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Expression of the Neuronal Adaptor Protein X11*α* Protects Against Memory Dysfunction in a Transgenic Mouse Model of Alzheimer's Disease

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Abstract

X11*α* is a neuronal-specific adaptor protein that binds to the amyloid-*β* protein precursor (A*β*PP). Overexpression of X11*α* reduces A*β* production but whether X11*α* also protects against A*β*-related memory dysfunction is not known. To test this possibility, we crossed X11*α* transgenic mice with A*β*PP-Tg2576 mice. A*β*PP-Tg2576 mice produce high levels of brain A*β* and develop age-related defects in memory function that correlate with increasing A*β* load. Overexpression of X11*α* alone had no detectable adverse effect upon behavior. However, X11*α* reduced brain A*β* levels and corrected spatial reference memory defects in aged X11*α*/A*β*PP double transgenics. Thus, X11*α* may be a therapeutic target for Alzheimer's disease.

Keywords

Amyloid-*β* protein precursor; axonal transport; Mint1; protein trafficking; X11*α*

INTRODUCTION

Altered processing of the amyloid-*β* protein precursor (A*β*PP) to increase production of A*β* is believed to be a key pathogenic event in Alzheimer's disease [1]. Lowering A*β* production thus represents one of the favored routes for therapeutic intervention. X11*α* (also known as mint-1) is a neuronal-specific adaptor protein that binds to the intracellular domain of A*β*PP [2-4]. Overexpression of X11*α* decreases production of A*β* [5-10]. This finding has prompted the suggestion that modulating X11*α* function or X11*α*-A*β*PP interactions might provide therapeutic targets for Alzheimer's disease [11]. However, more formal support for this notion requires the demonstration that X11*α* not only reduces cerebral A*β* load but also rescues A*β*-related defects in cognition. To test this possibility, we studied the effect of X11*α* on memory function and brain A*β* levels by crossing transgenic mice that overexpress

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X11*α* [9] with A*β*PP-Tg2576 transgenic mice. A*β*PP-Tg2576 mice express a familial Alzheimer's disease "Swedish" mutant A*β*PP and develop age-related defects in spatial reference memory that correlate with increasing levels of brain A*β* [12-15].

Crossing of X11*α* and A*β*PP-Tg2576 mice produced the predicted ratio of genotypes (one quarter non-transgenic (NTg), A*β*PPswedish mutant (A*β*PPswe), X11*α*, A*β*PPswe/X11*α*). Mice were studied at 3–4 and 16–18 months of age. At 3–4 months, A*β*PP-Tg2576 have low levels of A*β* and normal memory function, but at 16–18 months, cerebral A*β* levels are elevated and the mice display memory dysfunction [14,15]. There were no significant differences in gender ratios between the four genotypes in either the 3–4 or 16–18 month age groups (chi-square analyses) and so males and females were pooled for analyses.

We initially tested animals for sensorimotor defects using the SHIRPA primary screen [16]. No significant differences were detected between the four genotypes at either age demonstrating that X11*α* overexpression has no obvious effect on sensorimotor function in either the absence or presence of A*β*PPswe (data not shown). We then studied the effect of X11*α* on spatial reference memory function using the Morris water maze. For these studies we performed visible platform training followed by hidden platform testing with three rounds of probe trials as described previously by us and others for A*β*PP-Tg2576 mice [15,17,18]. All genotypes in both age groups learned the location of the visible platform within 3 days of training (Fig. 1A, B) and were similarly proficient swimmers (data not shown). However, 16–18 but not 3–4 month old A*β*PPswe and A*β*PPswe/X11*α* mice both had increased latencies to reach the platform compared with NTg and X11*α* littermates in the first two training blocks on day 1 (Fig. 1A, B). This result is consistent with previous studies which also showed that older but not young A*β*PP-Tg2576 mice have an initial delay in escape latency to the visible platform [15,17]. Despite this lag in reaching the visible platform by 16–18 month A*β*PPswe and A*β*PPswe/X11*α* mice, our finding that there were no significant differences between any genotype on days 2 and 3 argues against sensorimotor deficits as a potential explanation for any defective performance in determining the location of the hidden platform. Others have formed the same conclusion with similar data on A*β*PP-Tg2576 mice [15,17]. In addition, our findings that there were no significant differences between NTg and X11*α*, or between A*β*PPswe and A*β*PPswe/X11*α*, at either time studied argues that the phenotype is due to A*β*PPswe and is not influenced by the presence of X11*α*. We thus conclude that X11*α* has no effect on escape latency to reach the visible platform in the Morris water maze.

We then tested the abilities of the mice to reach the hidden platform in the Morris water maze. This involved analyses of escape latencies and of swimming patterns in probe trials where the platform was removed. At 3–4 months of age, all genotypes performed equally well in the trials (Fig. 1C and data not shown). However, at 16–18 months, A*β*PPswe mice displayed significant deficits compared to all other genotypes. This was revealed by an increase in escape latency to reach the hidden platform (Fig. 1D) and by reduced performance in the probe trials (Fig. 2). This age-dependent defect in the Morris water maze by A*β*PPswe transgenics is consistent with previous reports on A*β*PP-Tg2576 mice [15,17,18]. There were no detectable differences between X11*α* and NTg mice in any tests. However, 16–18 month A*β*PPswe/X11*α* mice displayed significant improvements in both escape latencies and mean probe trial scores compared to A*β*PPswe mice such that their performances were not significantly different from NTg or X11*α* mice (Figs 1D and 2).

Previous studies involving similar crossing of X11*α* with A*β*PP-Tg2576 transgenic mice have shown that overexpression of X11*α* reduces A*β* levels in the brains of 3 month old A*β*PPswe mice and reduces amyloid plaque numbers in older mice [9]. However, the effect of X11*α* on A*β* levels in older A*β*PPswe mice has not been reported. To determine whether

the rescue of spatial memory function in 16–18 month old AβPPswe/X11α compared to AβPPswe littermate mice was associated with changes in Aβ, we analyzed A$\beta_{1–40}$ and A$\beta_{1–42}$ levels in the brains of these animals. In young AβPP-Tg2576 mice, Aβ levels are mainly soluble in aqueous buffers but in older mice Aβ levels rise and require solubilization in agents such as formic acid [14,17,19]. We thus prepared Tris-HCl-soluble and formic acid-soluble fractions for analyses. Levels of Tris-HCl-soluble and formic acid-soluble A$\beta_{1–40}$ and A$\beta_{1–42}$ were significantly reduced in AβPPswe/X11α compared to AβPPswe littermates (Fig. 3). Thus, overexpression of X11α improves spatial reference learning and memory in AβPP-Tg2576 mice and this is associated with a decrease in cerebral Aβ load.

X11α decreased Aβ levels to approximately 60% and this produced a marked improvement in cognitive performance. This suggests that there is a threshold of Aβ concentration that impacts on memory. Alternatively, X11α may positively influence cognition by additional Alzheimer's disease-related mechanisms that do not involve Aβ (e.g., modifying AβPP function). Indeed, the precise mechanisms by which X11α lowers Aβ are far from clear, and there is conflicting evidence on the effect of loss of X11α on AβPP processing [20-22]. X11α has both pre- and post-synaptic functions [23-26] which may involve trafficking of synaptic cargoes including AβPP but also NMDA receptors since X11α also binds kinesin KIF17 [27]. Altered protein trafficking and axonal transport is seen in Alzheimer's disease [28]. Finally, X11α binds to the copper chaperone for superoxide dismutase-1 and so may play a role in copper homeostasis [29]. Defective copper metabolism is also implicated in Alzheimer's disease [30].

The X11s comprise three family members (X11α, X11β, and X11γ) [11] and overexpression of X11β also inhibits Aβ production and corrects memory dysfunction in AβPP-Tg2576 mice [17,19]. However, X11α and X11β have different functions [31-33], and there is evidence that they inhibit Aβ production by different mechanisms [34,35]. As such it is important to determine whether different X11s similarly protect against Aβ-related memory dysfunction and whether there are isoform-specific differences in any protection. This is especially the case since X11α and X11β are differentially expressed in the brain which may provide a route for targeting different regions for therapy ci36. Our findings reported here that X11α lowers brain Aβ and protects against Aβ-related cognitive dysfunction thus validate X11α as a potential therapeutic target for Alzheimer's disease.

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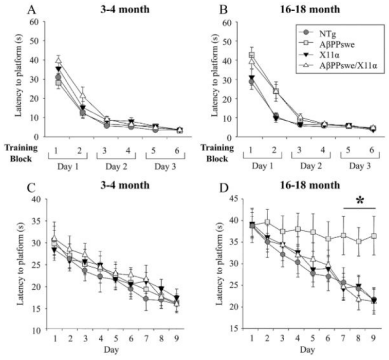

Fig. 1.

X11*α* improves acquisition of platform location in aged A*β*PPswe mice in the Morris water maze. Escape latencies in seconds (s) for NTg, A*β*PPswe, X11*α*, and A*β*PPswe/X11*α* mice were measured during visible and hidden platform training. (A and B) show escape latencies to the visible platform in 3–4 month and 16–18 month mice. No significant differences were detected between any of the genotypes in visible platform training at 3–4 months of age (two-way ANOVA). In 16–18 month animals, both A*β*PPswe and A*β*PPswe/X11*α* transgenics showed increased latencies in the first two blocks (day 1) of visible platform training compared with both X11*α* and NTg ($P < 0.05$). No differences were detected between NTg and X11*α* and between A*β*PPswe and A*β*PPswe/X11*α* at any time point (two-way ANOVA). (C and D) show escape latencies to the hidden platform in 3–4 month and 16–18 month mice. No significant differences were detected between any genotype in 3–4 month mice (two-way ANOVA). However, in 16–18 month mice, latencies were specifically increased in A*β*PPswe compared to all other genotypes on days 7, 8 and 9 of testing and this effect was rescued in A*β*PPswe/X11*α* mice (two-way ANOVA; $P < 0.01$ day 7; $P < 0.001$ days 8 and 9). *indicates significant differences between A*β*PPswe and all other genotypes. $n = 15–21$ for 3–4 month mice; $n = 12–16$ for 16–18 month mice. Error bars are ± SEM.

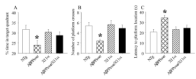

Fig. 2.

X11α improves retention of platform location in 16–18 month AβPPswe mice in the Morris water maze. Spatial memory retention was assessed in probe trials which determined the % of time spent in target quadrant (A), mean number of platform crosses (B), and latency to platform location (C). AβPPswe mice had reduced performance in all measures and this effect was rescued in AβPPswe/X11α mice (one-way ANOVA $P < 0.05$). *indicates significant differences between AβPPswe and all other genotypes. Error bars are ± SEM.

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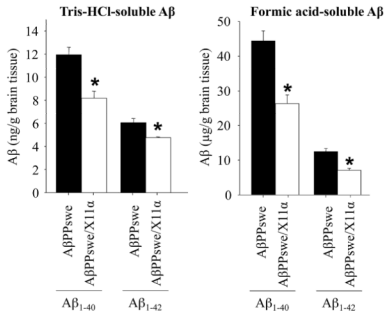

Fig. 3.

X11*α* reduces A*β* levels in total brain samples from A*β*PPswe mice. Histograms show Tris-HCl-soluble and formic-acid-soluble levels of A$\beta_{1–40}$ and A$\beta_{1–42}$ in 16–18 month A*β*PPswe and A*β*PPswe/X11*α* transgenic mice (n = 12–16). X11*α* significantly reduced the levels of both A*β* species. *indicates significant differences (t-test, $P < 0.05$). Error bars are ± SEM.