Altered Glutamate Receptor Signaling in Tuberous Sclerosis Complex

Abstract

Tuberous Sclerosis Complex (TSC) is a debilitating disease characterized by the development of benign tumors called hamartomas in multiple organ systems, including the brain. TSC is caused by dysregulation of the mammalian target of rapamycin (mTOR) pathway, a critical regulator of protein translation, cell growth, and synaptic plasticity. Although 80-90% of TSC patients suffer from seizures, the mechanisms of epilepsy in TSC are poorly understood, and seizures associated with TSC are often resistant to currently available anticonvulsants. Previous studies have shown that TSC patients exhibit altered expression of ionotropic glutamate receptors (iGluRs), which mediate the vast majority of excitatory neurotransmission. Given that the functional properties of an iGluR are determined by the particular combination of subunits that comprise the receptor, the main focus of this study was to assess iGluR subunit expression in relationship to alterations in mTOR signaling and seizure development in a mouse model of TSC. Western blotting demonstrated that the TSC mutant has an abnormal iGluR expression pattern that is conducive to increased network excitability. In particular, at postnatal day 8 (P8), the AMPA receptor (AMPAR) subunit GluRl was significantly higher in TSC mutants than in controls, and GluR2 showed a trend of decreased expression in mutants. These results suggested abnormally high levels of Ca2+-permeable AMPARs prior to the onset of spontaneous seizures at P10-14. Furthermore, taking into account all ages analyzed (P4, P8, Pl4, P2I, P29), the developmental expression profiles of the NMDA receptor subunits NR2B and NR3A were significantly different between TSC mutants and controls, with higher expression in mutants after P2l. Parallel immunocytochemistry experiments showed that this aberrant iGluR expression is regionally distributed, cell-specific, and highly correlated with mTOR pathway overactivation. Pilot data are also presented suggesting novel, seizure-modulated interactions between Rheb, a GTPase that controls the mTOR pathway, and NR3A in P10 Long-Evans rats. The studies presented provide further evidence of cell-autonomous mechanisms underlying the development of seizures in TSC, validate an important TSC mouse model for future studies, and suggest a direct link between seizure activity and the mTOR pathway via Rheb-NR3A interactions. A better understanding of the mTOR pathway's relationship to seizure activity and of the mechanisms underlying hyperexcitability in the TSC brain will allow potential future therapeutics to be identified not only for TSC, but also for epileptogenesis in general.