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Functional properties of human NMDA receptors associated with epilepsy-related mutations of GluN2A subunit

Sibarov D., Bruneau N., Antonov S., Szepetowski P., Burnashev N., Giniatullin R.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

© 2017 Sibarov, Bruneau, Antonov, Szepetowski, Burnashev and Giniatullin. Genetic variants of the glutamate activated N-methyl-D-aspartate (NMDA) receptor (NMDAR) subunit GluN2A are associated with the hyperexcitable states manifested by epileptic seizures and interictal discharges in patients with disorders of the epilepsy-aphasia spectrum (EAS). The variants found in sporadic cases and families are of different types and include microdeletions encompassing the corresponding GRIN2A gene as well as nonsense, splice-site and missense GRIN2A defects. They are located at different functional domains of GluN2A and no clear genotype-phenotype correlation has emerged yet. Moreover, GluN2A variants may be associated with phenotypic pleiotropy. Deciphering the consequences of pathogenic GRIN2A variants would surely help in better understanding of the underlying mechanisms. This emphasizes the need for functional studies to unravel the basic functional properties of each specific NMDAR variant. In the present study, we have used patch-clamp recordings to evaluate kinetic changes of mutant NMDARs reconstituted after co-transfection of cultured cells with the appropriate expression vectors. Three previously identified missense variants found in patients or families with disorders of the EAS and situated in the N-terminal domain (p.Ile184Ser) or in the ligand-binding domain (p.Arg518His and p.Ala716Thr) of GluN2A were studied in both the homozygous and heterozygous conditions. Relative surface expression and current amplitude were significantly reduced for NMDARs composed of mutant p.Ile184Ser and p.Arg518His, but not p.Ala716His, as compared with wild-type (WT) NMDARs. Amplitude of whole-cell currents was still drastically decreased when WT and mutant p.Arg518His-GluN2A subunits were co-expressed, suggesting a dominant-negative mechanism. Activation times were significantly decreased in both homozygous and heterozygous conditions for the two p.Ile184Ser and p.Arg518His variants, but not for p.Ala716His. Deactivation also significantly increased for p.Ile184Ser variant in the homozygous but not the heterozygous state while it was increased for p.Arg518His in both states. Our data indicate that p.Ile184Ser and p.Arg518His GluN2A variants both impacted on NMDAR function, albeit differently, whereas p.Ala716His did not significantly influence NMDAR kinetics, hence partly questioning its direct and strong pathogenic role. This study brings new insights into the functional impact that GRIN2A variants might have on NMDAR kinetics, and provides a mechanistic explanation for the neurological manifestations seen in the corresponding human spectrum of disorders.

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Keywords

Epilepsy, Genetic variants, GluN2A, GRIN2A, NMDA receptors

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