors, retinoblastoma protein, and signal transducer and activator of transcription 3 (STAT3) [27]. CLIP-170 regulates microtubule arrangements; ODC is a key enzyme in the polyamine biosynthetic pathway; HIF-1α is a transcription factor regulating a wide spectrum of biological processes such as angiogenesis, inflammation, bioenergetics, proliferation, motility, and apoptosis; lipin plays a role in lipid biosynthesis and might intervene in diacylglycerol production in the brain [67]; and STAT3 transduces signals from some interleukin (IL) cytokines (e.g., IL-6 and IL-10) to regulate expression of genes involved in cellular processes [68] (Fig. 2; Table 3).

mTORC2 Upstream Regulators and Downstream Targets

mTORC2 seems to lie downstream to PI3K signaling [69]; however, its exact mechanism of activation still remains to be clarified. Rheb showed negative and indirect effects on mTORC2 [70]. More recently, an EGFR mutation (EGFRvIII) was demonstrated to stimulate mTORC2 kinase activity and this effect was only partially suppressed by PTEN [71]. mTORC2 was found to be physically associated with ribosomal but not protein synthesis. PI3K signaling promoted this binding and it seemed to be physiologically relevant for both normal and cancer cells, with a particular emphasis on cell growth [72]. In addition, several downstream targets have been identified. The best characterized substrate is AKT kinase, which is phosphorylated. AKT belongs to the AGC kinase family and is one of the most important for cell survival. After phosphorylation, AKT regulates different cellular processes including cell growth, proliferation, apoptosis, and glucose metabolism [73]. Therefore, AKT (which is also activated by PI3K at the cell membrane) appears to be an upstream regulator of mTORC1 while also being a downstream target of mTORC2 (Fig. 1; Table 3). Based on this fact, the latter complex has recently gathered attention as a possible new anticancer drug target [27]. Protein kinase Cs are phosphorylated by mTORC2, and this is connected to modulation of protein translation and other intracellular targets to modulate actin organization and cell motility and possibly other functions [40, 74–76]. Also, Rho GTPases are regulated by mTORC2 and are involved in the actin cytoskeleton [40].

SGK1 regulates (by phosphorylation) processes such as ion transport and growth [77] and is positively modulated by mTORC2 [78]. SGKs have recently accumulated evidence for being important target mediators of mTORC2 signaling, but since their physiological and pathological relevance is still poorly understood, further studies are needed to identify their substrates and to better define their role and function in relation to mTORC2 [27, 79].

The mTOR Signaling Pathway: Relevance for Brain Function and Pathology

The role of mTOR signaling in the brain appears to be particularly important, since it is involved in processes contributing to nervous system physiology and pathology such as control of protein translation, local protein synthesis in dendrites and axons of neurons, autophagy, and microtubule dynamics (Table 4).

Several translation-inducing signals lead to mTOR-mediated phosphorylation of 4E-BP1, which causes eIF4E release, allowing for the formation of the functional eiF4F complex and initiation of translation [80]. Another initiation factor eIF4B, also mTOR dependent, needs to be phosphorylated by the p70S6K to be associated to the translation initiation complex [81]. However, p70S6K is better known for its kinase activity towards the S6 protein. Through phosphorylation, p70S6K increases the production rate of proteins involved in the regulation of translation processes. It has been demonstrated that phosphorylation of translation inhibitor eEF2K by p70S6K is sensitive to rapamycin [82]. In addition to protein synthesis, the rate of protein degradation depends on mTOR activity.

Autophagy (a form of sequestration and degradation of intracellular components) is an evolutionarily conserved process of catabolic cell response to poor extracellular nutrient conditions, employing the lysosomal pathway [83]. Activation of mTOR by trophic factors (TSC–RHEB pathway) or by increased amino acid availability (class III PI3K–RHEB pathway) results in the inhibition of autophagy; in contrast, inhibition of mTOR by decreased amino acid availability, energetic stress resulting in AMPK activation, or by rapamycin can lead to increased autophagy [84, 85]. Although early research primarily focused on the mTOR-dependent translation impact on synaptic and brain plasticity [86], later studies demonstrated mTOR involvement in neuronal development [87] and brain pathophysiology [88]. During neuronal development, mTOR may control

Table 4 Overview of the physiological and pathological role and function of the mTOR signaling pathway in the central nervous system (CNS)

CNS function or pathology	Role of mTOR	References
Alzheimer's disease (AD)	Activation of p70S6K, downstream of mTORC1, contributes to hyperphosphorylated tau accumulation in neurons with neurofibrillary tangles An increase in the level of phosphorylated mTOR and tau has been reported in the brain of AD patients Alteration of mTOR kinase levels in lymphocytes of AD patients correlates with memory and cognitive decline mTOR inhibition reduces the level of Aβ and improves the cognitive function in a mouse model of AD	[130–134]
Cortical malformations	mTOR was found to be activated in cytomegalic neurons of human cortical dysplasia, and rapamycin treatment was able to suppress seizures and neuronal hypertrophy Neuronal cells in gangliogliomas express components of the PI3K–mTOR signaling pathway in a higher percentage than cells in control cortex	[182, 184]
Depressive disorders	Deficits in the mTOR-dependent translation initiation, particularly via the p70S6K/eIF4B pathway, contribute to the molecular pathology in patients with major depressive disorder Ketamine, a faster-acting antidepressant, appears to act by activating mTOR and mTOR-dependent synapse formation	[144, 145]
Epilepsy and epileptogenesis	In TSC, it was demonstrated that mTOR hyperactivation is involved in neuronal hyperexcitability, promotes seizures and other neurological consequences Early treatment with rapamycin prevents the development of epilepsy in a $Tsc1^{GFAP}$CKO mouse model of TSC mTORC1 hyperactivation has an important role in GABAergic interneuron development, function, and migration Rapamycin and mTOR are involved in two models of TLE Rapamycin in NS-PTEN KO mice suppresses epileptiform activity and mossy fiber sprouting for several weeks Ketogenic diet inhibits the mTOR pathway hyperactivation after kainate-induced SE	[160–162, 168, 169, 171–175]
Food uptake	In the hypothalamus, mTOR acts as an energy sensor to control animal food intake and regulate body energy balance Leptin increases hypothalamic mTOR activity Hypothalamic mTORC1 regulates feeding behavior	[88, 115]
Hormonal effects	Centrally expressed mTOR controls the gonadotrophic axis and the onset of puberty, producing delayed vaginal opening and decreased LH and estradiol levels accompanied by ovarian and uterine atrophy in rats Inactivation of mTOR also blunts the positive effects of leptin on puberty onset in food-restricted females	[118]
Huntington's disease (HD)	mTOR is sequestered by aggregates of mutated huntingtin with expanded polyglutamine tracts Rapamycin attenuates huntingtin accumulation and cell death in models of HD and protects against neurodegeneration	[128, 129, 141]
Learning and memory	mTORC1 is required for late-phase LTP and is necessary for memory consolidation Stimuli that induce LTP activate mTOR. However, mTORC1 activation is required for memory formation; unregulated mTORC1 signaling can also disrupt memory function	[30, 93–98]
Neuronal development	mTOR controls protein expression at different levels, and other cellular processes such as neuronal survival and differentiation, as well as axon growth and navigation, dendritic arborization, and synaptogenesis	[87, 89, 90]
Parkinson's disease (PD)	Increasing mTORC1 activity, thereby silencing TSC2, reduces neurodegeneration Rapamycin treatment alleviates the dyskinesia side effects of L-DOPA	[137, 139]
Schizophrenia	Hypoactivity of upstream regulators of mTOR activity, AKT and PI3K, is correlated with schizophrenia	[142]
Tuberous sclerosis (TSC)	mTOR hyperactivation is responsible for most of the abnormal cell growth, proliferation, and tumorigenesis in TS	[122, 125, 159]
Others	mTOR responds to external light and regulates circadian clock neurons in the suprachiasmatic nuclei	[119]

protein expression at different levels and other cellular processes such as neuronal survival and differentiation, as well as axon growth and navigation, dendritic arborization, and synaptogenesis [87, 89, 90].

In the adult CNS, mTOR is crucial for many forms of synaptic plasticity such as long-term potentiation (LTP) in the hippocampus and, thereby, plays an important role in the process of learning and memory via protein synthesis-dependent strengthening of synapses [30]. Furthermore, the mTOR pathway is involved in synaptic plasticity by coordinating the timing and location for the synthesis of new proteins. Dendrites contain numerous mRNAs encoding proteins influencing synaptic function [91]. It has been shown that activation of mTOR at synapses promotes protein synthesis necessary to facilitate plasticity and may also modulate neural activity by suppressing translation of specific messages [92]. mTORC1 is required for late-phase LTP, since rapamycin application results in a reduction of this process and also blocks the synaptic potentiation induced by brain-derived neurotrophic factor (BDNF) [93]. Downstream of BDNF activation, there is an increase in mTOR-mediated mRNA translation and synaptic glutamate AMPA-type receptor (GluR1) subunit expression that is required for memory consolidation [94]. Furthermore, stimuli that induce LTP activate mTOR in a PI3K- and ERK/MAPK-dependent manner [95–98].

Potential molecular mechanisms by which mTOR can regulate synaptic plasticity are very broad [99]. Analysis of RNAs regulated by BDNF through mTOR [100] identified several proteins previously studied for their role in synaptic plasticity, learning, and memory, e.g., NMDA-type glutamate receptor subunit NR1, Homer2, Pyk2, LIMK-1, and e-NOS. Additional proteins regulating synaptic plasticity such as CamKIIα, PSD-95, Arc, and PKM-zeta were also reported to be expressed in an mTOR-dependent fashion [101–103].

There is evidence that molecular processes crucial for synaptic plasticity are also important for processes of learning and memory, suggesting that mTOR activity is used by the brain to monitor nutrient status and consolidate long-term memories. Protein synthesis is a well-established requirement for memory formation. In rodent models, ex novo protein synthesis is required to stabilize a short-term memory into a long-term memory [104]. It has been demonstrated that enhancing neuronal mTORC1 activity may improve memory function [105, 106], while administration of rapamycin disrupts this process in several behavioral paradigms [107]. Rapamycin has also been shown to affect consolidation of memories in different brain regions, including hippocampus-dependent spatial memory [105], auditory cortex-dependent memory [108, 109], gustatory cortex-dependent memory for taste aversion [110], and prefrontal cortex-dependent trace fear memory [111]. In contrast, increasing mTORC1 activity can also disrupt memory processing. An example of memory *deficits* associated with overactive mTORC1 are human patients and animal models of tuberous sclerosis (TS) [112]. In a transgenic mouse model of TS, rapamycin treatment rescued memory deficits [113], suggesting that memory impairments were due, at least in part, to abnormal mTOR signaling in adult neurons as opposed to the developmental effects of the disease. Finally, mTOR activation has also been shown to be involved in the development of memory deficits associated with Δ^9-tetrahydrocannabinol administration in mice, with rapamycin being able to abrogate its amnesic-like effects [114]. This effect was attributed to a transient modulation of the mTOR pathway in the hippocampus, by cannabinoid receptor type 1 activation, which was mediated through GABAergic interneurons and also required NMDA receptor activity [114]. Thus, while mTORC1 activation is required for memory formation, unregulated mTORC1 signaling can disrupt memory function, too.

In addition to its role in synaptic plasticity, mTOR is also involved in the control of food uptake. In the hypothalamus, mTOR acts as an energy sensor to control animal food intake and regulate body energy balance [88]. mTOR signaling is controlled by energy status in the arcuate nucleus of the hypothalamus. Central administration of leucine, known to induce mTOR activity, increases hypothalamic mTOR signaling and suppresses food intake and body weight. Infusion of rapamycin along with leucine removed this suppression. The peptide hormone leptin that has pro-anorectic effects increases hypothalamic mTOR activity, and inhibition of mTOR by rapamycin reduces leptin's anorectic effect [88]. Thus, hypothalamic mTORC1 activity can coordinate multiple signals to regulate feeding behavior. Further studies demonstrated that hypothalamic mTORC1 regulates feeding behavior, at least in part, through S6K, a marker for mTORC1 activity [115]. Studies examining hypothalamic mTOR have shown that acute mTOR activity responds to sufficient nutrient levels and signal cessation of feeding behavior, while chronic elevation of mTORC1 activity can contribute to complications associated with metabolic disorders and homeostatic imbalance [88, 116, 117].

Centrally expressed mTOR also controls the gonadotrophic axis and the onset of puberty [118]. Central activation of mTOR can stimulate luteinizing hormone (LH) secretion, and the blockade of central mTOR signaling by rapamycin causes inhibition of the gonadotrophic axis at puberty, producing delayed vaginal opening and decreased LH and estradiol levels accompanied by ovarian and uterine atrophy in rats. Inactivation of mTOR also blunts the positive effects of leptin on puberty onset in food-restricted females [118].

The mTOR signaling pathway also contributes to other potential brain-specific functions, e.g., it responds to external light and regulates circadian clock neurons in the

suprachiasmatic nuclei [119]. Since many functions of the brain require neurons to rapidly alter their firing properties in response to stimuli, more immediate functional roles of neuronal mTOR signaling will most likely be identified in the future. Because of its complex roles in CNS physiology, it is not surprising that disruption of mTOR signaling may contribute to the pathophysiology of various neurological diseases.

Recent studies have revealed altered mTOR signaling in many brain tumors as well as a variety of cortical malformations: TSC, cortical dysplasia, traumatic brain injury, and neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's diseases [23, 120, 121]. TSC patients with benign brain tumors frequently present mental disorders such as intellectual disability, autism, and epilepsy [122]. The epileptic phenotype is probably linked to hyperactive mTOR in neurons, as suggested by epilepsy mouse models deficient for PTEN (see below) or TSC1 [123, 124]. The intellectual disability is probably related to the role of mTOR in learning and memory formation as described above [125]. Different studies suggest that mTOR signaling could be deregulated in several neurodegenerative diseases via its role in autophagy [21, 85]. Neurodegenerative disorders such as Alzheimer's (AD), Parkinson's (PD), and Huntington's (HD) disease are characterized by a gross neuronal loss in certain brain areas and aberrant accumulation of misfolded proteins, leading to neuronal death and consequently involuntary tremors, dementia, and loss of memory and language function [126]. A common theme in these disorders is aggregation of pathological aggregate-prone proteins such as β-amyloid (Aβ) and hyperphosphorylated tau, α-synuclein, and polyglutamine-expanded huntingtin in AD, PD, and HD brains, respectively [126, 127]. The process of abnormal intracellular protein aggregate clearance from neurons can be accelerated by increasing the rate of autophagy by mTOR inhibition in experimental models of HD [128, 129].

Studies on mTOR involvement in AD have concentrated on the control of protein synthesis and the cell cycle reentrance. The activation of p70S6K, downstream of mTORC1, has been identified as a contributor to hyperphosphorylated tau accumulation in neurons with neurofibrillary tangles [130]. An increase in the level of phosphorylated mTOR and tau has been reported in brain sections of AD patients [131] leading to the hypothesis that hyperactivation of mTOR signaling might be one of the important components of Alzheimer's pathology. Alteration of mTOR kinase levels in lymphocytes of AD patients correlates with memory and cognitive decline [132]. Studies on neural cell cultures examined the roles of mTOR on Aβ; however, contrasting results were reported [29, 133]. More recently, mTOR inhibition by rapamycin was shown to reduce the level of Aβ and improve the cognitive function in a mouse model of AD [134]. Studies examining tau are also conflicting. As stated above, mTOR activity can increase levels of tau protein production, suggesting that activation of mTORC1 may be upstream of tau pathology. Conversely, in a *Drosophila* fly model of tauopathy, neurodegeneration was accompanied by mTORC1 activity induction [135]. Rapamycin treatment of flies expressing mutant tau prevented the abnormal cell cycle activation and neuronal death suggesting that elevated mTORC1 activity may drive neurodegeneration in AD [135].

A complex relationship between PD and mTORC1 activity, through a stress response protein RTP801, has been reported. RTP801 is a negative regulator of mTOR, working downstream of AKT and upstream of TSC2 to inhibit mTOR activity [136], and is highly induced in several animal models of PD and in dopaminergic neurons of Parkinson's patients [137]. PD-associated stresses induce RTP801, which, as a consequence, triggers neuronal degeneration and death by suppressing activation of mTOR. Increasing the mTORC1 activity, thereby silencing TSC2, reduces neurodegeneration, suggesting that increasing mTORC1 activity could be neuroprotective [137]. Furthermore, several data have suggested that rapamycin treatment might be beneficial for Parkinson's patients and that it might be useful to alleviate dyskinesia side effects of L-DOPA, a common treatment for Parkinson's [138, 139].

Studies in animal models as well as HD patients' tissues revealed that mTOR is sequestered by aggregates of mutated huntingtin with expanded polyglutamine tracts [128]. The reduced mTOR activity is accompanied by an upregulation of autophagy and represents a defense mechanism. The key role of mTOR signaling in the regulation of autophagy was established a long time ago [140] and confirmed by extensive investigations [141]. Rapamycin can enhance the autophagic clearance of intracellular proteins with long polyglutamines and a polyalanine-expanded protein and reduces their toxicity [129]. In line with these observations, rapamycin also attenuates huntingtin accumulation and cell death in models of HD and protects against neurodegeneration in a fly model of HD [129, 141].

It has been shown that hypoactivity of upstream regulators of mTOR activity, AKT, and PI3K (reduced activation/phosphorylation of one or both kinases reduces upstream mTOR activation; see Fig. 1) is correlated with psychiatric disorders such as schizophrenia [142]. Dysregulation of mTOR signaling may also be involved in the pathophysiology of depression [143]. It was hypothesized that deficits in the mTOR-dependent translation initiation, particularly via the p70S6K/eIF4B pathway, contribute to the molecular pathology in patients with major depressive disorder and that a rapid reversal of these abnormalities may underlie antidepressant activity [144]. Furthermore, ketamine, a faster-acting antidepressant, appears to act by activating

mTOR and mTOR-dependent synapse formation [145]. It is worth noting that the clinical use of rapamycin (sirolimus) and other synthetic "rapalog" derivatives (e.g., everolimus (RAD001) and temsirolimus (CCI-779)) in oncology and organ transplantation has been correlated with several adverse side effects (e.g., mucositis, skin rash, hyperglycemia, and other metabolic changes). Quite surprisingly, among these, only a few are directly based on the CNS, such as attention and working memory impairment, development of tremor and somnolescence/depression, and these have a very low incidence [146–149]. However, in a clinical study in adult maintenance heart transplant recipients, everolimus improved memory, concentration, and overall psychiatric symptoms when patients were switched from calcineurin inhibitors (i.e., cyclosporin A) to everolimus [150]. In view of the difficulty of extrapolating side effect data from critically ill patients such as these, taking mTOR inhibitors, it would be of great value to have clinical studies also considering positive or negative effects of these drugs on brain function. For example, in clinical trials with mTOR inhibitors, a psychiatric and/or neurologic evaluation by using appropriate questionnaires would certainly indicate whether these drugs have an effect on mood or memory function. Any type of patient with an indication for the use of such drugs could be easily studied. However, studies on patients with a specific pathology (i.e., major depression) would be the first choice for testing the effects of mTOR inhibitors for every indicated disease.

As evidenced by the data reviewed above (Table 4), an increasing interest in mTOR has been observed in the last few years, with particular attention to its possible role in several CNS pathologies. A considerable amount of data have been accumulated and the development of mTOR modulating drugs has also been improved (Table 2). However, in most cases, the exact picture of the most plausible target and the consequence of its modulation are unclear. Nevertheless, the actual sketch is very promising and more research is certainly warranted.

Another important field of application is epilepsy and epileptogenesis, which share many commonalities with other neurological disorders. Very often, epilepsy represents a common manifestation of other CNS pathologies or vice versa: many neurological and psychiatric disorders are comorbid diseases associated with epilepsy [151–153]. In the next final section, the role of mTOR in epilepsy and epileptogenesis as currently understood will be reviewed.

Molecular Role and Studies on the Involvement of mTOR in Epilepsy and Epileptogenesis

Epilepsy represents one of the oldest neurological disorders. In recent years, several advances have been achieved in the field and several new drug targets have been suggested [7, 19]. However, the introduction of new AEDs has failed to significantly improve seizure control in refractory patients [154]. mTOR represents one of the most promising molecular targets in this area, and its involvement in epilepsy (chronic spontaneous seizures in established disease) and epileptogenesis (the process leading to the appearance of chronic spontaneous seizures and epilepsy establishment) is now certain (see "Introduction") [7, 155]. One particular condition has drawn considerable attention. TSC is an inherited autosomal dominant disorder in which benign tumors can develop in multiple organs of the body (brain, skin, kidneys, liver, heart, lung) and represents one of the most common genetic causes of intractable epilepsy, even though it has a population prevalence of ~0.01 % [7, 156]. TSC is now known to be caused by an inactivating mutation in either the TSC1 or TSC2 genes, which encode the tuberous sclerosis complex-1 and −2 gene products TSC1 and TSC2, respectively [157]. The first evidence for a role of mTOR in TSC dates back to 2002, when it was demonstrated that in TSC2-null murine neuroepithelial cells (a model for human tuberous giant cells), an overactivation of the mTOR pathway was present [158]. In the same year, many other reports demonstrated the inhibitory effects TSC1 and TSC2 on mTOR activity [48–50] (see above). This discovery led to the study of the mTOR signaling pathway in TSC and to the current acquired knowledge that mTOR hyperactivation was responsible for most of the abnormal cell growth, proliferation, and tumorigenesis [159]. mTOR has been strongly linked to tumorigenesis in TSC, and more recently, it was demonstrated that its hyperactivation might be involved in neuronal hyperexcitability, promoting seizures and other neurological consequences [160–162]. Interestingly, it was previously demonstrated that mouse models of TSC were not more susceptible to convulsants (acutely administered pentylenetetrazole and flurothyl-induced seizures), whereas they were prone to chemically induced kindling, indicating an altered synaptic plasticity with a propensity to develop seizures. Therefore, epileptogenesis in TSC might not only be linked to the development of focal hamartomatous lesions [163, 164]. The role of mTOR in these earlier studies was not analyzed, but it was very likely involved in cell differentiation, synaptic plasticity, and epileptogenesis. It was later demonstrated that treatments with mTORC1 inhibitors such as rapamycin and its hydroxyethyl derivative RAD001 (everolimus) in a Tsc1-ablated mouse model of TSC were able to improve phenotype survival and function, normalizing neurofilament abnormalities, myelination, and cell enlargement but not dysplastic neuronal features and dendritic spine density and length [161]. More recently, a new Tsc1-loss mouse model for TSC was developed involving doxycycline prenatal treatment and postnatal treatment with rapamycin, which completely reversed the

animal phenotype, rescuing the mutants from epilepsy and premature death [165]. In heterozygous $Tsc2^{+/-}$ mice, the abnormal long-term potentiation observed in the CA1 region of the hippocampus and the deficit in hippocampal-dependent learning were restored [113]. Furthermore, it was very recently demonstrated that a single-dose prenatal treatment with rapamycin in a fetal brain model of TSC rescued the lethality of the mutant mice and a continued postnatal treatment extended survival; however, treated animals developed enlarged brains with an increased number of brain cells accompanied with developmental delay [166]. Finally, Zeng et al. [162] demonstrated that an early treatment with rapamycin (starting at P14) prevented the development of epilepsy in every animal of the treated group in a $Tsc1^{GFAP}$CKO mouse model of TSC (where the Tsc1 gene was conditionally inactivated in glia), also prolonging survival from 4 to 6 months. Treatment was started at P14 before the onset of seizures. When the treatment was started at 6 weeks of age, seizure frequency was also dramatically reduced after 1 week of treatment with rapamycin, and after several weeks, most of the mice were seizure free (four out of eight and the remaining had a significant reduction), with improved survival and no deaths during treatment [162]. The early treatment and, to a certain extent, the late treatment were also able to prevent the progressive astrogliosis, to normalize brain size, and to preserve the structure and organization of hippocampal pyramidal neurons. Furthermore, rapamycin treatment normalized the levels of astrocyte glutamate transporters (Glt-1 and GLAST) whose expression is reduced in this animal model and might be contributing to seizures and epileptogenesis [162, 167]. The role of astrocyte glutamate transporters in epileptogenesis in this model has been recently confirmed. Increased expression of transporters in presymptomatic TSC mice reduced neuronal death and seizure frequency and increased survival. However, their increased expression in TSC mice phenotypically expressing seizures had no effects [168]. Very recently, a role for mTORC1 in a TSC mouse model was demonstrated on the regulation of the GABAergic system. Fu et al. [169] generated conditional knockout mice with a selective deletion of the Tsc1 gene in GABAergic interneuron progenitor cells and found an increase in mTORC1 signaling accompanied by enlarged cortical and hippocampal interneurons, a decreased number in the cortex with a differential reduction of specific GABAergic subtypes, and an impaired migration. Mice also showed a decreased threshold for flurothyl-induced seizures (flurothyl is a halogenated inhalational convulsant). The authors concluded that the Tsc1 gene and, as a consequence, mTORC1 hyperactivation have an important role in GABAergic interneuron development, function, and migration [169]. In agreement with these latter findings, it was very recently demonstrated that rapamycin suppresses axon sprouting by somatostatin interneurons in green fluorescent protein (GFP)-expressing inhibitory neuron mice treated systemically with pilocarpine to induce acute status epilepticus (SE) (and then after 2 months, spontaneous chronic seizures), which represents a validated model of temporal lobe epilepsy [170]. Rapamycin suppressed axon sprouting by promoting the survival of the somatostatin/GFP-positive interneurons. The authors concluded that the mTOR signaling pathway might represent a useful target for modulating GABAergic reorganization during epileptogenesis [170].

In the same animal model of temporal lobe epilepsy (TLE), it was previously demonstrated that rapamycin dose-dependently suppressed mossy fiber sprouting in mice when administered continuously for 2 months, starting 24 h after pilocarpine-induced SE; however, rapamycin treatment did not affect seizure development or seizure frequency [171]. In the same report, the authors observed a rapamycin-dependent suppression of dentate gyrus hypertrophy by reducing granule cell enlargement and not by inhibiting granule cell proliferation, whereas no effects where observed on hilar neuron loss and the generation of ectopic granule cells [171]. In a previous study, rapamycin was continuously infused intrahippocampally, starting within 3 and 10 h after pilocarpine-induced SE up to 2 months [172]; mTOR inhibition by rapamycin suppressed mossy fiber sprouting time-dependently; however, this effect was reversed after cessation of drug administration, with mossy fiber sprouting approaching levels similar to those of untreated animals. This suggests that inhibition of mTOR-dependent mossy fiber sprouting is not permanent and may require continuous treatment. Furthermore, rapamycin treatment was unable to reverse established mossy fiber sprouting and did not prevent hilar neuron loss [172]. In this latter study, no data on seizure parameters were presented; however, it was later demonstrated that the mTOR pathway is hyperactivated in rats with chronic spontaneous seizures after pilocarpine-induced SE [173]. Rapamycin treatment (5 mg/kg/day i.p. for three consecutive days, followed by treatment on every other day) for 3 weeks suppressed seizure activity (−93 %); the effects were already observable after a few days, with a reduction of both seizure frequency and severity, although after treatment suspension (in a time window of 3 weeks), seizure parameters returned to control levels of epileptic animals not treated with rapamycin, in agreement with the nonpermanent effects of rapamycin on mossy fiber sprouting [172, 173]. The above results obtained with rapamycin in the pilocarpine models of TLE and related epileptogenesis indicate to some extent a role for mTOR both during the epileptogenic process and in the control of chronic spontaneous seizures; this evidence is less compelling than that obtained in the TSC models. Actually, more promising are some newer data. In a very recent study, it was demonstrated that a single course of

rapamycin (10 mg/kg i.p. for 5 days a week during the fourth and fifth postnatal weeks) in neuron subset-specific-phosphatase and tensin homolog conditional knockout mice was able to temporarily suppress epileptiform activity and mossy fiber sprouting for several weeks (up to 4–7 weeks after treatment ended); however, ~5 weeks after drug cessation, epilepsy recurred [174]. Notably, re-exposure to the drug every 5 weeks prevented seizure recurrence; this long-term intermittent treatment protocol also increased survival without compromising growth [174]. In another animal model of TLE, following kainate-induced SE (which shares many commonalities with the pilocarpine TLE model), opposite and more convincing results on mTOR mediation of epileptogenesis were obtained [168, 175]. It was demonstrated that also in this model, the mTOR pathway was activated by kainate-induced seizures, but in a biphasic manner—an early activation phase terminating within the first 6 h after kainate-induced seizures and a later activation after 2–3 days, which peaked after 5 days and gradually decreased over the following few weeks returning to baseline levels after 5 weeks. Furthermore, the later increased mTOR activation was only observed in the hippocampus and not in the neocortex as observed for the early phase, and the latter was further related to the seizures themselves and not to kainic acid administration [175]. Rapamycin pretreatment for three consecutive days before kainate injection, without affecting seizure parameters, was able to decrease cell death, neurogenesis, mossy fiber sprouting, and reduced seizure frequency (−96 %) with three out of eight mice being seizure free [175]. In contrast, rapamycin treatment started 24 h after kainate-induced SE for six consecutive days and then every other day until the end of the study blocked the delayed activation of mTOR and inhibited mossy fiber sprouting but had no effects on neuronal death and neurogenesis; it also reduced seizure frequency (−88 %) but less efficaciously than pretreatment [175]. Finally, it was later reported that rapamycin administration within 1 h after kainate injection induced paradoxical effects causing a higher level of mTOR activation and this was associated with a greater neuronal death. Thus, it appears that rapamycin has the potential to have either neuroprotective or exacerbating effects on neuronal death [168]. This confirms the complexity and variable activation status of the mTOR signaling pathway and is in agreement with other findings such as the contrasting pro- or anti-apoptotic effects of mTOR activation [5, 176, 177]. In a very recent report [178], the lack of efficacy of posttreatment with rapamycin in preventing epileptogenesis in the amygdala stimulation model of temporal lobe epilepsy and epileptogenesis was demonstrated, suggesting that mTOR might not have a universal role in this phenomenon and might therefore be limited to certain types of epilepsy and epileptogenic processes. In this study, chronic seizures developed secondarily to SE induced by electrical stimulation of the amygdala, and the authors did not find any difference between treated (rapamycin 6 mg/kg i.p.) and untreated rats in any seizure parameter. However, there were no measures of mTOR expression in the brain, and only the quantification of S6 ribosomal protein phosphorylation, which is a downstream of mTOR, was found to be increased by SE and this increase was abolished by rapamycin treatment. Furthermore, mossy fiber sprouting was not modified by the treatment [178]. This contrasting set of results is not surprising, considering the complexity of the epileptogenic process (see above), but this particular model certainly deserves to be further investigated by also measuring brain mTOR expression. It would also be very interesting to know if rapamycin pretreatment can influence the *onset* of SE, and moreover, studies starting treatment at different times after SE (where rapamycin was injected starting 24 h after SE induction) would also be warranted, since it has previously been demonstrated that rapamycin effects are highly influenced by the beginning of treatment also giving opposite results in another model of TLE (see above [168, 175]). In this light, in another recent study, it was demonstrated that the mTOR pathway was already activated by pentylenetetrazol (PTZ)-induced acute seizures 1 h after PTZ injection, and it remained elevated for up to 16 h and therefore long after seizures were terminated [179]. This is at odds with the observations made in the kainate model, where a secondary increase in mTOR activity was observed several days after seizures. According to Zhang and Wong, this might justify the lack of cell death in this model. Furthermore, they tested the effects of wortmannin, a PI3K/Akt pathway inhibitor which inhibits mTOR upstream, and found no effects on seizures, but only on mTOR activation [179]. It would have been very interesting to know if rapamycin had any effect against PTZ-induced seizures and, since an alteration in the pathway was observed, whether this phenomenon could be correlated with studies on the PTZ-kindling model.

The mTOR signaling pathway and its inhibition have also been suggested as potential therapeutic targets in focal and other cortical malformations such as human cortical dysplasia which, similar to TSC, is associated with intractable epilepsy, representing 25 % of cases of medically refractory partial epilepsy [180, 181]. mTOR was found to be activated in cytomegalic neurons of human cortical dysplasia [182], and rapamycin treatment was able to suppress seizures and neuronal hypertrophy [124]. Within malformations of cortical development, gangliogliomas represent the most frequent type of neoplasms in pediatric medically intractable epilepsy [183]. Neuronal cells in gangliogliomas express components of the PI3K–mTOR signaling pathway in a higher percentage than cells in control cortex [184]. Finally, everolimus therapy on 28 patients affected by serial growth of subependymal giant cell astrocytomas was

associated with a significant reduction of the astrocytoma volume and seizure frequency [185]. Very recently, it was also demonstrated that the ketogenic diet, an effective non-drug treatment for epilepsy, inhibits the mTOR pathway activation both in the hippocampus and liver of rats and blocked the hippocampal hyperactivation that occurs after kainate-induced SE [186].

Taken altogether, these data strongly demonstrate a role for mTOR in convulsive epileptic syndromes and epileptogenesis and warrant further experiments and research in order to better clarify its real potentiality. mTOR inhibitors might then have potential beneficial effects both during the epileptogenic process and by suppressing certain types of established seizures. Regarding their exact mechanism of action, which obviously could include all their effects reported in this review, some others could also be considered and it seems clear that more detailed studies on the mechanism(s) by which mTOR interferes with neuronal excitability are needed.

Possible Future Challenges and Developments

The role of mTOR in epilepsy was also confirmed in a recent transcriptome analysis of the hippocampus in rats following pilocarpine-induced SE [155]. In this study, 34,000 transcripts of rat genes were studied and 1,400 were differentially expressed during the course of epileptogenesis. Within these, a group of 128 genes was found to be consistently hyperexpressed throughout epileptogenesis. Regarding signaling pathways, those corresponding to hyper- and hypoexpressed genes included MAPK, jak-STAT, phosphatidylinositol, transforming growth factor-beta (TGF-β), and mTOR [155]. Moreover, rapamycin itself was reported to alter gene expression, morphology, and electrophysiological properties of rat hippocampal neurons [187]. The authors reported no effects on cell size and dendrite length on cultured hippocampal neurons, whereas rapamycin exposure after 3 days of culture significantly altered the expression of 13 distinct neuronal mRNAs within the cytoskeletal element, growth factor, transcription factor, neurotransmitter, and ion channel gene families. After 14 days of treatment, altered expression of 18 mRNAs was seen. The expression of nestin, aFGF, BMP6, CREB, c-jun, OTX1, EAAC1, Kir 4.1 and 5.1, c-ret, PDGFRβ, TGF-β2, and TGFRβ1 was reduced, while erbβ4, netrin 1, mGluR4 and 5, LIF, IL-6, LIFR, IGF-1, GLT-1, HES, KCNQ2, and Kir 1.1 expressions were increased. Rapamycin (200 nM), however, did not alter voltage-dependent Na^+ or K^+ currents underlying neuronal action potential generation and only slightly decreased spontaneous firing or network excitability [187]. Furthermore, it was previously reported that inhibition of the mTOR pathway by rapamycin reduced the synaptic expression of GluR2/3 subunits of glutamate AMPA receptors in cultured cortical neurons, suggesting an involvement of this pathway in surface receptor expression [188]. All these data might suggest a direct or indirect role for mTOR in the regulation of neuronal excitability and synaptic transmission; however, this is still elusive and deserves further clarification.

mTOR is also a negative modulator of autophagy and rapamycin administration can induce it [84, 85] (see above). The role of autophagy has been studied in some neurological disorders, but only few data regarding autophagy in epilepsy have been reported. It was previously demonstrated that after kainate treatment in mice, autophagy was only transiently induced, and in the same study, an increase in mTOR phosphorylation from 6 to 16 h was observed indicating that a potential negative feedback loop might exist to prevent excessive stimulation of autophagic stress [189]. Similarly, autophagy was increased in the rat pilocarpine-induced SE model [190]. Both the role of autophagy in epilepsy and that of mTOR in this context clearly require further clarification.

Finally, there is a rapidly growing body of evidence that supports the involvement of inflammatory mediators—released by brain cells and peripheral immune cells—in both the origin of individual seizures and the epileptogenic process [191]; however, it remains to be seen whether inflammation is the cause or if it is caused by seizures [192]. Inflammatory mediators are produced in the brain by parenchymal cells such as microglia, astrocytes, endothelial cells of the blood brain barrier, and leukocytes; these contribute to epilepsy directly by affecting neuronal excitability and by transcriptional activation of dependent genes involved in brain functions such as synaptic, molecular, and cellular plasticity (for a review, see [191]). Direct evidence of mTOR signaling pathway modulation by inflammation in epilepsy is still lacking; however, it is known that mTOR is essential for the survival, cytokine production, and migration of neutrophils and mast cells [60, 193–195]. The mTOR pathway has been shown to selectively control microglial activation in response to pro-inflammatory cytokines and appears to play a crucial role in microglial viability; mTOR inhibitors may, therefore, represent a useful tool in controlling neuroinflammation [196]. Activation of the mTOR pathway enhances STAT3 activity and IL-10 but reduces pro-inflammatory molecules and NF-κB activation, whereas inhibition of mTOR with rapamycin has reciprocal effects [197]. When considering the effects of rapamycin in epilepsy and epileptogenesis models, the question of whether the observed effects might have been mediated by its modulation of inflammatory responses or by some other unknown remains completely unanswered.

Conclusions

The overall impression regarding the increasing interest in the role of mTOR in epilepsy/epileptogenesis and other

neurological disorders is very positive and intriguing, and the high effectiveness of rapamycin and the involvement of the mTOR pathway in so many pathologies are certainly noteworthy. However, the most recent results in a range of epilepsy models have shown that mTOR activation in epilepsy is not a universal phenomenon and might therefore be relevant for only some models and experimental situations [178, 179]. More experiments are also warranted by the continued lack of information regarding the exact mechanisms involved in the epileptogenic process and the complete definition of the mTOR pathway role in the CNS. Finally, there is currently a complete lack of "rapalog" molecules which might selectively act in the brain, thus avoiding other serious side effects which are generally associated with treatment with rapamycin or other mTOR inhibitors, such as immunosuppression. Therefore, human use in clinical trials of such drugs for the management of established refractory epilepsy syndromes or for the prevention of epilepsy development after brain insult or injury (epileptogenesis) still seems difficult, and in any case, caution in their use might be advisable.

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