CHARACTERISATION OF THE SUBSTANTIA NIGRA PARS COMPACTA IN THE ABSENCE OF NEURTURIN

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Purpose Neurturin, a member of the GDNF family, is a potent neurotrophic factor for dopaminergic cells and is currently being trialled as a therapy for Parkinson’s disease patients. Previous analysis of neonatal neurturin knockout mice reported no gross abnormalities in the brain or any overt difference in tyrosine hydroxylase staining, however these analyses were qualitative. Here we show quantitative data on dopaminergic neurons of the substantia nigra in mice lacking neurturin.

Methods Sections from 12-14 week old neurturin knockout mouse brains and their wildtype littermates (n=3) were sequentially labelled through the composition of the nigral dopaminergic neurons in the absence of neurturin, namely, promoting a TH+/calbindin+ cell phenotype in the substantia nigra pars compacta.

Results No significant difference in the overall number of TH+ cells was found between knockout and wildtype mice. However, there was a significant increase in the number of TH+/calbindin+ cells in the knockout mice as compared with their wildtype littermates. Conclusion TH+/calbindin+ dopaminergic neurons of the substantia nigra have an increased survival potential in Parkinson’s disease (Murase and McKay, 2006). Our results suggest a change in the composition of the nigral dopaminergic neurons in the absence of neurturin, namely, promoting a TH+/calbindin+ cell phenotype in the substantia nigra pars compacta.

TORSINA, A PROTEIN ASSOCIATED WITH EARLY ONSET DYSTONIA, INTERACTS WITH COLLAPSING RESPONSE MEDIATOR PROTEIN

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The dystonias represent a heterogeneous group of neurological disorders characterised by involuntary muscle contraction and twisting, repetitive movements. Mutation of the torsinA gene (DYT1) causes early-onset general torsion dystonia, the most common and severe form of dystonia. While previous studies have indentified several torsinA interacting proteins and suggested potential roles for torsinA in nuclear membrane morphology and protein transport, the function of torsinA is currently unknown. For this reason we have searched for novel binding partners of torsinA to determine the mechanism by which mutation of torsinA might produce a neurological phenotype and to investigate the molecular pathways disrupted in dystonia. Methods: Potential interactors of torsinA were identified using an unbiased proteomics approach. TorsinA was immunoprecipitated from human cortex and associated proteins were identified by LC-MSMS. TorsinA-interacting proteins were further confirmed by co-immunoprecipitation and localisation studies. Results: We have identified the collapsin response mediator protein (CRMP2) as a torsinA interacting protein. The interaction was confirmed by in vivo co-immunoprecipitation analysis of endogenous and exogenous CRMP2 in both HEK-293 and human neuroblastoma cells (BE-M17) using two independent torsinA antibodies. Furthermore, we examined the effect of the dystonia specific mutation (ΔE302/303) and mutations within functional domains of torsinA on the interaction between torsinA and CRMP2. Conclusion: We have identified CRMP2 as a novel protein that interacts with torsinA. CRMP2 has been associated with neurological disorders such as Alzheimers disease is thought to be involved in neuronal microtubule assembly and axon outgrowth. Our results suggest that disruption of the interaction between torsinA and CRMP2 may contribute to the development of dystonia.

UNLOCKING THE SECRETS OF FAMILIAL EPILEPSY: A WHOLE GENOME ANALYSIS

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In the past ten years a number of familial epilepsies have been associated with single ion channel gene mutations. In a bid to understand fundamentals of disease genesis in familial epilepsy, we generated gene targeted knock-in mouse models harbouring human ion channel mutations found in families with epilepsy. Two such models have been associated with single ion channel mutation models. In addition, our findings have identified ion channels exhibiting an age-genotype interaction. In R43Q model a 2 way ANOVA identified ion channels exhibiting an age-genotype interaction (p<0.01). Conclusion: Our results suggest that and P40) and homozygote mice (examined at P14 only due to premature death at P21); wild type litter mates (n=3) were controls for each group. Total RNA was isolated and hybridised to the Affymetrix mouse all exon array. Differential expression was analysed using gene summary data by Partek Genomics Suite (Version 6.3) and GOMiner. Results: At P14 in the C121W model, a number of cell adhesion molecules is increased in knock-in mice compared to controls (p<0.01). In R43Q model a 2 way ANOVA identified ion channels exhibiting an age-genotype interaction (p<0.001). Conclusion: Genes already implicated by their association with ion channel function or epilepsy, were found differentially expressed in these single ion channel mutation models. In addition, our findings have revealed pathways that may underlie seizure genesis with implications for diagnosis and therapy.

MUTATIONS IN PARKIN AND PACRG IN DYSTONIA

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Mutations in parkin are the most common genetic cause of early onset Parkinson’s disease (EO-PD). Parkin functions in the ubiquitin-proteasomal system (UPS) as an E3 ubiquitin ligase and disruption of this pathway has been linked to the pathogenesis of PD. PACRG shares a bi-directional promoter with parkin and the two genes are co-regulated (West et al., 2003). The function of PACRG is unknown however we hypothesise that parkin and PACRG interact and function in a common pathway. PURPOSE: To confirm an interaction between parkin and PACRG and investigate its molecular consequences on PACRG localisation and function. METHODS: BE(M17) neuroblastoma cells stably overexpressing PACRG or parkin were generated and co-immunoprecipitation (co-IP) analysis was performed. Truncated parkin constructs were generated to identify the parkin domain mediating the interaction. The functional consequences of the interaction were investigated by performing ubiquitination assays, immunohistochemistry and microtubule stability studies. RESULTS: Co-IP studies demonstrated that parkin and PACRG interact in vivo, through the RING2 domain of parkin. Furthermore, parkin mediates the ubiquitination of PACRG utilising both K48 and K63 ubiquitin linkages. The interaction and ubiquitilation of PACRG by parkin promoted the recruitment of PACRG to aggresomes. PACRG-positive aggresomes were observed in 82.0%±13 of M17 cells overexpressing parkin compared with 46.7%±11.8 of parental M17 cells (p=0.02, n=3) and were resistant to microtubule destabilisation by nocodazole. CONCLUSION: Our results suggest that parkin and PACRG function in a common pathway, potentially involving microtubule mediated aggresome formation. Aggresomes may represent a cellular defence mechanism against the toxic effects of misfolded proteins and disruption of this process may contribute to the pathogenesis of PD.