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Increased Dendrite Branching in A β PP/PS1 Mice and Elongation of Dendrite Arbors by Fasudil Administration

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Abstract

Amyloid- β (A β) overproduction and dendrite arbor atrophy are hallmarks of Alzheimer's disease. The RhoA GTPase (Rho) signals through Rho kinase (ROCK) to control cytoskeletal dynamics and regulate neuron structure. Hyperactive Rho signaling destabilizes neurons leading to dendritic regression that can be rescued by genetic or pharmacological reduction of ROCK signaling. To understand what effect reduced ROCK signaling has on the dendrite arbors of mice that overproduce A β , we administered the ROCK inhibitor fasudil to A β PP/PS1 transgenic mice. We report that increased dendrite branching occurs in A β PP/PS1 mice and that fasudil promotes lengthening of the dendrite arbors of CA1 pyramidal neurons.

Keywords

Amyloid- β protein precursor; dendrites; fasudil; hippocampus; intracerebroventricular infusion; presenilin-1; Rho GTPase; Rho kinase

INTRODUCTION

Alzheimer's disease (AD) is characterized by overproduction of amyloid- β (A β) as well as loss of synapses and reduction of dendritic arbors in specific brain regions [1–3]. Pyramidal neurons in the CA1 region of the hippocampus are particularly affected in AD, displaying a loss of total dendrite length from both their apical and basal trees [4].

The Rho GTPase (Rho) signals through its effector Rho kinase (ROCK) to coordinate cytoskeletal dynamics and regulate neuron morphology [5]. ROCK controls cytoskeletal dynamics, in part, by activating LIM kinase leading to phosphorylation and inhibition of the actin-severing protein cofilin [6]. Neuron function requires proper regulation of Rho activity, and hyperactive Rho signaling antagonizes dendrite stability in a variety of contexts. For example, transfection of hippocampal slices with a constitutively active Rho mutant reduces dendrite arbors in a ROCK-dependent manner [7]. Similarly, genetic

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perturbation of upstream regulators that antagonize Rho activity causes simplification of CA1 pyramidal neurons, which can be rescued by genetic attenuation of ROCK signaling [8]. Finally, increased Rho activity has been reported in AD model mouse brain [9] and has been hypothesized to contribute to AD pathogenesis [10].

Rho/ROCK signaling is activated in response to ischemia and spinal cord injury [11–14]; administration of the ROCK inhibitor fasudil improves neurological recovery after acute insult [15,16]. We administered fasudil via intracerebroventricular infusion to $A\beta$ PP/PS1 mice that overproduce $A\beta$ [17] and analyzed the resulting neuron morphology. We found that CA1 pyramidal neurons are more branched in $A\beta$ PP/PS1 mice and that fasudil stabilizes dendrite arbors in both wild type (WT) and $A\beta$ PP/PS1 mice.

MATERIALS AND METHODS

Intracerebroventricular infusion

Two days prior to surgery, micro-osmotic pumps with brain infusion assemblies (Alzet Pump Model 1004 and Brain Infusion Kit 3; DURECT, Cupertino, CA) were aseptically filled with fasudil (LC Laboratories, Woburn, MA) dissolved in artificial cerebrospinal fluid (aCFS) or aCSF mock treatment. Fresh aCSF was prepared by mixing equal parts solution A (296 mM NaCl, 6 mM KCl, 2.7 mM $CaCl_2$, 1.6 mM $MgCl_2$) and solution B (1.6 mM Na_2HPO_4 , 0.39 mM NaH_2PO_4). Pumps were incubated in phosphate-buffered saline (PBS) at 37°C until surgery.

Pumps were surgically implanted into naive 3-month-old WT and $A\beta$ PP/PS1 mice (courtesy of Stephen Strittmatter) of mixed genetic background (C57BL/6, 129/SvJ, C3H/HeJ). Animals were allowed free access to analgesic (Metacam; Boehringer Ingelheim, St. Joseph, MO) for one day before and two days after the procedure. Anesthesia was induced with 4% isoflurane and maintained with 2% isoflurane in oxygen delivered by vaporizer (Model 100; SurgiVet, Waukesha, WI). Animals were surgically prepared and positioned in a stereotaxic frame with mouse adaptor (Dual Lab Standard; Stoelting, Wood Dale, IL). For each animal, a small midline incision was made across the scalp, a burr hole was drilled in the skull, the cannula was stereotaxically positioned to target the left lateral ventricle (ML –1.1 mm, AP –0.6 mm, DV –2.5 mm from bregma [18]), and the head mount was affixed to the skull using Loctite 454 adhesive (Loctite, Rocky Hill, CT). The pump canister was placed subcutaneously on the animal's back, and the wound was closed using nylon sutures. Fasudil was delivered at a rate of 0.6 mg/kg/day for 24–26 days prior to morphological analysis. The following antibodies were used for protein phosphorylation analysis: phosphocofilin (Ser-3) and cofilin (CP1151 and CP1131; ECM Biosciences, Versailles, KY), phospho-Erk1/2 (Thr202/Tyr204) and Erk1/2 (4370 and 9102; Cell Signaling Technology, Danvers, MA). All procedures were compliant with federal regulations and approved by the Yale University Animal Care and Use Committee.

Neuron staining and morphometric analysis

Neuron staining and morphometric analysis were performed according to our previously described protocol [8]. Briefly, individual live CA1 pyramidal neurons in 400 μ m hippocampal slices were injected with a 4% biocytin solution, fixed in 4% paraformaldehyde, cry-protected in 30% sucrose, resectioned at 40 μ m, and resolved using avidin-horseradish peroxidase (HRP) stain. Individually-labeled neurons were traced by an experimenter blind to genotype/treatment, reconstructed using NeuroLucida software, and enumerated using NeuroExplorer software. Branchpoint number and total dendrite length were analyzed by two-factor (genotype \times treatment) analysis of variance (ANOVA); Sholl intersections were analyzed by three-factor (genotype \times treatment \times shell radius) ANOVA

with repeated measures (RM) followed by pair-wise Student's *t*-tests. Values that fell more than two standard deviations from the mean were excluded or replaced with the group mean as needed for RM analysis.

RESULTS

Intracerebroventricular fasudil infusion in A β PP/PS1 mice

We wanted to test whether fasudil treatment could prevent dendritic regression in A β PP/PS1 mice. Previous studies indicated that these animals show subtle behavioral deficits at 4 months of age [19], so we delivered treatment to mice via osmotic pump with intracerebroventricular infusion for 24–26 days prior to analysis at 4 months (Fig. 1A). We labeled and reconstructed 116 individual CA1 pyramidal neurons from WT and A β PP/PS1 mice given fasudil or mock treatment (Fig. 1B) and performed quantitative morphological analysis of the resulting apical and basal dendrite arbors (Fig. 1C). To determine the biochemical effects of drug treatment, we analyzed protein phosphorylation in drug-treated animals (Fig. 1D). Consistent with inhibition of ROCK activity, cofilin phosphorylation was reduced by 70% ($p < 0.01$), while phosphorylation of unrelated Erk proteins was unaffected (Fig. 1E).

A β PP/PS1 transgenes promote dendrite branching and fasudil increases dendrite arbor length

While there were no significant differences in branching of apical dendrite arbors, basal dendrite arbor branchpoint number was elevated significantly in A β PP/PS1 mice (Fig. 2A; main effect of genotype $F_{(1,111)} = 7.29$, $p < 0.01$). Extended fasudil treatment conferred increased total dendrite length for both WT and A β PP/PS1 mice with significant main effects of treatment in both apical and basal dendrite arbors (Fig. 2B; $F_{(1,106)} = 4.20$, $p = 0.04$; $F_{(1,106)} = 5.93$, $p = 0.02$, respectively). For both branchpoint number and total dendrite length, no interactions were detected between treatment and genotype.

We performed Sholl analysis to assess the distribution of segments throughout the dendritic tree. Within the apical arbors, differences could be seen at the 475–550 μm shells where mock-treated WT animals were significantly reduced (Fig. 1C; $p < 0.05$ for each shell relative to all other groups). Within the basal arbors of fasudil-treated A β PP/PS1 mice, there was a pronounced increase in dendrite intersections with shells of 50–150 μm radii (Fig. 1D; $p < 0.05$ for each shell relative to mock-treated WT animals). Thus, it appears that the A β PP/PS1 transgenes promote branching throughout the basal dendrite arbor, while fasudil elongates dendrite branches.

DISCUSSION

Neuropathological findings in AD patients include dendritic regression in brain regions supporting cognitive functions compromised during this disease [3]. Integrity of the underlying cytoskeleton sustains neuron function and requires precise regulation by diverse signaling mechanisms. Elevated Rho/ROCK signaling contributes to dendritic regression [7,8] and has been noted in another mouse model of AD [9]. Clinically-approved in Japan for treatment of cerebral vasospasm [20], the ROCK inhibitor fasudil confers neuroprotective effects against multiple neuronal challenges [15,16]. We observed that hippocampal dendrite arbors are more highly branched in A β PP/PS1 mice and that fasudil promotes dendrite length in both WT and A β PP/PS1 mice.

Increased dendrite branching has been observed in a subset of neurons in humans with AD [21]. Furthermore, low concentrations of oligomeric A β have been seen to induce neurite formation via p75 neurotrophin receptor in cultured neurons [22]. While it is unclear why

increased branching occurs in A β PP/PS1 mice, one possibility is that toxic A β species present in these mice damage neurons, resulting in neurite sprouting as a neuroplastic response. In an effort to maintain synaptic homeostasis, this response may help preserve hippocampal circuit connectivity and delay deficits in hippocampal-dependent functions, such as spatial learning and reference memory [19].

Rho signaling specifically controls dendrite branch length: increased Rho activity leads to branch shortening while decreased Rho activity leads to branch elongation [23]. In addition to total dendrite length, we found that dendrite length per branch is also increased in fasudil-treated animals (data not shown). We did not observe dendritic regression in mock-treated transgenic mice possibly owing to young age, homeostatic compensatory mechanisms, or incomplete recapitulation of disease etiology.

Although preliminary, our results warrant further investigation of the potential benefits fasudil may have for AD. Long-term fasudil treatment was well-tolerated by mice in our study, and others have shown that ROCK inhibition improves spatial learning and working memory in aged rats [24]. Thus, in addition to the stabilization of dendritic arbors, fasudil may also promote functional recovery in AD.

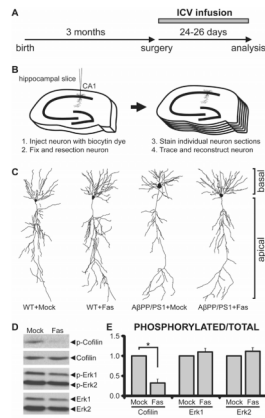
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**Fig. 1.**

Intracerebroventricular fasudil infusion in $A\beta$ PP/PS1 mice and resulting dendrite morphology and biochemical effects. A) Mice were treated via osmotic pump with intracerebroventricular infusion for 24–26 days and analyzed at 4 months of age. B) Individual CA1 pyramidal neurons were dye-filled in thick hippocampal sections followed by fixation, resectioning, and staining. Neurons were traced and reconstructed using NeuroLucida. C) Neuron traces from WT and $A\beta$ PP/PS1 mice given mock or fasudil treatment. D) Representative immunoblots of 80 μ g hippocampal protein lysate from WT mice given mock or fasudil treatment for nine days. E) Quantification of protein phosphorylation levels. Bars represent phosphorylation level means \pm SE in drug-treated animals normalized to mock-treated littermate controls. Kruskal-Wallis test, $*p < 0.01$. For biochemical analysis: Mock, $n = 3$ mice; Fas, $n = 4$ mice.

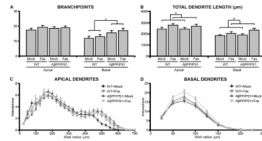


Fig. 2.

Increased dendrite branching in $A\beta$ PP/PS1 mice and stabilization of dendrite arbors by fasudil administration. A) Branchpoint number and (B) total dendrite length for apical and basal dendrite arbors from WT and $A\beta$ PP/PS1 mice given mock or fasudil treatment. Bars represent means \pm SE. Two-factor ANOVA main effects, $*p < 0.05$. Sholl analysis of (C) apical and (D) basal dendrite arbors from WT and $A\beta$ PP/PS1 mice given mock or fasudil treatment. Sholl analysis measures dendrite distribution by quantifying the number of intersections that the arbor makes with concentric spheres centered on the neuron cell body. Number of intersections with a shell is plotted versus the shell radius. Symbols represent means \pm SE. For morphometric analysis: WT+Mock, $n = 9$ mice, 27 neurons; WT+Fas, $n = 9$ mice, 27 neurons; $A\beta$ PP/PS1+Mock, $n = 11$ mice, 30 neurons; $A\beta$ PP/PS1+Fas, $n = 9$ mice, 32 neurons.