

Impaired Neurotransmitter Release in Alzheimer's and Parkinson's Diseases

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Key Words

Synapse · Age-related neurodegeneration · Presenilin · Parkin · Nicastrin

Abstract

Mutations in several causative genes have been linked to monogenic forms of Alzheimer's disease (AD) or Parkinson's disease (PD). To look for possible common pathogenic mechanisms underlying age-related neurodegeneration in AD and PD, we employed genetic approaches to investigate systematically the roles of these gene products (e.g. presenilins (PS) for AD; Parkin, DJ-1, PINK1 and LRRK2 for PD) in the mouse brain, especially in neural circuits that are particularly vulnerable in AD or PD. Our series of genetic studies revealed that PS play cell type-specific roles in the developing brain with the most prominent function in the maintenance of neural progenitor cells. In the adult cerebral cortex, where the pathogenesis of AD occurs, loss of PS results in progressive memory impairment and age-related neurodegeneration. Specifically, PS are involved in the regulation of long-term potentiation and NMDA receptor functions. Interestingly, our further genetic dissection in the hippocampal Schaeffer collateral pathway highlighted the importance of presynaptic PS in the activity-dependent regulation of glutamate release and long-term potentiation induction via

modulation of calcium release from intracellular stores. Intriguingly, our independent genetic analysis of Parkin, DJ-1, PINK1 and LRRK2 showed a common defect in activity-dependent dopamine release caused by PD-linked mutations in these genes. Together, our genetic studies suggest that presynaptic dysfunction might be a converging early pathogenic event before neurodegeneration in AD and PD.

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Alzheimer's disease (AD) is the most common form of dementia and neurodegenerative disorder. AD is characterized clinically by progressive memory loss and deterioration of cognitive functions, and neuropathologically by extracellular amyloid plaques, intracellular neurofibrillary tangles, and synaptic and neuronal loss. Presenilins (PS1 and PS2) are the major causative genes of early-onset (<65 years of age) familial AD and harbor ~90% of FAD mutations identified to date. PS are essential components of γ -secretase, a multi-subunit protease complex that catalyzes the intramembranous cleavage of a number of type I transmembrane proteins, including Notch and the amyloid precursor protein. Notch is a key physiological substrate of γ -secretase, as evidenced by similar developmental phenotypes exhibited by PS and Notch mutant mice [reviewed in 1], and the dependence of Notch

signaling on the γ -secretase-mediated release of its intracellular domain [2, 3].

PS play important roles during embryonic development. *PS1*^{-/-} mice exhibit perinatal lethality and somitogenesis defects [4, 5], while mice deficient for both PS1 and PS2 die before embryonic day 9.5 and display early patterning defects [6]. Our investigations of PS function in neural development revealed essential roles for PS in maintenance of neural progenitor cells, differentiation and migration of postmitotic neurons and generation of radial glia [1, 4, 7–9]. PS1 also regulates neuronal migration in a non-cell-autonomous manner by controlling proliferation of meningeal fibroblasts, which in turn affects the survival of Cajal Reizus neurons, pioneer neurons that are important for proper neuronal migration [8]. PS regulate these processes primarily through the Notch signaling pathway [1–3, 7, 10]. However, it is unclear whether Notch is the key functional mediator of PS in the adult brain.

To investigate the role of PS in synaptic function in the adult cerebral cortex, which is the most relevant experimental system for the investigation of the pathogenesis of AD, we generated a viable *PS1* conditional knockout, in which expression of PS1 is selectively eliminated in excitatory pyramidal neurons of the forebrain beginning at postnatal day ~18 [11]. PS is normally expressed highly in pyramidal neurons of the cerebral cortex. This hypomorphic *PS* mutant mouse exhibits a specific but mild deficit in spatial memory [11]. Synaptic transmission and plasticity in the hippocampal CA3-CA1 synapse, however, are normal [11]. Analysis of conditional *PS*-null mice lacking both PS1 and PS2 in the postnatal forebrain revealed impairments in hippocampal memory and long-term potentiation (LTP) prior to any neuropathological changes, demonstrating a requirement for PS in normal synaptic plasticity and memory [12]. More specifically, we found a selective reduction in NMDA receptor-mediated responses and synaptic levels of NMDA receptors and α CaMKII in mutant mice. Furthermore, in the absence of PS, levels of CBP and transcription of CREB/CBP target genes are reduced [12, 13], even though subsequently we found that CREB-mediated transcription is regulated indirectly by PS [14]. Strikingly, *PS* cDKO mice develop in an age-dependent manner synaptic, dendritic and neuronal degeneration with accompanying astrogliosis and hyperphosphorylation of tau, demonstrating an essential role for PS in neuronal survival [12, 15]. Furthermore, PS promote memory and neuronal survival in a γ -secretase-dependent manner, as conditional inactivation of nicastrin, another component of the γ -secretase

complex, in the adult cerebral cortex similarly resulted in progressive memory impairment and neurodegeneration [16]. Based on these in vivo findings and a large number of reports on the effects of FAD-linked mutations in culture and in vitro systems as well as in *C. elegans*, we proposed that PS mutations may cause dementia and neurodegeneration in AD via a partial loss-of-function mechanism [17]. The fact that synaptic impairments precede progressive neurodegeneration suggests that synaptic dysfunction caused by loss of PS function promotes subsequent neuronal degeneration.

To determine the precise synaptic site of PS function, we performed a systematic genetic analysis through the restriction of PS inactivation to hippocampal CA1 or CA3 neurons [18]. This strategy permitted analysis of the effects of PS inactivation in either presynaptic or postsynaptic neurons of the Schaeffer collateral pathway. We found that LTP induced by theta burst stimulation is decreased after presynaptic but not postsynaptic deletion of PS. Moreover, presynaptic but not postsynaptic inactivation of PS impairs short-term plasticity and synaptic facilitation. The probability of evoked glutamate release, measured with the open-channel NMDA receptor antagonist MK-801, is reduced by presynaptic inactivation of PS. Strikingly, depletion of calcium internal stores by thapsigargin or inhibition of calcium release from these stores by ryanodine receptor inhibitors mimics and occludes the effects of presynaptic PS inactivation. Collectively, our genetic and electrophysiological studies demonstrate that loss of PS function impairs LTP induction and glutamatergic neurotransmitter release by a presynaptic mechanism. These findings, which distinguish unequivocally between presynaptic and postsynaptic functions of PS, raise the possibility that presynaptic mechanisms may play a primary role in AD pathophysiology. In support of this hypothesis, PS are localized to presynaptic terminals [18], and amyloid precursor protein C-terminal fragments, precursors of A β , accumulate in presynaptic terminals of *PS1* conditional knockout mice [19].

Parkinson's disease (PD) is the most common movement disorder characterized by resting tremor, rigidity and bradykinesia. These clinical features are thought to result from reduced dopaminergic input to the striatum, which is caused by the loss of dopaminergic neurons in the substantia nigra. The occurrence of PD is largely sporadic, but clinical syndromes resembling sporadic PD have been linked to mutations in at least 5 distinct genes (*α -synuclein*, *parkin*, *DJ-1*, *PINK1* and *LRRK2*). The recessive inheritance mode of the mutations and the existence of large deletions in the *parkin*, *DJ-1* and *PINK1*

genes indicate a loss-of-function pathogenic mechanism. Parkin can function as an E3 ubiquitin ligase [20–23] or as a transcription regulator [24]. DJ-1 itself can be oxidized, thus protecting cells from oxidative damage [25, 26]. PINK1 is a protein kinase localized in the mitochondrion and other subcellular compartments [27–30]. Our previous generation and multidisciplinary analysis of *parkin*^{-/-}, *DJ-1*^{-/-} and *PINK1*^{-/-} mice have demonstrated that each of these gene products is required for normal dopaminergic function and evoked dopamine release in nigrostriatal terminals [31–34]. Furthermore, inactivation of each or all three of these recessive PD genes does not cause dopaminergic neurodegeneration [31, 33–35]. In addition, our studies have further shown that loss of PINK1 or Parkin impairs mitochondrial function [36, 37]. A role for PINK1 and Parkin in mitochondria is also supported by genetic studies in *Drosophila* [38–42]. Thus, two converging cellular pathogenic mechanisms have emerged from genetic studies of recessive parkinsonism

[43]. Specifically, presynaptic dopaminergic dysfunction in evoked release may be a central pathogenic precursor before leading to frank dopaminergic neuron loss in PD.

In summary, our genetic approaches to the studies of AD and PD [44] have uncovered a novel pathogenic mechanism, suggesting that defects in presynaptic neurotransmitter release may represent a convergent mechanism leading to neurodegeneration in affected circuits in AD and PD. Therapeutic strategies directed toward restoring normal neurotransmitter release may be effective in combating circuit dysfunction and neurodegeneration in these disorders.

Acknowledgements

The author would like to thank Adair Swain for assistance. This work was supported by grants from the NINDS.

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