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Oligomeric amyloid- β peptide disrupts phosphatidylinositol-4,5-bisphosphate metabolism

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Abstract

Synaptic dysfunction caused by oligomeric assemblies of amyloid- β peptide (A β) has been linked to cognitive deficits in Alzheimer's disease. Here we found that incubation of primary cortical neurons with oligomeric A β decreases the level of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂), a phospholipid that regulates key aspects of neuronal function. The destabilizing effect of A β on PtdIns(4,5)P₂ metabolism was Ca²⁺-dependent and was not observed in neurons that were derived from mice that are haploinsufficient for *Synj1*. This gene encodes synaptojanin 1, the main PtdIns(4,5)P₂ phosphatase in the brain and at the synapses. We also found that the inhibitory effect of A β on hippocampal long-term potentiation was strongly suppressed in slices from *Synj1*^{+/-} mice, suggesting that A β -induced synaptic dysfunction can be ameliorated by treatments that maintain the normal PtdIns(4,5)P₂ balance in the brain.

Alzheimer's disease is the most common form of late-onset dementia. Although the molecular mechanisms responsible for the development of the idiopathic cases of Alzheimer's disease (that is, without any obvious genetic basis) are unknown in the majority of patients, growing evidence indicates that cerebral elevation and accumulation of A β peptide mediate many aspects of Alzheimer's disease pathogenesis. A β peptide is produced by the sequential proteolytic cleavage of amyloid precursor protein (APP) by the membrane-bound proteases β - and γ -secretase^{1–3}. Intramembrane cleavage of the COOH terminus of A β peptide by presenilin, the active component of the γ -secretase complex, gives rise to two major species, A β 1–40 and A β 1–42. A β 1–40 is the predominant cleavage product, although the longer and less abundant product A β 1–42 (A β 42) is more amyloidogenic and is thus believed to be a key cytotoxic agent in Alzheimer's disease. Although Alzheimer's disease brains generally contain amyloid plaques that consist of insoluble aggregates of A β , mounting evidence indicates that different conformations of A β , such as A β oligomers and fibrils, may contribute to Alzheimer's disease pathogenesis via distinct mechanisms at different stages of the disease². Notably, accumulation of soluble oligomeric forms of A β in the extracellular space closely correlates

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AUTHOR CONTRIBUTIONS

D.E.B. designed, coordinated and carried out the bulk of experiments, and contributed to the writing of the manuscript. C.D. performed the fluorescent PtdIns(4,5)P₂/DAG probe experiments and image analysis. S.V.V. carried out the Western blot analysis, lipid measurements in *Synj1*^{+/-} neurons and ATP measurements. H.Z. and O.A. contributed the electrophysiology experiments. L.B.J.M. and A.Z.M. prepared and analyzed synthetic and cell-derived A β species. A.S. and O.A. contributed the behavioral experiments. T.-W.K. and G.D.P. conceived and supervised the project, wrote the manuscript and contributed equally to this work.

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with cognitive decline and/or disease progression in animal models and Alzheimer's disease patients². Consistent with these studies, oligomers of A β , particularly A β 42, have been shown to directly affect synaptic plasticity and trigger the loss of synaptic dendritic spines^{2,4–6}. Although we are beginning to understand A β -induced synaptic alterations that lead to cognitive decline, the molecular mechanisms underlying these defects are still unclear. Important clues have been provided by studies showing that A β can modulate cell surface levels of AMPA and NMDA receptors and affect calcium homeostasis, suggesting that a fundamental mode of action of A β is to alter the signaling properties and permeability of synaptic membranes^{2,4–7}. Whether A β directly alters lipid bilayers by forming pores that ultimately modify their conductance^{8–10} or acts through proteinaceous receptors (for example, α 7 nicotinic acetylcholine receptor)¹¹ is a matter of intense debate. Similarly, the intracellular signaling pathways perturbed by A β at synapses are under extensive investigation, as their elucidation may pave the way for the identification of suitable drug targets and the development of new therapeutic interventions.

Phosphoinositides (that is, phosphorylated derivatives of phosphatidylinositol) are major signaling phospholipids in cellular membranes that regulate many aspects of physiology. Of the seven known phosphoinositides, PtdIns(4,5)P₂ and PtdIns(3,4,5)P₃ are the most relevant for processes occurring at the cell surface, as they are concentrated at the cytosolic leaflet of the plasma membrane and control signal transduction, actin dynamics, exo-endocytosis and the permeability of ion channels^{12–15}. Although a potential link between Alzheimer's disease and phosphoinositides was first suggested by a study that found reduced levels of inositol lipids in the brains of individuals with Alzheimer's disease¹⁶, our recent studies have demonstrated that familial Alzheimer's disease (FAD) mutations in the genes encoding presenilin 1 and presenilin 2 (*PSEN1* and *PSEN2*, respectively) result in a perturbation of the metabolism of PtdIns(4,5)P₂, which, in turn, affects Ca²⁺ currents through transient receptor potential channels as well as the biogenesis of A β ¹⁷. More specifically, this study showed that downregulating the levels of PtdIns(4,5)P₂, either indirectly by expression of the presenilin FAD mutants or directly by overexpressing a PtdIns(4,5)P₂ 5-phosphatase, enhances the production of A β 42, suggesting that an imbalance in PtdIns(4,5)P₂ may underlie some aspects of FAD pathogenesis¹⁷. However, the mechanism by which presenilin mutations destabilize PtdIns(4,5)P₂ metabolism is unclear.

Because elevated A β levels are believed to underlie key pathogenic aspects of both idiopathic and FAD, we set out to expand our studies on the link between Alzheimer's disease and phosphoinositide metabolism by investigating whether A β *per se* can affect the metabolism of PtdIns(4,5)P₂, and if so, whether this potential effect is pathophysiologically relevant, particularly with respect to Alzheimer's disease–linked synaptic dysfunction. We found that A β oligomers destabilize the metabolism of PtdIns(4,5)P₂ both acutely and chronically in primary cortical cultures and that preventing this destabilization by genetic means suppresses some of the synapse-impairing actions of A β oligomers.

RESULTS

Synthetic A β 42 oligomers decrease phosphoinositide levels

To test whether soluble oligomers of A β 42 affect PtdIns(4,5)P₂ metabolism in neurons, we prepared primary cultures from mouse neonatal cortices, allowed them to differentiate for 15 d and incubated them with a crude oligomer preparation made from synthetic A β 42 peptides (oA β 42) (Supplementary Fig. 1 online). We determined the effects of this preparation after either an acute (0–120 min) or a subchronic treatment (72 h), under conditions where we observed no obvious cell death or changes in ATP levels (Supplementary Fig. 2 online). The levels of phosphoinositides, as well as a variety of other anionic phospholipids, including phosphatidic acid (PtdA), cardiolipin (or diphosphatidylglycerol, DPtdG) and

phosphatidylserine (PtdS), were measured and quantified in neuronal extracts using high-performance liquid chromatography with suppressed conductivity detection^{17,18}.

oA β 42, which was used at 200 nM (that is, a concentration that is lower than that typically used in neurotoxicity and cell-death studies), induced a rapid and progressive decrease in the levels of PtdIns(4,5)P₂, which stabilized at approximately 60% of control (vehicle) levels after 120 min (Fig. 1a). After a trend toward a transient increase, the levels of PtdIns4P also decreased in response to oA β 42, but the effect was more subtle than that observed for PtdIns(4,5)P₂ and was found only after a 120-min treatment (Fig. 1a). No changes were observed in the levels of most other lipids, although we found a trend for an increase in PtdA, but with a higher variability than all the other anionic phospholipids (Fig. 1a). We did not observe an effect on PtdIns(4,5)P₂ or the other lipids when experiments were done using a control peptide that contained the inverse sequence (A β 42Rev) or with a preparation of the shorter (1–38) and noncytotoxic A β peptide, A β 38, which was processed for oligomerization similarly to oA β 42 (Fig. 1b). Notably, A β -induced PtdIns(4,5)P₂ deficiency was rescued by preincubating oA β 42 with the antibody 6E10 (Fig. 1b), which has been shown to neutralize the toxic effects of A β oligomers¹⁹. The antibody alone had no effect on PtdIns(4,5)P₂ levels (Fig. 1b). Oligomers of A β 42 also exerted a sustained decrease in the levels of PtdIns(4,5)P₂ after 3 (Fig. 1c) or 7 d (Supplementary Fig. 2) of treatment, suggesting that the effects of oligomers on this lipid are long-lasting. In contrast to the acute time-course experiments, prolonged exposures to oA β 42 failed to cause a decrease in the levels of PtdIns4P, indicating that the long-lasting effect was specific for PtdIns(4,5)P₂. When A β 42-containing medium was replaced with control medium after either 3 or 7 d of treatment, the levels of PtdIns(4,5)P₂ returned to normal levels 3 d later (Fig. 1d and Supplementary Fig. 2), demonstrating that the peptide effect is reversible. Finally, oA β 42-induced alterations of the levels of PtdIns(4,5)P₂ occurred in the absence of major changes in the expression levels of the two main enzymes regulating the synthesis and elimination of PtdIns(4,5)P₂, PtdInsP kinase type 1- γ and synaptojanin 1, respectively (Supplementary Fig. 3 online).

Oligomers, unlike monomers or fibrils, lower PtdIns(4,5)P₂

In light of recent studies suggesting that soluble oligomers of A β 42 are probably the most cytotoxic species of the A β family with respect to synaptic function, we next addressed whether different assembly states of A β 42 differentially affect PtdIns(4,5)P₂ metabolism.

We found that the effect of A β 42 on primary cortical neurons was markedly selective for crude oligomer preparations, as crude monomer and fibril preparations did not affect PtdIns(4,5)P₂ levels after a 3-d treatment (Fig. 2a and Supplementary Fig. 1). To further establish the role of oligomers as phosphoinositide-destabilizing agents, we capitalized on the existence of a naturally occurring hexahydroxy alcohol, referred to as *scyllo*-inositol, which has been shown to antagonize the neurotoxic effects of soluble A β 42 oligomers^{19–21}. Notably, we did not observe oA β 42-induced PtdIns(4,5)P₂ deficiency when neurons were incubated for 3 d with both the peptide and 5 μ M *scyllo*-inositol (Fig. 2b). The effect was specific for *scyllo*-inositol, as another inositol enantiomer that is devoid of A β 42 oligomer-neutralizing properties, *chiro*-inositol, did not show any protective action on PtdIns(4,5)P₂. When added alone, neither of the two compounds had any effect on PtdIns(4,5)P₂ levels (Fig. 2b). Together, these results suggest that oligomeric assemblies of A β 42 may be particularly potent at destabilizing PtdIns(4,5)P₂ metabolism compared with the monomers and the fibrils.

Cell-derived A β decreases phosphoinositide levels

Next, to test whether cell-derived A β also affects PtdIns(4,5)P₂ levels, we used cells expressing the ‘Swedish’ mutant of APP (APP^{sw}) because of the well-described increase in A β production associated with this mutant²². Primary neurons were prepared from the cortex of transgenic

mice expressing APP^{sw} (Tg(*App*^{sw}) neurons) under the control of the prion protein promoter²². After 2 weeks in culture, we observed a 58% decrease in the levels of PtdIns(4,5)P₂ of Tg(*App*^{sw}) neurons compared with controls (Supplementary Fig. 4 online). This biochemical deficiency was also seen in the N2a neuroblastoma cell line expressing the same mutant (Supplementary Fig. 4). Although these data are consistent with an effect of Aβ on phosphoinositide metabolism, the overexpression of APP itself, along with other potential 'intrinsic' properties of the cells expressing the transgene, may affect phosphoinositide metabolism independently of Aβ. To specifically address the role of cell-derived secreted Aβ on PtdIns(4,5)P₂ metabolism, we used conditioned media from control and APP^{sw}-expressing N2a cells.

Immunoprecipitation of Aβ from concentrated media, followed by western blot analysis of the immunoprecipitated peptide separated by SDS-PAGE, showed that APP^{sw}-expressing N2a cells secrete Aβ and that a fraction of this peptide in the media is present in the form of trimers and tetramers (Supplementary Fig. 1). These oligomeric assemblies comigrate with the better-characterized oligomer species secreted by Chinese hamster ovary (CHO) cells coexpressing human APP and the ΔE9 FAD mutation of *PSEN1* (ref. 23), as well as with trimers and tetramers derived from synthetic preparations (Supplementary Fig. 1). Conditioned media from APP^{sw}-expressing N2a cells (diluted 1:4) induced a decrease in the levels of PtdIns(4,5)P₂ after a 3-d treatment of control neuroblastoma cells, whereas the media derived from control cells had no effect (Fig. 2c). The deficiency of PtdIns(4,5)P₂ was triggered by cell-derived secreted Aβ, as preincubation of conditioned media from the Swedish cells with 6E10 antibody abolished this effect (Fig. 2c), as was previously shown in the synthetic peptide experiment (Fig. 1b). Because the levels of cell-derived Aβ measured in the media by ELISA were below the nanomolar range (data not shown), we conclude that naturally secreted Aβ is particularly potent at destabilizing PtdIns(4,5)P₂ and that it can achieve this effect at concentrations that theoretically fall in the pathophysiological range.

Aβ-induced decrease in PtdIns(4,5)P₂ is Ca²⁺ dependent

Having observed an Aβ oligomer-induced PtdIns(4,5)P₂ deficiency, we wanted to elucidate the signaling mechanisms underlying this effect. On the basis of evidence suggesting that soluble oligomers of Aβ trigger Ca²⁺ dyshomeostasis^{7,21}, we tested whether Aβ-induced PtdIns(4,5)P₂ deficiency is associated with Ca²⁺ mobilization. Cortical neurons were treated with oAβ42 for 60 min in the presence or absence of the cell-impermeable Ca²⁺ chelator ethylene glycol tetraacetic acid (EGTA, 2 mM). Chelation of extracellular Ca²⁺ prevented the oAβ42-induced decrease in PtdIns(4,5)P₂ levels, whereas EGTA had no effect on the levels of this lipid in the absence of the oligomers (Fig. 3a). The cell-permeable Ca²⁺ chelator 1,2-bis-(o-aminophenoxy)-ethane-*N,N,N',N'*-tetraacetic acid, tetraacetoxymethyl ester (BAPTA-AM) also blocked the action of oAβ42, although sequestration of both extracellular and intracellular Ca²⁺ by this compound led to a twofold increase in the levels of PtdIns(4,5)P₂ in both vehicle-treated and oAβ42-treated cortical neurons (data not shown). In parallel experiments, treatment of neurons with the Ca²⁺ ionophores ionomycin and A23187 (2 μM) caused PtdIns(4,5)P₂ levels to decrease, although the latter effects were more notable compared with those of oAβ42 (Fig. 3a). Together, these experiments suggest that Aβ-induced PtdIns(4,5)P₂ deficiency involves Ca²⁺ dyshomeostasis.

Next, we used a pharmacological approach to test whether the actions of oAβ42 on PtdIns(4,5)P₂ are mediated through specific Ca²⁺-permeable glutamate receptor channels. The NMDA and AMPA receptors are likely candidates on the basis of previous studies showing that Aβ42 decreases the cell surface levels of these channels, thereby promoting synaptic changes resembling long-term depression^{5,6,21}. Additionally, Aβ-induced neuronal damage can be rescued by an NMDA receptor antagonist^{21,24}. Blockade of the AMPA receptor with the

selective inhibitor 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) had no effect on PtdIns(4,5)P₂ in the absence of oAβ42 and did not prevent oAβ42 from destabilizing this lipid when neurons were incubated with the peptide (Fig. 3b). However, blockade of NMDA with the selective inhibitor D(-)-2-amino-5-phosphonovaleric acid (AP5) caused a partial rescue of the PtdIns(4,5)P₂ deficiency (Fig. 3b), suggesting that oAβ42 in part requires functional NMDA receptors to exert its effects on PtdIns(4,5)P₂.

Aβ-induced PtdIns(4,5)P₂ reduction is PLC dependent

A large pool of PtdIns(4,5)P₂ is concentrated at the plasma membrane, as determined by studies of the localization of genetically encoded probes for this lipid, such as the pleckstrin homology domain of phospholipase C δ1 (PH_{PLCδ1})²⁵ and of PtdIns(4)P5 kinases, the main PtdIns(4,5)P₂-synthesizing enzymes¹². To test whether oAβ42 induces a deficiency of PtdIns(4,5)P₂ at the plasma membrane, we transfected the pheochromocytoma cell line PC12 with a construct encoding a GFP fusion of the PH_{PLCδ1}. After 16–24 h, we visualized the transfected PC12 cells by confocal microscopy and observed that the fluorescence of GFP-PH_{PLCδ1} appeared as a rim that borders the cells and is thus concentrated at the plasma membrane, as expected²⁵ (Fig. 4a). In minutes, treatment of cells with oAβ42 triggered a partial loss of probe fluorescence from the plasma membrane and a corresponding increase of fluorescence in the cytoplasm, which appeared as a more diffuse signal. This effect was mimicked by a treatment with ionomycin, suggesting that it reflects hydrolysis of PtdIns(4,5)P₂ via activation of the PLC pathway at the plasma membrane (Fig. 4b, and see below). No change in the probe localization was observed when cells were exposed to Aβ42Rev or to Aβ38 (Fig. 4b,c). Similar to the experiments that we carried out in primary cortical neurons (Fig. 2b), preincubation of oAβ42 with *scyllo*-inositol, but not *chiro*-inositol, was able to prevent the disappearance of the PtdIns(4,5)P₂ probe from the plasma membrane (Fig. 4d).

The PLC pathway, which involves a family of lipid enzymes that hydrolyzes PtdIns(4,5)P₂ to diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (Ins(1,4,5)P₃), is a major pathway activated by elevated intracellular calcium. Thus, Aβ-induced PtdIns(4,5)P₂ deficiency may be caused by an activation of PLCs. To address this, we treated PC12 cells with oAβ42 for 120 min in the presence or absence of the PLC inhibitors U73122 or edelfosine (0.5 μM). The decrease in PtdIns(4,5)P₂ levels that was mediated by oAβ42 treatment was abolished in the presence of these compounds (Fig. 4c), suggesting that oAβ42 reduces the levels of PtdIns(4,5)P₂, at least in part, by promoting its hydrolysis through the PLC pathway. When incubated with the drugs alone, PtdIns(4,5)P₂ fluorescence at the plasma membrane was not affected (Fig. 4c). Metabolites derived from this reaction may be detectable in cells exposed to the peptide, further indicating that oAβ42 promotes PtdIns(4,5)P₂ hydrolysis through an activation of PLC. One of these metabolites, DAG, can be recognized by a genetically encoded probe, the C1 domain of protein kinase Cγ (C1-PKCγ), which we fused to GFP²⁵. Treatment of PC12 cells with oAβ42 led to a partial and rapid redistribution of this probe from the cytoplasm to the cell surface, indicating that the peptide application resulted in increased DAG levels at the plasma membrane (Fig. 4e). Together with the PLC blocker experiments and the disappearance of PtdIns(4,5)P₂ from the plasma membrane, these data suggest that oAβ42 activates the PLC pathway.

Synj1 haploinsufficiency protects against Aβ42 oligomers

If PtdIns(4,5)P₂ deficiency is an important aspect of oAβ42-induced synaptic dysfunction, reducing PtdIns(4,5)P₂ catabolism may diminish or inhibit the action of this peptide. To test this hypothesis, we used mouse *Synj1* mutants, as previous work has shown that neurons lacking synaptojanin 1 have higher levels of PtdIns(4,5)P₂²⁶. Because homozygous mutants are not viable²⁶, we used heterozygous animals (*Synj1*^{+/-}), which show a 50% decrease in the levels of synaptojanin 1 (Fig. 5a) and a reduced PtdIns(4,5)P₂ phosphatase activity in the brain

(S.V.V. and G.D.P., unpublished data), to produce primary cortical neurons. Incubation of *Synj1*^{+/+} neurons with oAβ42 for 2 h caused the usual decrease in the levels of phosphoinositides (Fig. 5b). In contrast, neurons derived from *Synj1*^{+/-} cortices were resistant to oAβ42 treatment, as we did not observe the decrease in PtdIns(4,5)P₂ levels under the same conditions. The reduced sensitivity of *Synj1*^{+/-} neurons to oAβ42 appeared to be specific for PtdIns(4,5)P₂, as the levels of PtdIns4P in heterozygous neurons were found to be decreased to virtually the same extent as was seen in wild-type neurons during the acute exposure to the peptide (data not shown). These data suggest that the haploinsufficiency of *Synj1* may offer a resistance to the neurotoxic actions of oAβ42, potentially by compensating for the PtdIns(4,5)P₂ destabilizing action of the peptide.

If the PtdIns(4,5)P₂-destabilizing effect of oAβ42 is an important aspect of oAβ42-induced synaptic dysfunction, the haploinsufficiency of *Synj1* may also confer a resistance against the peptide with respect to neurophysiology. To test this, we carried out experiments addressing the effects of oAβ42 on synaptic plasticity in adult hippocampal slices from wild-type and *Synj1* heterozygous mice²⁶. As a premise to these experiments, oAβ42 was tested for its ability to affect phosphoinositide metabolism in hippocampal slices under the same conditions as were used for the electrophysiology experiments. We saw that oAβ42 decreased the levels of PtdIns(4,5)P₂ by approximately 40% compared with vehicle treatment (Supplementary Fig. 5 online). Consistent with the data that we obtained from neuronal cultures (Fig. 1a), we observed a subtle downregulation (≈20%) of PtdIns4P in oAβ42-treated slices, with no changes being detected in any of the other anionic phospholipids that we analyzed by high-performance liquid chromatography (Supplementary Fig. 5). Basal neurotransmission was normal in *Synj1*^{+/-} slices in the absence of peptide (Supplementary Fig. 6 online). Similarly, we induced paired-pulse facilitation in the CA1 region of the hippocampus through stimulation of the Schaeffer collateral pathway and observed no differences between the two genotypes over the whole interstimulus interval range (Supplementary Fig. 6). Furthermore, long-term potentiation (LTP) in *Synj1*^{+/+} slices was comparable to that obtained in *Synj1*^{+/-} slices in the presence of vehicle (Fig. 5c). Notably, although oAβ42 partially impaired LTP in control slices, as has been previously reported^{27–29}, the effect of this crude oligomer preparation on LTP was strongly suppressed in *Synj1*^{+/-} slices (Fig. 5c). These results indicate that the haploinsufficiency of *Synj1* may protect neurons against the deleterious effects of soluble oligomers of Aβ on synaptic function. Together with behavioral data indicating that the performance of *Synj1*^{+/-} mice was indistinguishable from that of wild type mice in tasks assessing associative memory (that is, fear conditioning; see Supplementary Fig. 7 online), as well as spatial learning and loco motor activity (that is, the Morris water maze; see Supplementary Fig. 7), our data indicate that partial blockade of synaptojanin 1 function may confer neurophysiological and neurobehavioral benefits on elevation of brain levels of Aβ, particularly the oligomeric forms.

DISCUSSION

Our data provide robust evidence that Aβ affects the metabolism of phosphoinositides in neurons. Of the variety of anionic phospholipids that we tested, PtdIns(4,5)P₂ and, to a lesser extent, PtdIns4P, appeared to be downregulated by acute applications of Aβ42. However, prolonged applications of Aβ42 (tested up to a week) resulted in the selective deficiency of PtdIns(4,5)P₂, which was observed solely when the peptide was delivered as a low molecular-weight oligomer preparation, rather than as monomer- or fibril-enriched preparations. PtdIns(4,5)P₂ deficiency does not simply reflect increased cell death or poor viability, as the effect of Aβ42 was reversible and was not accompanied by global changes in DNA pyknosis and ATP levels. Pharmacological dissection of the Aβ signaling pathway indicated that the destabilizing effect of the peptide on PtdIns(4,5)P₂ requires extracellular Ca²⁺, full functionality of the NMDA receptor and at least two PtdIns(4,5)P₂-consuming pathways: via

PLC, which hydrolyzes PtdIns(4,5)P₂ into DAG and Ins(1,4,5)P₃, and the inositol-5 phosphatase synaptojanin 1, which dephosphorylates this phosphoinositide on the 5' position of the inositol ring.

Taking into consideration the multiple effects of A β reported to impact cell physiology raises the fundamental question of whether the biochemical changes triggered by the peptide are pathophysiologically meaningful or are simply part of compensatory responses with little relevance for the phenotypic manifestations of Alzheimer's disease. We reasoned that a powerful approach to test for the relevance of the PtdIns(4,5)P₂ change induced by A β was to exploit a genetic model in which a major pathway involved in the downregulation of phosphoinositides, and PtdIns(4,5)P₂ in particular, is defective. We used mice that are haploinsufficient for *Synj1*. The use of the null mutant was precluded by the previously described early postnatal lethality of these animals²⁶ and the critical importance of synaptojanin 1 in synaptic vesicle recycling^{26,30,31} and receptor internalization³². However, mice containing approximately half the amount of synaptojanin 1 showed no obvious abnormalities and performed similarly to wild-type mice in tasks assessing various forms of learning and memory, consistent with the lack of electrophysiological phenotypes observed in the hippocampus of *Synj1*^{+/-} mice. When incubated with A β oligomer concentrations leading to, and previously reported to induce, a disruption of LTP in control hippocampal slices, preparations from *Synj1*^{+/-} mice showed normal LTP. Notably, neurons derived from heterozygous mice did not undergo substantial changes in PtdIns(4,5)P₂ levels in response to A β , suggesting that *Synj1* haploinsufficiency protects against the neurotoxic effects of the peptide. Because the protective effect was observed for PtdIns(4,5)P₂, but not for PtdIns4P (which is also affected by A β on acute treatments and may be a physiological substrate of the NH₂-terminal phosphatase domain of synaptojanin 1, Sac1), a plausible interpretation is that A β -triggered hydrolysis of PtdIns(4,5)P₂ may be part of the signaling cascade that ultimately leads to the suppression of hippocampal LTP (see below). Whether changes in other phosphoinositides that have been previously implicated in neurophysiology and are potentially affected by A β (for example, PtdIns(3,4,5)P₃) participate in the described effects remains unclear, however. Similarly, a dysregulation of the PtdIns(4,5)P₂ metabolites Ins(1,4,5)P₃ and DAG may also account for some of the observed effects.

A fundamental question that is raised by our study is how the haploinsufficiency of *Synj1* exerts its protective effect on oA β 42-mediated downregulation of PtdIns(4,5)P₂ and impairment of synaptic function. Under normal conditions, *Synj1* haploinsufficiency does not appear to affect neurophysiology and learning behavior *per se*. However, under conditions where PtdIns(4,5)P₂ is excessively catabolized through the PLC pathway (as in the presence of A β), reducing the function of synaptojanin 1, and thus the further catabolism of PtdIns(4,5)P₂, may facilitate the regeneration of this lipid. The resulting normalization of PtdIns(4,5)P₂ levels in the presence of A β may prevent some of the molecular and cellular changes that occur in response to the neurotoxic peptide at synapses, such as the internalization of glutamate receptors and the remodeling of actin^{6,27,29,33–36}. It remains to be determined whether A β treatment promotes the activation of synaptojanin 1 (alongside PLC) as part of the neurotoxic signaling pathway that impairs synaptic function (for example, suppression of LTP and induction of long-term depression)^{6,27,29,33–35}. Stimulation of synaptojanin 1 may occur via its dephosphorylation by calcineurin^{37–39}, a major protein phosphatase that is regulated by Ca²⁺/calmodulin, which has been previously shown to be activated by A β ^{5,21,40}. Regardless of how *Synj1* haploinsufficiency mediates its protective effect against A β , our study suggests that treatments using inhibitors of synaptojanin 1 and, potentially, specific PLC isozymes to prevent A β -induced PtdIns(4,5)P₂ breakdown may improve synaptic function in individuals with Alzheimer's disease and offers a previously unknown avenue for therapeutic approaches to Alzheimer's disease.

Finally, our study may have important implications for trisomy 21, also known as Down syndrome. Indeed, individuals with Down syndrome develop the pathology of Alzheimer's disease after the third decade of their lives, at least in part because they overexpress the chromosome 21 gene *APP*⁴¹. Because *Synj1* is also present on this chromosome, its overexpression in individuals with Down syndrome may contribute to their early Alzheimer's disease pathology by rendering neurons more sensitive to A β insults, in contrast to the protective effect conferred by its haploinsufficiency. Altogether, neurons in the brains of individuals with Down syndrome may undergo a dual 'hit' on PtdIns(4,5)P₂, accounted for by *Synj1* trisomy and A β elevation, which could accelerate the onset of cognitive decline in middle-aged adults with Down syndrome.

METHODS

Animals

Synj1 wild-type and heterozygous mice (B6C3Sn background) of 3–4 months of age were used for behavior and electrophysiology. All experiments were in accordance with protocols approved by the Institutional Animal Care and Use Committee of Columbia University.

Cell Culture

PC12 cells were maintained as previously described⁴². Transfections of PC12 cells with constructs encoding GFP fused with PH_{PLC δ 1} or C1-PKC γ ²⁵ were carried out with Lipofectamine 2000 (Invitrogen). Primary cortical neurons were generated from newborn wild-type mice as previously described⁴³. Some experiments were carried out using transgenic mice overexpressing the Swedish mutant of *APP* (Tg2576)²² and *Synj1*^{+/-} mice²⁶, along with their wild-type littermates. Treatments with A β 42 were carried out after 15 days *in vitro*, and incubation time with oA β 42 was 60 min unless otherwise specified. Drugs were added to the cultures 30 min before the addition of A β . We preincubated 6E10 antibody (Signet Laboratories/Covance, 1:200 final dilution) with the oA β 42 solution for 2 h at 20–23 °C before adding it to the cultures in the neutralization assay.

Peptide preparation

To solubilize A β peptide, we allowed synthetic A β (1–42) peptide (American Peptide) to equilibrate at 20–23 °C for 30 min before it was resuspended and diluted to 1 mM in 1,1,1,3,3,3-hexafluoro-2-propanol. After evaporation for 2 h, peptide films were dried in a Speed Vac and stored at –20 °C. Peptide films were resuspended to 1 mM in dimethyl sulfoxide (DMSO) using bath sonication for 10 min. The solution, which was stored at –20 °C, was used within 2 weeks of dilution in DMSO. To form the oligomers, we diluted the 1 mM DMSO solution to 100 μ M in cold phosphate-buffered saline (PBS), vortexed it for 30 s and incubated it overnight at 4 °C. Before use, the A β -PBS solution was further diluted in culture media. To form the monomers, we immediately diluted the 1 mM DMSO solution in culture media to the final concentration following bath sonication. To form the fibrils, we diluted the 1 mM DMSO solution to 100 μ M in 10 mM HCl, vortexed it for 30 s and incubated it overnight at 37 °C. We diluted the solution in culture media and vortexed briefly before use⁴⁴.

Immunoisolation of cell-derived oligomers

N2a cells and N2a cells stably overexpressing APP with the Swedish mutation (APP^{SW}) were cultured as previously described⁴⁵. CHO cells stably expressing human APP (7WD4, a kind gift from D. Selkoe, Harvard University) were transfected with a construct encoding *Psen1* FAD mutant Δ E9 to generate the stable line, CHO-7WD4 *Psen1* Δ E9, that was used in this study. Conditioned media was collected after 4 d and centrifuged at 130g to remove cellular debris. Cleared media was concentrated about 40-fold with Amicon Ultra-3K nominal

molecular weight limit device and subjected to immunoprecipitation as previously described^{27,34} with the 6E10 antibody. Immunoprecipitate was eluted in 2× NuPAGE sample buffer and 2× NuPAGE sample reducing agent (Invitrogen).

Western blot analysis

SDS-PAGE was carried out according to the manufacturer's protocols (Invitrogen). For the analysis of A β , samples of the final dilution in culture media were prepared with NuPage LDS sample buffer and loaded onto 4–12% Bis-Tris NuPage gels; separation and electroblotting were carried out using MES running and transfer buffers (Invitrogen). Samples were transferred to PVDF membranes (Bio-Rad). Membranes were boiled for 5 min in PBS before blocking in a solution of 50% commercial blocking buffer (Li-Cor) and 50% Tris-buffered saline. Membranes were incubated with the primary antibody 6E10 and visualized using an infrared imaging system (Odyssey Infrared Imaging System, Li-Cor). The immunoblotting of brain extracts from *Synj1* mutant mice and of neuronal cultures was carried out as previously described²⁶ using rabbit polyclonal antibodies to the COOH terminus of synaptojanin 1 (OCK) and the COOH terminus of PIPK1 γ (Zola) (kind gifts from P. De Camilli, Yale University) and a mouse monoclonal antibody to α -tubulin (B-5-1-2, Sigma).

Lipid measurements

To determine the phospholipid content in cortical primary cultures, N2a cells and brain tissue, we carried out anion-exchange high-performance liquid chromatography as previously described^{17,18}.

Confocal microscopy

To analyze GFP-PH_{PLC δ 1} domain dissociation from the plasma membrane, we incubated PC12 cells with 200 nM A β 42 oligomers, 2 μ M ionomycin (Sigma-Aldrich), 200 nM of A β 42Rev (inverted peptide) or 200 nM of A β 38 24 h after transfection with GFP-PH_{PLC δ 1} domain. Cells were then washed in phosphate buffer and fixed with 4% paraformaldehyde (wt/vol). Confocal z-stack images (0.5 μ m) of PC12 were obtained using a Nikon EZC1.2.30 confocal microscope with a 100× oil-immersion objective. We calculated GFP intensity using the ImageJ software (US National Institutes of Health). For each cell in a given image, a line intensity profile across the cell was obtained. The relative decrease in plasma membrane localization was calculated as the ratio between the plasma membrane fluorescence intensity and the average cytosolic fluorescence intensity. To analyze GFP-C1-PKC γ translocation to the plasma membrane, we incubated PC12 cells with oA β 42 for 10 min 24 h after transfection with GFP-C1_{PKC γ} and fixed them. After 30 min in blocking solution (5% donkey serum (vol/vol) from SIGMA in PBS) cells were stained for 30 min with Wheat Germ Agglutinin–Alexa 594 (WGA, 5 μ g ml⁻¹). As for GFP-PH_{PLC δ 1}, the relative increase in plasma membrane localization was calculated as the ratio between the plasma membrane fluorescence intensity and the average cytosolic fluorescence intensity. WGA–Alexa 594 was used as a plasma membrane marker to select the coordinates for determining the external peaks for the quantitative analysis.

Electrophysiology

Transverse hippocampal slices (400 μ m) were cut with a tissue chopper (EMS) and maintained in an interface chamber at 29 °C for 90 min before recording, as previously reported⁴⁶. CA1 field-excitatory postsynaptic potentials were recorded by placing both the stimulating and the recording electrodes in CA1 stratum radiatum. LTP was induced using a θ -burst stimulation (four pulses at 100 Hz, with bursts repeated at 5 Hz and each tetanus including three 10-burst trains separated by 15 s). oA β 42 was applied for 20 min before the θ burst.

Behavioral analysis

Morris water-maze experiments were carried out according to a published procedure^{47,48}. Fear conditioning was performed as previously described⁴⁸.

Statistical analysis

Statistical analysis of the biochemical experiments was carried out using ANOVA and a paired or unpaired Student's *t*-test (Prism Software). The electrophysiological data were analyzed by two-way ANOVA. The behavioral data were analyzed using two-way ANOVA with repeated measures, one-way ANOVA and a Bonferroni test for *post hoc* comparisons. The significance level was established at $P < 0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Tanzi RE, Bertram L. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. *Cell* 2005;120:545–555. [PubMed: 15734686]
2. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid- β peptide. *Nat. Rev. Mol. Cell Biol* 2007;8:101–112. [PubMed: 17245412]
3. Marjaux E, Hartmann D, De Strooper B. Presenilins in memory, Alzheimer's disease and therapy. *Neuron* 2004;42:189–192. [PubMed: 15091335]
4. Selkoe DJ. Alzheimer's disease is a synaptic failure. *Science* 2002;298:789–791. [PubMed: 12399581]
5. Snyder EM, et al. Regulation of NMDA receptor trafficking by amyloid- β . *Nat. Neurosci* 2005;8:1051–1058. [PubMed: 16025111]
6. Hsieh H, et al. AMPAR removal underlies A β -induced synaptic depression and dendritic spine loss. *Neuron* 2006;52:831–843. [PubMed: 17145504]
7. Demuro A, et al. Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. *J. Biol. Chem* 2005;280:17294–17300. [PubMed: 15722360]
8. Kaye R, et al. Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. *J. Biol. Chem* 2004;279:46363–46366. [PubMed: 15385542]
9. Sokolov Y, et al. Soluble amyloid oligomers increase bilayer conductance by altering dielectric structure. *J. Gen. Physiol* 2006;128:637–647. [PubMed: 17101816]
10. Arispe N, Diaz JC, Simakova O. A β ion channels. Prospects for treating Alzheimer's disease with A β channel blockers. *Biochim. Biophys. Acta* 2007;1768:1952–1965. [PubMed: 17490607]
11. Wang DH, Lee RC, Davis CB, Shank RP. Amyloid peptide A β (1–42) binds selectively and with picomolar affinity to $\alpha 7$ nicotinic acetylcholine receptors. *J. Neurochem* 2000;75:1155–1161. [PubMed: 10936198]
12. Di Paolo G, De Camilli P. Phosphoinositides in cell regulation and membrane dynamics. *Nature* 2006;443:651–657. [PubMed: 17035995]

13. Yin HL, Janmey PA. Phosphoinositide regulation of the actin cytoskeleton. *Annu. Rev. Physiol* 2003;65:761–789. [PubMed: 12471164]
14. Suh BC, Hille B. Regulation of ion channels by phosphatidylinositol 4,5-bisphosphate. *Curr. Opin. Neurobiol* 2005;15:370–378. [PubMed: 15922587]
15. Hilgemann DW, Feng S, Nasuhoglu C. The complex and intriguing lives of PIP2 with ion channels and transporters. *Sci. STKE* 2001;29:RE19. [PubMed: 11734659]
16. Stokes CE, Hawthorne JN. Reduced phosphoinositide concentrations in anterior temporal cortex of Alzheimer-diseased brains. *J. Neurochem* 1987;48:1018–1021. [PubMed: 3029323]
17. Landman N, et al. Presenilin mutations linked to familial Alzheimer's disease cause an imbalance in phosphatidylinositol 4,5-bisphosphate metabolism. *Proc. Natl. Acad. Sci. USA* 2006;103:19524–19529. [PubMed: 17158800]
18. Nasuhoglu C, et al. Nonradioactive analysis of phosphatidylinositides and other anionic phospholipids by anion-exchange high-performance liquid chromatography with suppressed conductivity detection. *Anal. Biochem* 2002;301:243–254. [PubMed: 11814295]
19. Townsend M, et al. Orally available compound prevents deficits in memory caused by the Alzheimer amyloid- β oligomers. *Ann. Neurol* 2006;60:668–676. [PubMed: 17192927]
20. McLaurin J, Golomb R, Jurewicz A, Antel JP, Fraser PE. Inositol stereoisomers stabilize an oligomeric aggregate of Alzheimer amyloid β peptide and inhibit abeta -induced toxicity. *J. Biol. Chem* 2000;275:18495–18502. [PubMed: 10764800]
21. Shankar GM, et al. Natural oligomers of the Alzheimer amyloid- β protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. *J. Neurosci* 2007;27:2866–2875. [PubMed: 17360908]
22. Hsiao K, et al. Correlative memory deficits, A β elevation, and amyloid plaques in transgenic mice. *Science* 1996;274:99–102. [PubMed: 8810256]
23. Townsend M, Shankar GM, Mehta T, Walsh DM, Selkoe DJ. Effects of secreted oligomers of amyloid- β protein on hippocampal synaptic plasticity: a potent role for trimers. *J. Physiol. (Lond.)* 2006;572:477–492. [PubMed: 16469784]
24. De Felice FG, et al. A β oligomers induce neuronal oxidative stress through an *N*-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. *J. Biol. Chem* 2007;282:11590–11601. [PubMed: 17308309]
25. Hurley JH, Meyer T. Subcellular targeting by membrane lipids. *Curr. Opin. Cell Biol* 2001;13:146–152. [PubMed: 11248547]
26. Cremona O, et al. Essential role of phosphoinositide metabolism in synaptic vesicle recycling. *Cell* 1999;99:179–188. [PubMed: 10535736]
27. Walsh DM, et al. Naturally secreted oligomers of amyloid- β protein potently inhibit hippocampal long-term potentiation *in vivo*. *Nature* 2002;416:535–539. [PubMed: 11932745]
28. Walsh DM, Selkoe DJ. A β oligomers—a decade of discovery. *J. Neurochem* 2007;101:1172–1184. [PubMed: 17286590]
29. Gong B, et al. Ubiquitin hydrolase Uch-L1 rescues β -amyloid-induced decreases in synaptic function and contextual memory. *Cell* 2006;126:775–788. [PubMed: 16923396]
30. Kim WT, et al. Delayed reentry of recycling vesicles into the fusion-competent synaptic vesicle pool in synaptotagmin 1 knockout mice. *Proc. Natl. Acad. Sci. USA* 2002;99:17143–17148. [PubMed: 12481038]
31. Mani M, et al. The dual phosphatase activity of synaptotagmin 1 is required for both efficient synaptic vesicle endocytosis and reavailability at nerve terminals. *Neuron* 2007;56:1004–1018. [PubMed: 18093523]
32. Irie F, Okuno M, Pasquale EB, Yamaguchi Y. EphrinB-EphB signaling regulates clathrin-mediated endocytosis through tyrosine phosphorylation of synaptotagmin 1. *Nat. Cell Biol* 2005;7:501–509. [PubMed: 15821731]
33. Lambert MP, et al. Diffusible, nonfibrillar ligands derived from A β 1–42 are potent central nervous system neurotoxins. *Proc. Natl. Acad. Sci. USA* 1998;95:6448–6453. [PubMed: 9600986]
34. Klyubin I, et al. Amyloid beta protein immunotherapy neutralizes A β oligomers that disrupt synaptic plasticity *in vivo*. *Nat. Med* 2005;11:556–561. [PubMed: 15834427]

35. Kim JH, Anwyl R, Suh YH, Djamgoz MB, Rowan MJ. Use-dependent effects of amyloidogenic fragments of (β)-amyloid precursor protein on synaptic plasticity in rat hippocampus *in vivo*. *J. Neurosci* 2001;21:1327–1333. [PubMed: 11160403]
36. Maloney MT, Bamburg JR. Cofilin-mediated neurodegeneration in Alzheimer's disease and other amyloidopathies. *Mol. Neurobiol* 2007;35:21–44. [PubMed: 17519504]
37. Marks B, McMahon HT. Calcium triggers calcineurin-dependent synaptic vesicle recycling in mammalian nerve terminals. *Curr. Biol* 1998;8:740–749. [PubMed: 9651678]
38. Bauerfeind R, Takei K, De Camilli P. Amphiphysin I is associated with coated endocytic intermediates and undergoes stimulation-dependent dephosphorylation in nerve terminals. *J. Biol. Chem* 1997;272:30984–30992. [PubMed: 9388246]
39. Lee SY, Wenk MR, Kim Y, Nairn AC, De Camilli P. Regulation of synaptojanin 1 by cyclin-dependent kinase 5 at synapses. *Proc. Natl. Acad. Sci. USA* 2004;101:546–551. [PubMed: 14704270]
40. Mulkey RM, Endo S, Shenolikar S, Malenka RC. Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. *Nature* 1994;369:486–488. [PubMed: 7515479]
41. Lott IT, Head E. Down syndrome and Alzheimer's disease: a link between development and aging. *Ment. Retard. Dev. Disabil. Res. Rev* 2001;7:172–178. [PubMed: 11553933]
42. Greene LA, Tischler AS. Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Proc. Natl. Acad. Sci. USA* 1976;73:2424–2428. [PubMed: 1065897]
43. Banker, GA.; Goslin, K. *Culturing Nerve Cells*. MIT Press, Cambridge; Massachusetts: 1991.
44. Dahlgren KN, et al. Oligomeric and fibrillar species of amyloid- β peptides differentially affect neuronal viability. *J. Biol. Chem* 2002;277:32046–32053. [PubMed: 12058030]
45. Thinakaran G, Teplow DB, Siman R, Greenberg B, Sisodia SS. Metabolism of the “Swedish” amyloid precursor protein variant in neuro2a (N2a) cells. Evidence that cleavage at the “ β -secretase” site occurs in the golgi apparatus. *J. Biol. Chem* 1996;271:9390–9397. [PubMed: 8621605]
46. Puzzo D, et al. Amyloid- β peptide inhibits activation of the nitric oxide/cGMP/cAMP responsive element-binding protein pathway during hippocampal synaptic plasticity. *J. Neurosci* 2005;25:6887–6897. [PubMed: 16033898]
47. Morris RG. Synaptic plasticity and learning: selective impairment of learning rats and blockade of long-term potentiation *in vivo* by the *N*-methyl-d-aspartate receptor antagonist AP5. *J. Neurosci* 1989;9:3040–3057. [PubMed: 2552039]
48. Gong B, et al. Persistent improvement in synaptic and cognitive functions in an Alzheimer mouse model after rolipram treatment. *J. Clin. Invest* 2004;114:1624–1634. [PubMed: 15578094]

Figure 1.

Oligomeric Aβ42 peptide causes a decrease in the levels of PtdIns(4,5)P₂. **(a)** The acute effect of soluble Aβ42 oligomers (200 nM) on anionic phospholipids in 2-week-old primary cortical neuronal cultures is shown ($n = 6$ for each time point). **(b)** We observed a decrease in the level of PtdIns(4,5)P₂ after 60 min of treatment with oAβ42 ($n = 12$, $P < 0.01$), but not with vehicle ($n = 10$), inverted peptide Aβ42Rev ($n = 6$, $P = 0.858$), shorter nonamyloidogenic peptide Aβ38 ($n = 6$, $P = 0.871$) or oAβ42 pre-incubated with monoclonal antibody 6E10 ($n = 6$, $P = 0.778$). 6E10 antibody by itself did not affect PtdIns(4,5)P₂ levels ($n = 6$, $P = 0.804$). **(c)** Lipid levels after a 3-d exposure of cortical neurons to 200 nM oAβ42. PtdIns(4,5)P₂ levels remained depressed after subchronic exposure to oAβ42 ($P < 0.01$), whereas no further changes were observed in the levels of DPtdG, phosphatidylserine and PtdIns4P ($n = 6$). There was also a trend for an increase in PtdA levels ($P = 0.091$). **(d)** The oAβ42-induced PtdIns(4,5)P₂ reduction was reversed when the peptide-containing medium of cells treated with oAβ42 for 3 d was replaced with medium from age-matched cortical cultures (not containing peptide) and treated for an additional 3 d ($n = 6$, $P < 0.01$). Error bars here and in the rest of the figures denote mean $\pm$ s.e.m. * indicates $P < 0.05$ and ** indicates $P < 0.01$.

Figure 2.

Oligomers of A β are potent destabilizers of PtdIns(4,5)P₂. **(a)** Oligomeric (O, $n = 6$, $P = 0.016$), but not monomeric (M, $n = 6$, $P = 0.748$) or fibrillary (F, $n = 6$, $P = 0.127$), forms of A β 42 reduced PtdIns(4,5)P₂ levels in cortical neurons after a 72-h exposure. **(b)** Effects of oA β 42 on PtdIns(4,5)P₂ were prevented after a 3-d incubation with the *scyllo* enantiomer of inositol (*scyllo* + oA β 42, $n = 6$, $P = 0.787$), but not with the inactive stereoisomer *chiro*-inositol (*chiro* + A β 42, $n = 6$, $P < 0.01$). *Scyllo*- and *chiro*-inositol alone did not have any effect on basal PtdIns(4,5)P₂ levels ($n = 6$, $P = 0.689$ and $P = 0.733$, respectively). **(c)** A 3-d incubation of wild-type (WT) N2a cells with conditioned medium (1:4 dilution) from the neuroblastoma N2a cell line expressing the APP^{sw} mutation containing naturally secreted A β oligomers triggered PtdIns(4,5)P₂ breakdown in WT N2a cells (APP^{sw} on WT, $n = 6$, $P < 0.05$); addition of the 6E10 antibody to the conditioned media abolished the effect (APP^{sw} + 6E10, $n = 6$, $P = 0.879$).

Figure 3.

oAβ42-triggered PtdIns(4,5)P₂ deficits are calcium and NMDA receptor dependent. **(a)** PtdIns(4,5)P₂ levels decreased after 60 min in the presence of the calcium ionophores A23187 (2 μM; *n* = 6, *P* < 0.01) and ionomycin (iono, 2 μM; *n* = 6, *P* < 0.01), but not when the calcium chelator EGTA (2 mM) was incubated with oAβ42 (*n* = 3, *P* = 0.5243). No effect on basal PtdIns(4,5)P₂ was observed when EGTA alone was added to the cultures (*n* = 6, *P* = 0.9023). **(b)** Aβ-induced PtdIns(4,5)P₂ deficiency was partially rescued by incubation of the cultures with the NMDA receptor antagonist AP5 (10 μM) and oAβ42 (AP5 + oAβ42, *n* = 6, *P* < 0.05 compared with control or oAβ42). Addition of the AMPA receptor antagonist CNQX (10 μM) had no effect on the oAβ42-induced PtdIns(4,5)P₂ reduction (CNQX + oAβ42, *n* = 6, *P* = 0.741 compared to oAβ42 alone).

Figure 4.

Analysis of fluorescent PtdIns(4,5)P₂ and DAG probes after treatment with oAβ42 in PC12 cells. **(a)** Representative intensity profiles of the GFP-PH_{PLCδ1} probe before (control) and after the addition of oAβ42 or ionomycin are shown. The relative decrease in plasma membrane fluorescence was calculated as a ratio between the plasma membrane fluorescence intensity (average between the two external peaks) and the average cytosolic fluorescence intensity (dashed line). **(b)** Graph showing the decrease in plasma membrane fluorescence of GFP-PH_{PLCδ1} after oAβ42 ($n = 32$) or ionomycin ($n = 15$) treatments. The control peptide Aβ42Rev had no effect ($n = 17$). **(c)** PLC inhibitors U73122 (0.5 μM) and edelfosine (edel, 0.5 μM) blocked the effect of oAβ42 after 120 min ($n = 17$, $P < 0.01$ compared with oAβ42 alone) and had no effects in the absence of the peptide ($n = 17$). Aβ38 had no effect on GFP-PH_{PLCδ1} translocation ($n = 17$). **(d)** Scyllo-inositol blocked the effect of oAβ42 (oAβ42 + scyllo, $n = 17$), whereas chiro-inositol had no effect (oAβ42 + chiro, $n = 17$, $P < 0.01$). The inositol stereoisomers had no effect when added alone ($n = 17$). **(e)** Left, PC12 cells were transfected with GFP-C1-PKCγ and analyzed after 10 min of oAβ42 treatment for DAG translocation. Alexa 594–WGA was used as a plasma membrane marker. Right, quantification of the oAβ42 effect on GFP-C1-PKCγ localization, showing a significant translocation of the probe from the cytoplasm to the membrane ($n = 17$, $P < 0.01$).

Figure 5.

Hippocampi from mice lacking one copy of *Synj1* show normal LTP in the presence of oAβ42. (a) Left, representative western blot analysis of synaptojanin 1 and tubulin in adult *Synj1*^{+/+} and *Synj1*^{+/-} brain extracts (50 μg). Right, quantitative analysis of the blots ($n = 4$, $P < 0.05$). (b) PtdIns(4,5)P₂ levels in cortical cultures from *Synj1*^{+/+} and *Synj1*^{+/-} mice after a 2-h treatment with 200 nM oAβ42. Although levels of PtdIns(4,5)P₂ were reduced by oAβ42 in *Synj1*^{+/+} cultures ($P < 0.05$), the levels of this lipid in *Synj1*^{+/-} neurons were unaffected by the peptide ($P = 0.853$) ($n = 8$ and $n = 6$ in control and oAβ42-treated cultures from *Synj1*^{+/+} mice, respectively; $n = 9$ and $n = 6$ in control and oAβ42-treated cultures from *Synj1*^{+/-} mice, respectively). (c) Although *Synj1*^{+/+} slices ($n = 7$) showed a reduction of LTP following bath application of 200 nM oAβ42 ($F_{1,15} = 8.556$, $P = 0.0104$, relative to vehicle), *Synj1*^{+/-} slices ($n = 7$) showed normal LTP in the presence of the peptide ($F_{1,16} = 0.121$, $P = 0.73$, relative to vehicle). LTP was normal in *Synj1*^{+/-} slices ($n = 11$) compared to *Synj1*^{+/+} slices ($n = 10$) in the presence of vehicle ($F_{1,19} = 0.026$, $P = 0.87$). Basal synaptic transmission was not affected in the *Synj1*^{+/-} mice (Supplementary Fig. 6). fEPSP, CA1 field-excitatory postsynaptic potential. The bar represents the time of bath application of oAβ42. The three arrows represent the θ-burst stimulation used to induce potentiation. Animals were 3–4 months old.