Published in final edited form as:

Acta Physiol (Oxf). 2018 June ; 223(2): e13046. doi:10.1111/apha.13046.

Presenilin PS1 E9 disrupts mobility of secretory organelles in rat astrocytes

Matjaž Stenovec^{1,2}, Saša Trkov Bobnar^{1,2}, Tina Smoli², Marko Kreft^{1,2,3}, Vladimir Parpura⁴, and Robert Zorec^{1,2,+}

¹Celica BIOMEDICAL, Tehnološki park 24, 1000 Ljubljana, Slovenia, EU

²Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia, EU

³CPAE, Department of Biology, Biotechnical Faculty, University of Ljubljana, Ve na pot 111, 1000 Ljubljana, Slovenia, EU

⁴Department of Neurobiology, Civitan International Research Center and Center for Glial Biology in Medicine, Evelyn F. McKnight Brain Institute, Atomic Force Microscopy & Nanotechnology Laboratories, 1719 6th Avenue South, CIRC 429, University of Alabama at Birmingham, Birmingham, AL 35294-0021, USA

Abstract

Aim—Alzheimer disease (AD) is largely considered a neuron-derived insult, but also involves failure of astroglia. A recent study indicated that mutated presenilin 1 (PS1M146V), a putative endoplasmic reticulum (ER) Ca²⁺ channel with decreased Ca²⁺ conductance, impairs the traffic of astroglial peptidergic vesicles. Whether other pathogenically relevant PS1 mutants, such as PS1 E9, which code for ER channel with putative increased Ca²⁺ conductance, similarly affect vesicle traffic, is unknown.

Methods—Here, we co-transfected rat astrocytes with plasmids encoding mutant PS1 E9 and atrial natriuretic peptide or vesicular glutamate transporter 1 tagged with fluorescent proteins (pANP.emd or pVGLUT1-EGFP, respectively), to microscopically examine whether alterations in vesicle mobility and Ca²⁺-regulated release of gliosignalling molecules manifest as a general vesicle-based defect; control cells were transfected to co-express exogenous or native wild-type PS1 and pANP.emd or pVGLUT1-EGFP. The vesicle mobility was analyzed at rest and after ATP stimulation that increased intracellular calcium activity.

Results—In PS1 E9 astrocytes, spontaneous mobility of both vesicle types was reduced ($P<0.001$) when compared to controls. Post-stimulatory recovery of fast vesicle mobility was hampered in PS1 E9 astrocytes. The ATP-evoked peptide release was less efficient in PS1 E9 astrocytes than in the controls ($P<0.05$), as was the pre-stimulatory mobility of these vesicles.

*Correspondence: Professor Robert Zorec, Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia. Tel.: +386 1 5437080, fax: +386 1 5437036, robert.zorec@mf.uni-lj.si.

Conflict of Interest

The authors declare no conflict of interest.

Conclusion—Although the PS1 mutants PS1M146V and PS1 E9 differently affect ER Ca^{2+} conductance, our results revealed a common, vesicle type indiscriminate trafficking defect in PS1 E9 astrocytes, indicating that reduced secretory vesicle-based signalling is a general deficit in AD astrocytes.

Keywords

ANP.emd; astrocytes; presenilin 1; regulated exocytosis; vesicle mobility; VGLUT1-EGFP

Introduction

Alzheimer disease (AD) is a progressive, irreversible and ultimately fatal neurodegenerative disease that affects cognition and is the most common cause of dementia in the elderly. AD predominantly occurs sporadically, with an age of onset of ~65 years and older, while in 25 to 40% of AD patients, genetic factors are involved. In some cases AD segregates as an autosomal dominant trait in at least three generations in families (FAD; 0.5% of all cases) and affects individuals before the age of 65 years¹. Approximately 50% of the FAD patients carry mutations in presenilin 1 (PS1), presenilin 2 (PS2), and amyloid precursor protein (APP). Notably, patients with PS1 mutations have the earliest age of disease onset; 43±9 years¹. Presenilins (PSs), 50 kDa proteins with nine transmembrane domains², reside in the endoplasmic reticulum (ER)³ and putatively act as passive low conductance ER Ca^{2+} leak channels⁴; FAD mutations in PSs may result in deranged Ca^{2+} signalling⁵. Currently 219 FAD-causing (pathogenic) PS1 mutations have been identified (<http://www.molgen.vib-ua.be/ADMutations>), whereby most are simple missense mutations that result in single amino-acid substitutions in PS1, while some are more complex, like small deletions, insertions or splice mutations. The most severe PS1 mutation is a donor–acceptor splice mutation that causes two amino-acid substitutions and an in-frame deletion of exon 9 (PS1 E9)⁶.

Although crucial, the phenotypic alterations caused by diverse PS1 mutants in FAD-affected brain cells are largely unknown. In particular, we lack insights into astrocyte dysfunction underlying expression of particular PS1 mutant, which is critical, since neurodegeneration in AD may not begin exclusively as a neuron derived insult⁷, but also as a failure in astroglia⁸, cells that otherwise maintain multifaceted brain homeostasis⁹. In theory, each PS1 mutant could give rise to a uniquely altered cellular phenotype or alternatively, diverse PS1 mutations result in just a few (or even one) phenotypic alterations in PS1 mutants expressing cells.

In 3×Tg-AD mouse model that mimics histopathology of AD¹⁰, neonatal cortical astrocytes expressing PS1-M146V, a mutant putative cation-channel with decreased Ca^{2+} conductance⁴, displayed impaired traffic of exo- and endocytotic vesicles, and reduced secretion of the signalling peptides¹¹. In this AD mouse model, cognitive impairments were manifested at age of 4 months, while synaptic dysfunction, including long-term potentiation deficits occurred prior to the appearance of extracellular Aβ deposits that first became evident at age of ~6 months¹². In another AD mouse model expressing the Swedish mutation of amyloid precursor protein (APP^{Swe}) and PS1 E9, a putative ER cation-channel with increased Ca^{2+}

conductance⁴, deficits in transient long-term potentiation were observed at the age of 3 months, while A β deposits appeared at the age of 6 months¹³. These results question the view whether calcium homeostasis disrupted by intra-neuronal accumulation of amyloid-beta (A β) is the sole initiating factor of synaptic dysfunction^{5, 14, 15}. Moreover, astrocyte dysfunction underlying expression of mutated PS1 E9 was never examined. We thus studied phenotypic alterations in peptidergic and glutamatergic vesicle traffic and Ca²⁺-regulated release of gliosignalling molecules in neonatal cortical rat astrocytes transfected to express PS1 E9 in conjunction with fluorescent markers of peptide-laden (ANP.emd) or glutamate-laden (VGLUT1-EGFP) secretory vesicles. Double transfected cells were maintained *in vitro* and compared to the age-matched controls transfected to express exogenous or native wild-type PS1 and ANP.emd or VGLUT1-EGFP. Our results revealed reduced spontaneous mobility of both secretory vesicles, hampered post-stimulatory recovery of vesicle mobility and diminished release of gliosignalling peptides from PS1 E9 astrocytes.

Results

Reduced spontaneous mobility of peptidergic vesicles in PS1 E9 astrocytes

Mobility of ANP-laden peptidergic secretory vesicles was imaged first and the motion patterns were analyzed in time sequences of confocal micrographs acquired in transfected controls, PS1wt and PS1 E9 astrocytes (Figures 1A and B) from five different cultures. Mobile vesicles exhibited directional and non-directional mobility (as indicated by the contorted and by the elongated vesicle tracks; see also¹⁶). In control astrocytes (Figure 1C, see also Figure_1_SuppInfo_ANP_control) more elongated tracks were generally observed than in PS1 E9 astrocytes (Figure 1D, see also Figure_1_SuppInfo_ANP_PS1 E9), indicating reduced directional mobility of peptidergic vesicles. To quantify differences in spontaneous vesicle mobility, four parameters (the track length (TL), the maximal displacement (MD), the directionality index (DI = MD/TL), and the speed) were determined in 15-s epochs. The mobility of 1468 vesicles in controls ($N=10$), 1648 vesicles in PS1wt ($N=10$) and 1375 vesicles in PS1 E9 cells ($N=10$) was analyzed. In comparison to controls, the vesicle TL was significantly reduced by ~9% ($N=10$, Figure 1E; $P<0.001$), the MD by ~18% ($N=10$, Figure 1F; $P<0.001$), the DI by ~13% ($N=10$, Figure 1G, $P<0.001$) and the vesicle speed by ~9% in PS1 E9 astrocytes ($N=10$, Figure 1H; $P<0.001$). When compared to PS1wt astrocytes, the same mobility parameters were significantly reduced by ~12% ($N=10$, Figure 1E; $P<0.001$), by ~16% ($N=10$, Figure 1F; $P<0.001$), by ~4% ($N=10$, Figure 1G; $P<0.05$) and by ~12% in PS1 E9 astrocytes ($N=10$, Figure 1H; $P<0.001$). With the sole exception of DI, other mobility parameters did not differ significantly in controls and PS1wt astrocytes. Due to high similarity of peptidergic vesicle mobility observed in PS1wt astrocytes transfected to express exogenous PS1wt and ANP.emd, and in transfected controls expressing native PS1 and ANP.emd, the later cells were used as controls in all subsequent experiments. Overall, the spontaneous mobility of peptidergic vesicles was diminished in PS1 E9 astrocytes. Additionally we also examined the relationship between the size of vesicles (given by their area) and the speed of their motions in controls and in PS1 E9 astrocytes. As revealed by the Pearson correlation coefficient (R) of -0.01 in controls ($P=0.915$) and -0.04 ($P=0.602$) in PS1 E9 astrocytes, the size of individual peptidergic vesicles ($N=153$) was not correlated with their speed of motion at rest (data not shown).

Reduced spontaneous mobility of glutamatergic vesicles in PS1 E9 astrocytes

Next, we examined the spontaneous mobility of glutamatergic vesicles (VGLUT1-EGFP-positive) in controls and PS1 E9 astrocytes (Figures 2A and B) from two different cultures. At rest, glutamatergic vesicles appeared less mobile than peptidergic vesicles (compare Figures 2C and D with Figures 1C and D). In both controls and PS1 E9 astrocytes, the vesicle tracks appeared rather contorted (Figures 2C and D, see also Figure_2_SupInfo_VGLUT1_control and Figure_2_SupInfo_VGLUT1_PS1 E9). To gain detailed insight into spontaneous mobility of these vesicles, the mobility of 870 glutamatergic vesicles in controls ($N=11$) and 850 vesicles in PS1 E9 cells ($N=11$) was further compared. In PS1 E9 astrocytes, the vesicle TL was significantly reduced by ~8% ($N=11$, Figure 2E; $P<0.001$), the MD by ~24% ($N=11$, Figure 2F; $P<0.001$), the DI by ~19% ($N=11$, Figure 2G; $P<0.001$) and the vesicle speed by ~8% ($N=11$, Figure 2H; $P<0.001$) in comparison with controls. As further revealed by the Pearson correlation coefficient of -0.15 in controls ($P=0.196$) and 0.40 in PS1 E9 astrocytes ($P=0.002$), the size of individual glutamatergic vesicles ($N=80$) was either not or only weakly correlated with their speed of motion (data not shown).

ATP-evoked reduction in directional mobility of peptidergic vesicles is larger, while the post-stimulation recovery of directional mobility is slower in PS1 E9 astrocytes

The alteration of vesicle mobility due to ATP-evoked increase in $[Ca^{2+}]_i$ was evaluated by measuring the time-dependent changes in vesicle TL and MD 1 min before and 2 min after application of $100 \mu M$ ATP to the cells (Figures 3A–D). In controls, during the first post-stimulation minute, TL was reduced ($P<0.001$) by ~48% and by ~38% during the second minute of recording, whereas the MD was reduced ($P<0.001$) by ~66% during the first minute and by ~54% during the second minute after ATP application. In PS1 E9 astrocytes, there was a lesser reduction in these trafficking parameters mainly due to a smaller initial values at rest (compare Figures 3A and B for TL, and Figures 3C and D for MD), along with a similar “flooring” in the ATP-evoked reduction of mobility in both cells types. Thus, TL was reduced ($P<0.001$) by ~42% during the first and by ~37% during the second post-stimulation minute, whereas the MD was reduced ($P<0.001$) by ~61% during the first and by ~54% during the second minute after ATP application. To gain insight into the ATP-evoked diminishment of directionality of vesicle motions, we plotted the relationship between TL and MD, and fitted a linear function $[MD=MD_0+a \times (TL)]$ to the data. The slope (a) of the function corresponds to the directionality index (DI). During the first post-stimulation minute, this parameter decreased from 0.71 ± 0.01 (not shown) to 0.59 ± 0.01 in control cells and even more, from 0.64 ± 0.01 (not shown) to 0.49 ± 0.01 in PS1 E9 cells ($P<0.001$), while during the second post-stimulation minute it slightly decreased to 0.57 ± 0.01 in controls and increased to 0.54 ± 0.01 in PS1 E9 cells ($P<0.001$) (Figures 3E and F), indicating altered recovery of directional vesicle mobility in PS1 E9 astrocytes. Thus we examined the tendency for recovery of vesicle mobility during continuous ATP stimulation. The linear function $[y=y_0 + a \times (x)]$ was fitted to all mobility parameters (during ATP stimulation, 0–120 s) to obtain the slope (a), indicating the speed of recovery of the selected parameter. During 2 min post-stimulation period, vesicle mobility partially recovered towards the initial values. The tendency for recovery of vesicle mobility was slower in PS1 E9 ($P<0.01$ and $P<0.05$) than in control cells (Figures 4A–D).

ATP-evoked reduction in directional mobility of glutamatergic vesicles is larger, while the post-stimulation recovery of mobility is slower in PS1 E9 astrocytes

As observed in peptidergic vesicles, the post-stimulatory mobility of glutamatergic vesicles was also reduced (Figures 5A–D). In control astrocytes, TL was reduced ($P<0.001$) by ~13% during the first and by ~8% during the second minute post-stimulation, whereas the MD was reduced ($P<0.001$) by ~42% during the first and by ~31% during the second minute of ATP application. In PS1 E9 astrocytes, TL was reduced ($P<0.001$) by ~6% during the first and by ~2% during the second post-stimulation minute, whereas the MD was reduced ($P<0.001$) by ~20% during the first and by ~11% during the second minute of ATP application. Thus, as with peptidergic vesicles, there was a lesser reduction in MD and TL for glutamatergic vesicles in PS1 E9 astrocytes, owing to retarded initial trafficking values at rest (compare Figures 5A and B for TL, and Figures 5C and D for MD), again, along with a similar “flooring” in the ATP-evoked reduction of mobility in both cell types. The diminishment in directionality of vesicle motions was larger in PS1 E9 ($P<0.001$) than in control astrocytes. During the first post-stimulation minute, the slope (a) of the linear function [$MD=MD_0+a\times(TL)$] decreased from 0.35 ± 0.02 (not shown) to 0.19 ± 0.01 in controls and similarly, from 0.26 ± 0.02 (not shown) to 0.19 ± 0.01 in PS1 E9 cells, while during the second post-stimulation minute the slope increased ($P<0.001$) to 0.31 ± 0.02 in controls, but remained low in PS1 E9 cells (Figures 5E and F), suggesting the absence of rapid recovery of more directional mobility in PS1 E9 astrocytes. Correspondingly, the glutamatergic vesicles did not display tendency for rapid post-stimulatory recovery of their mobility (0–120 s) in comparison to controls, that displayed an increase ($P<0.05$) in MD (Figures 6A–D).

ATP-evoked vesicular peptide release is reduced in PS1 E9 astrocytes, along with the traffic delivering vesicles towards the plasma membrane for fusion and cargo release

Finally, we examined whether the Ca^{2+} -dependent peptide secretion is affected in PS1 E9 astrocytes. The individual vesicular secretory events (Figure 7A) manifested as a sudden decrease in vesicle fluorescence were observed for 4 min after the application of 100 μ M ATP to astrocytes. The normalized, time-dependent plots of fluorescent cargo release from vesicles, revealed a step-wise fluorescence decrease to a level comparable with the background (auto)fluorescence, and indicated a similar rate of cargo release in control, PS1wt and PS1 E9 astrocytes (Figure 7B). A rapid and complete release of ANP.emd, indicative of full-collapse vesicle fusion¹⁷ was accomplished between the two successive image frames (Figure 7A), consistent with previous observations in rat and mouse astrocytes^{11, 18}. The rate of peptide release from individual vesicles was equal in controls, PS1wt and PS1 E9 astrocytes as confirmed by the measurements of $\tau_{20-80\%}$; 0.53 ± 0.02 s in controls, 0.56 ± 0.02 s in PS1wt astrocytes and 0.51 ± 0.03 s in PS1 E9 astrocytes, respectively (data not shown; $P=0.880$, $P=0.516$ and $P=0.451$). In contrast, the number of ATP-evoked elementary secretion events per cell and unit time was substantially diminished in PS1 E9 astrocytes; it was 20 ± 6 in controls ($N=8$), 22 ± 5 in PS1wt astrocytes ($N=8$) and 8 ± 2 in PS1 E9 ($N=8$) astrocytes, respectively ($P<0.05$; Figure 7C). Overall, the Ca^{2+} -dependent exocytotic secretion of ANP was reduced in PS1 E9 astrocytes. The plots of relative frequency distributions and the relative cumulative frequency distributions (Figures 7D–F) revealed similar time profile of ATP-evoked secretory events (Kolmogorov-Smirnov test, $P=0.317$, $P=0.206$, $P=0.234$) in all groups of astrocytes. The majority of exocytotic secretory

events were observed during the first half of the 4-minute cell stimulation with ATP, where intracellular calcium activity was likely higher. It thus appears that reduced Ca^{2+} -dependent secretion of ANP from PS1 E9 astrocytes (Figure 7C) underlies less effective delivery of exocytotic peptidergic vesicles (Figure 1) towards the plasmalemma.

Discussion

The studies of mutated presenilins are crucial for understanding the pathogenesis of FAD, since presenilins are involved in aberrant APP processing^{19, 20}, derailed Ca^{2+} signalling⁴, and altered intracellular vesicle transport required for cell-to-cell signalling in neurons^{21, 22} and also in astrocytes¹¹. Although the latter cells communicate with neighbouring brain cells in a slow time domain via the vesicular release of gliosignalling molecules that relies on intact transport providing delivery of vesicle material towards the plasmalemma, little is known about how expressions of FAD-linked PS1 mutants affect astrocyte vesicle-based secretome.

Dynamics of astrocytic secretory vesicles is reduced by the expression of mutated PS1 E9

Astrocytic peptidergic and glutamatergic vesicles represent two vesicle populations that differ in size²³ and mobility^{16, 18}. Although peptidergic vesicles move faster (range $0.04\text{--}0.45\ \mu\text{m s}^{-1}$) than glutamatergic vesicles ($0.03\text{--}1.08\ \mu\text{m s}^{-1}$), both astrocytic vesicles move slower than neuronal vesicles ($0.8\text{--}3.5\ \mu\text{m s}^{-1}$ ²⁴). The negligible correlation between the size (area) and vesicle mobility suggests that smaller and larger vesicles can be effectively delivered towards the plasmalemma for either docking or fusion. The bi-directional vesicle motions (towards the cell periphery and the interior) indicate that these vesicles likely associate with both kinesin and dynein-like molecules. The maximum force generated by the single kinesin molecule of $5.4\text{--}6.7\ \text{pN}$ ^{25, 26} and by the cytoplasmic dynein molecule of $1.1\ \text{pN}$ ²⁷ is unlikely sufficient to drag a single synaptic like vesicle, with the diameter of $\sim 42\ \text{nm}$ and estimated mass of $26.4 \pm 5.8 \times 10^{-18}\ \text{g}$ ($15.9 \pm 3.5\ \text{MDa}$ ²⁸, throughout the cytoplasm²⁹. It is thus likely that mobile astrocytic vesicles bind few (1–2) kinesin and up to a dozen (6–12) of dynein motors to enable their bi-directional motions along the microtubules³⁰.

The decreased vesicle speed in PS1 E9 astrocytes (Figures 1H and 2H) may indicate that vesicles were arrested more frequently along the cytoskeleton or alternatively, were less effectively dragged by the motor proteins during progressive “walking” without dissociating from the cytoskeleton track³¹. The mechanistic reason for reduced vesicle mobility in PS1 E9 astrocytes is unclear, but PS1 E9 mutation was reported to increase activation of glycogen synthase kinase 3β (GSK3 β), which increases the phosphorylation of kinesin, thereby leading to reduced binding of microtubules associated motor proteins to the membrane-bound cargo^{21, 22} and reduced kinesin driven motility³². Moreover, inhibition of serine/threonine protein phosphatases in rat hepatocytes increased the phosphorylation of the cytoplasmic dynein and inhibited dynein ATPase activity, essential to generate force from the hydrolysis of ATP powering the cargo transport along microtubules³³.

Diminished vesicle mobility observed in our study (Figures 1 and 2) corroborates the earlier morphological observations^{34–36} indicating a reduced surface area and volume of astroglia

measured as GFAP- and glutamine synthetase-positive astroglial profiles in the affected brain regions as early as in 1-month-old AD mice. Morphological re-modelling of astrocytes may contribute to memory impairment³⁷ through altered synaptic coverage and deficient homeostatic support, which together may lead to synaptic dysfunction³⁸, affecting neuronal information processing. Small changes in vesicle mobility may thus lead to long-term changes in astrocyte function contributing to the development of neurodegeneration in AD, a process taking decades^{39, 40}.

Altered calcium homeostasis differentially affects vesicle mobility in control and PS1 E9 astrocytes

The ATP stimulation reduced directional mobility of both secretory vesicle types, and was more pronounced in PS1 E9 astrocytes. The latter may indicate that secretory vesicles in PS1 E9 astrocytes more frequently disconnect from the cytoskeleton tracks to be transported by the Brownian motion in the cell cytosol. In addition to changes in microtubules associated motor protein activity and their binding to vesicles, impaired Ca^{2+} homeostasis may also account for reduced mobility in PS1 E9 astrocytes at rest (Figures 1 and 2) and especially after cell stimulation (Figures 3–6). In accordance with this possibility, transient reduction in mobility of astrocytic vesicles correlated well with stimulus-evoked increase in astrocytic $[\text{Ca}^{2+}]_i$ ^{31, 41}. The tendency for post-stimulatory recovery of vesicle mobility was slower in PS1 E9 than in control astrocytes (Figures 4 and 6), which may indicate that ATP evoked more prolonged elevation in $[\text{Ca}^{2+}]_i$ in PS1 E9 astrocytes than in the controls. In human SH-SY5Y neuroblastoma cells, carbachol stimulation gave rise to a greater increase in $[\text{Ca}^{2+}]_i$ in PS1 E9-expressing cells, which took longer to return to the basal levels when compared to control cells expressing wild type PS1. The long tail-off effect was mediated by inositol trisphosphate receptor and ryanodine receptors in PS1 E9-expressing SH-SY5Y cells that also had an enhanced basal phosphoinositide hydrolysis, and increased $[\text{Ca}^{2+}]_i$ when compared to controls⁴².

In neurons PS1 associates with microtubules and microfilaments⁴³, and with cytoskeleton-binding proteins, including the microtubule-associated protein tau⁴⁴ and the actin-binding protein filamin⁴⁵. FAD-linked PS1 mutants M146V, I143T, and E9 display reduced PS1 cytoskeletal binding, diminish the nuclear translocation of the Notch1 intracellular domain, and deregulate neurite growth⁴³. On the other side, pharmacological depolymerization of astrocyte cytoskeleton⁴⁶ and an agonist-evoked increase in $[\text{Ca}^{2+}]_i$ may also affect vesicle mobility, by compromising structural integrity of the cytoskeleton, in particular of microtubules³¹. Since microtubule disassembly typically occurs at $[\text{Ca}^{2+}]_i > 1\text{--}4\text{ }\mu\text{M}$ ⁴⁷, whereas $100\text{ }\mu\text{M}$ ATP typically evokes transient increases in $[\text{Ca}^{2+}]_i$ that peak at $780 \pm 120\text{ nM}$ ⁴⁸, mechanisms discussed earlier are more likely to account for diminished vesicle mobility than disassembly of the microtubule cytoskeleton in PS1 E9 astrocytes.

Reduced secretory peptide release from PS1 E9 astrocytes

Expression of FAD-linked PS1 mutant apparently affected vesicle capacity to engage into secretory response (Figures 7C) at pre-, but not at post-fusion level of Ca^{2+} -regulated exocytosis, since pre-stimulatory mobility of secretory vesicles was reduced (Figures 1E–H), whereas cargo release kinetics from vesicles was not impaired in PS1 E9 astrocytes

(Figures 7A and B). Our results thus contrast findings of de Diego et al.⁴⁹ who measured single-amperometric exocytotic events in chromaffin cells from aged mice co-expressing the human PS1 mutant (A246E) and the APPSwe (and also from the control C57 mice), and found 45% shorter spike half-width, 60% smaller quantal size and 50% faster decay time in spikes in APP/PS1 cells indicating subtle differences in the last steps of exocytosis that could be related to synaptic dysfunction of the secretory machinery. The seemingly disparate findings may be a result not only of expression of PS1 E9 in our study as opposed to PS1A246E and APPSwe in the other study, but also of the use of different agonist and cells to evoke increase in $[Ca^{2+}]_i$. Chromaffin cells, the modified sympathetic neurons from adrenal medulla, which release catecholamines in response to high K^+ depolarization⁴⁹ cannot be directly compared to the astroglia releasing gliosignalling peptides in response to ATP stimulation that transiently mobilizes intra-/extracellular Ca^{2+} into the cytosol^{48, 50, 51} after activation of purinergic receptors⁵². Both examined cell types also express the non-identical SNARE proteins and SNARE-associated proteins involved in regulation of vesicle docking and fusion^{53, 54} and the fusion pore activity^{23, 55}, and are also affected by elevated $[Ca^{2+}]_i$ with dissimilar peak values and temporal profiles^{48, 56}; all could account for the differences observed in both studies. Notably, a role of presenilins in the activity-dependent regulation of transmitter release by modulation of intracellular Ca^{2+} was also reported in hippocampal neurons⁵⁷.

In summary, our experiments revealed, that expression of mutated presenilin 1 (PS1 E9) in cultured astrocytes reduces vesicle dynamics, hampers post-stimulatory recovery of vesicle mobility and reduces evoked release of the signalling molecule ANP; these alterations diminish efficacy of gliosignalling and likely reduce homeostatic performance of astroglia *in vivo*.

Materials and methods

Cell culture

Primary astrocyte cultures were prepared from neocortices of 3-day-old female Wistar rats (obtained from Medical Experimental Centre at the Institute of Pathology, University of Ljubljana, Slovenia) as described⁵⁸. The care for experimental animals was in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe No 123, Strasbourg 1985). Cells were grown in high-glucose (25 mM) DMEM containing 10% fetal bovine serum (Biochrom AG, Berlin, Germany), 1 mM sodium pyruvate, 2 mM L-glutamine, 5 U ml⁻¹ penicillin / 5 µg ml⁻¹ streptomycin in an atmosphere of 5% CO₂/95% air. To purify isolated cells, sub-confluent cultures were shaken at 225 rpm overnight with three subsequent medium changes. Before the experiments, the cells were trypsinized, sub-cultured onto poly-L-lysine-coated coverslips and maintained at 37°C, in an atmosphere of 5% CO₂/95% air. On the next day, cultured cells were transfected with the plasmids as described below.

Cell transfection

To visualize individual secretory vesicles and to examine the effect of mutated presenilin1 (PS1 E9) on their dynamics, cultured astrocytes were co-transfected with: 1) the plasmid

encoding atrial natriuretic peptide C-terminally tagged with emerald green fluorescent protein (ANP.emd; a gift from Dr. Ed Levitan, University of Pittsburgh, Pittsburgh, PA, USA; ^{59, 60}) and with the plasmid encoding PS1 E9 (kindly provided by Dr. Ilya Bezprozvanny, UT Southwestern Medical Center at Dallas, Dallas, TX, USA; ⁴) or 2) with the plasmid encoding vesicular glutamate transporter 1 tagged with enhanced fluorescent protein (VGLUT1-EGFP; kindly provided by Dr. Salah El Mestikawy, McGill University, Montreal, Canada) and with the plasmid encoding PS1 E9 using Lipofectamine LTX Reagent (Thermo Fisher Scientific, Waltham, MA, USA). For double-transfection, DNAs encoding either ANP.emd ($0.4 \mu\text{g } \mu\text{l}^{-1}$) or VGLUT1-EGFP ($0.4 \mu\text{g } \mu\text{l}^{-1}$) and PS1 E9 ($0.4 \mu\text{g } \mu\text{l}^{-1}$) were mixed with $1 \mu\text{l}$ Plus Reagent, diluted in $50 \mu\text{l}$ serum-free DMEM; $2 \mu\text{l}$ of Lipofectamine LTX Reagent was diluted in $50 \mu\text{l}$ of serum-free culture medium. Both solutions were mixed and incubated for 5 min at room temperature ($22\text{--}24^\circ\text{C}$). Astrocytes were supplemented with $900 \mu\text{l}$ of serum-free culture medium and $100 \mu\text{l}$ of the lipofection mixture was pipetted onto the cells. Control cells expressing wild-type PS1 were transfected with pANP.emd ($0.8 \mu\text{g } \mu\text{l}^{-1}$) or pVGLUT1-EGFP ($0.8 \mu\text{g } \mu\text{l}^{-1}$). To independently verify that single transfected cells represent valid controls, we conducted a separate set of experiments, in which we co-transfected cells with plasmids encoding exogenous wild-type PS1 (PS1wt) ($0.4 \mu\text{g } \mu\text{l}^{-1}$) and ANP.emd ($0.4 \mu\text{g } \mu\text{l}^{-1}$). Transfected cells were incubated for 3 h at 37°C in an atmosphere of 5% CO_2 /95% air and then $30 \mu\text{l}$ of Ultrosor G (Pall Corporation, Port Washington, NY, USA) was added to the culture medium. The medium was exchanged for fresh culture medium the next day. Transfected cells were observed after 48–72 h.

Solutions

Extracellular solution (ECS) consisted of (in mM): 130 NaCl, 5 KCl, 2 CaCl_2 , 1 MgCl_2 , 10 D-glucose, and 10 HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), pH 7.2 with NaOH. Unless stated otherwise, all chemicals were of the highest grade of purity available (Sigma-Aldrich, Munich, Germany).

Analysis of vesicle mobility and cargo secretion

Astrocyte-loaded coverslips were mounted into the recording chamber and transferred to a confocal microscope (LSM 780; Zeiss, Jena, Germany) equipped with a plan-apochromatic oil-immersion objective ($63\times/\text{NA } 1.4$). ANP.emd and VGLUT1-EGFP were excited by a 488-nm argon laser line and emission fluorescence was filtered with a bandpass filter 495–550 nm. Time-lapse images were acquired every $\sim 0.5 \text{ s}$ for 1 min before and 4 min after stimulation with $100 \mu\text{M}$ ATP. The mobility of peptidergic (ANP.emd) and glutamatergic (VGLUT1-EGFP) vesicles was analyzed by ParticleTR software (Celica, Ljubljana, Slovenia) in exported tiff files ¹⁶. Typically, $\sim 20\text{--}40$ randomly selected mobile vesicles were tracked per cell and the track length (TL; the pathway that individual vesicles travelled), the maximal displacement (MD; the farthest translocation of a vesicle), the directionality index ($\text{DI} = \text{MD}/\text{TL}$), and the speed were determined; all parameters were estimated for 15-s epochs. Analysis of the vesicle mobility was performed before and 2 min after the onset of ATP stimulation ($100 \mu\text{M}$).

Individual exocytotic cargo (ANP_{emd}) secretion events⁶⁰ were identified visually in time-lapse confocal images of transfected cells and analyzed as described previously^{11, 61}. For statistical evaluation, the mean number of release events per cell within 4 min after ATP application was compared in control, PS1^{wt} and PS1^{E9} astrocytes. The rate of cargo release from individual vesicles was measured by custom-written MATLAB software (MathWorks, Natick, MA, USA) as the time necessary for a vesicle fluorescence decrease from 80% to 20% of the maximal fluorescence ($t_{80-20\%}$).

Statistical analysis

The parameters of vesicle mobility (TL, MD, DI and speed) and the number of elementary secretory events were expressed as means $\pm$ SEM. The relationship between the apparent size and speed of individual vesicles was quantified by calculating the Pearson product moment correlation coefficient. Statistical significance was determined with the Mann-Whitney *U* test using SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA) and with ANCOVA using MATLAB (MathWorks).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge the financial support from the Slovenian Research Agency (research core funding #P3 310, and the projects J3-3632, J3-4051, J3-6790, J3-7605). V.P.'s work is supported by the National Institutes of Health (HD078678).

References

1. Shea YF, Chu LW, Chan AO, Ha J, Li Y, Song YQ. A systematic review of familial Alzheimer's disease: Differences in presentation of clinical features among three mutated genes and potential ethnic differences. *J Formos Med Assoc.* 2015; 115:67–75. [PubMed: 26337232]
2. Laudon H, Hansson EM, Melen K, Bergman A, Farmery MR, Winblad B, Lendahl U, von Heijne G, Naslund J. A nine-transmembrane domain topology for presenilin 1. *J Biol Chem.* 2005; 280:35352–35360. [PubMed: 16046406]
3. Annaert WG, Levesque L, Craessaerts K, Dierinck I, Snellings G, Westaway D, George-Hyslop PS, Cordell B, Fraser P, De Strooper B. Presenilin 1 controls gamma-secretase processing of amyloid precursor protein in pre-golgi compartments of hippocampal neurons. *J Cell Biol.* 1999; 147:277–294. [PubMed: 10525535]
4. Tu H, Nelson O, Bezprozvanny A, Wang Z, Lee SF, Hao YH, Serneels L, De Strooper B, Yu G, Bezprozvanny I. Presenilins form ER Ca²⁺ leak channels, a function disrupted by familial Alzheimer's disease-linked mutations. *Cell.* 2006; 126:981–993. [PubMed: 16959576]
5. Smith IF, Green KN, LaFerla FM. Calcium dysregulation in Alzheimer's disease: recent advances gained from genetically modified animals. *Cell Calcium.* 2005; 38:427–437. [PubMed: 16125228]
6. De Strooper B. Loss-of-function presenilin mutations in Alzheimer disease. Talking Point on the role of presenilin mutations in Alzheimer disease. *EMBO Rep.* 2007; 8:141–146. [PubMed: 17268505]
7. Verkhratsky A, Parpura V. Astroglial pathology in neurological, neurodevelopmental and psychiatric disorders. *Neurobiol Dis.* 2015; 85:254–261. [PubMed: 25843667]
8. Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol.* 2010; 9:119–128. [PubMed: 20083042]

9. Verkhatsky A, Marutle A, Rodriguez-Arellano JJ, Nordberg A. Glial Asthenia and Functional Paralysis: A New Perspective on Neurodegeneration and Alzheimer's Disease. *Neuroscientist*. 2014; 21:552–568. [PubMed: 25125026]
10. Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kaye R, Metherate R, Mattson MP, Akbari Y, LaFerla FM. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron*. 2003; 39:409–421. [PubMed: 12895417]
11. Stenovec M, Trkov S, Lasic E, Terzieva S, Kreft M, Rodriguez Arellano JJ, Parpura V, Verkhatsky A, Zorec R. Expression of familial Alzheimer disease presenilin 1 gene attenuates vesicle traffic and reduces peptide secretion in cultured astrocytes devoid of pathologic tissue environment. *Glia*. 2015; 64:317–329. [PubMed: 26462451]
12. Billings LM, Oddo S, Green KN, McGaugh JL, LaFerla FM. Intraneuronal Abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. *Neuron*. 2005; 45:675–688. [PubMed: 15748844]
13. Jankowsky JL, Fadale DJ, Anderson J, Xu GM, Gonzales V, Jenkins NA, Copeland NG, Lee MK, Younkin LH, Wagner SL, Younkin SG, Borchelt DR. Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. *Hum Mol Genet*. 2004; 13:159–170. [PubMed: 14645205]
14. DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Ann Neurol*. 1990; 27:457–464. [PubMed: 2360787]
15. Scheff SW, Scott SA, DeKosky ST. Quantitation of synaptic density in the septal nuclei of young and aged Fischer 344 rats. *Neurobiol Aging*. 1991; 12:3–12. [PubMed: 2002880]
16. Potokar M, Kreft M, Pangrsic T, Zorec R. Vesicle mobility studied in cultured astrocytes. *Biochem Biophys Res Commun*. 2005; 329:678–683. [PubMed: 15737639]
17. Vardjan N, Stenovec M, Jorgacevski J, Kreft M, Grilc S, Zorec R. The fusion pore and vesicle cargo discharge modulation. *Ann N Y Acad Sci*. 2009; 1152:135–144. [PubMed: 19161384]
18. Trkov S, Stenovec M, Kreft M, Potokar M, Parpura V, Davletov B, Zorec R. Fingolimod—a sphingosine-like molecule inhibits vesicle mobility and secretion in astrocytes. *Glia*. 2012; 60:1406–1416. [PubMed: 22639011]
19. De Strooper B, Saftig P, Craessaerts K, Vanderstichele H, Guhde G, Annaert W, Von Figura K, Van Leuven F. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature*. 1998; 391:387–390. [PubMed: 9450754]
20. Wolfe MS, Xia W, Ostaszewski BL, Diehl TS, Kimberly WT, Selkoe DJ. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. *Nature*. 1999; 398:513–517. [PubMed: 10206644]
21. Gunawardena S, Yang G, Goldstein LS. Presenilin controls kinesin-1 and dynein function during APP-vesicle transport in vivo. *Hum Mol Genet*. 2013; 22:3828–3843. [PubMed: 23710041]
22. Pigino G, Morfini G, Pelsman A, Mattson MP, Brady ST, Busciglio J. Alzheimer's presenilin 1 mutations impair kinesin-based axonal transport. *J Neurosci*. 2003; 23:4499–4508. [PubMed: 12805290]
23. Gu ek A, Jorgacevski J, Singh P, Geisler C, Lisjak M, Vardjan N, Kreft M, Egner A, Zorec R. Dominant negative SNARE peptides stabilize the fusion pore in a narrow, release-unproductive state. *Cell Mol Life Sci*. 2016; 73:3719–3731. [PubMed: 27056575]
24. Grafstein B, Forman DS. Intracellular transport in neurons. *Physiol Rev*. 1980; 60:1167–1283. [PubMed: 6159657]
25. Meyhofer E, Howard J. The force generated by a single kinesin molecule against an elastic load. *Proc Natl Acad Sci U S A*. 1995; 92:574–578. [PubMed: 7831332]
26. Higuchi H, Muto E, Inoue Y, Yanagida T. Kinetics of force generation by single kinesin molecules activated by laser photolysis of caged ATP. *Proc Natl Acad Sci U S A*. 1997; 94:4395–4400. [PubMed: 9114000]
27. Mallik R, Carter BC, Lex SA, King SJ, Gross SP. Cytoplasmic dynein functions as a gear in response to load. *Nature*. 2004; 427:649–652. [PubMed: 14961123]
28. Takamori S, Holt M, Stenius K, Lemke EA, Grønborg M, Riedel D, Urlaub H, Schenck S, Brugger B, Ringler P, Müller SA, Rammner B, Gräter F, Hub JS, De Groot BL, Mieskes G, Moriyama Y,

- Klingauf J, Grubmüller H, Heuser J, Wieland F, Jahn R. Molecular anatomy of a trafficking organelle. *Cell*. 2006; 127:831–846. [PubMed: 17110340]
29. Holzwarth G, Bonin K, Hill DB. Forces required of kinesin during processive transport through cytoplasm. *Biophys J*. 2002; 82:1784–1790. [PubMed: 11916838]
 30. Hendricks AG, Perlson E, Ross JL, Schroeder HW 3rd, Tokito M, Holzbaur EL. Motor coordination via a tug-of-war mechanism drives bidirectional vesicle transport. *Curr Biol*. 2010; 20:697–702. [PubMed: 20399099]
 31. Stenovec M, Trkov S, Kreft M, Zorec R. Alterations of calcium homeostasis in cultured rat astrocytes evoked by bioactive sphingolipids. *Acta Physiol (Oxf)*. 2014; 212:49–61. [PubMed: 24825022]
 32. Morfini G, Szebenyi G, Elluru R, Ratner N, Brady ST. Glycogen synthase kinase 3 phosphorylates kinesin light chains and negatively regulates kinesin-based motility. *EMBO J*. 2002; 21:281–293. [PubMed: 11823421]
 33. Runneger MT, Wei X, Hamm-Alvarez SF. Increased protein phosphorylation of cytoplasmic dynein results in impaired motor function. *Biochem J*. 1999; 342(Pt 1):1–6. [PubMed: 10432292]
 34. Olabarria M, Noristani HN, Verkhratsky A, Rodríguez JJ. Concomitant astroglial atrophy and astrogliosis in a triple transgenic animal model of Alzheimer's disease. *Glia*. 2010; 58:831–838. [PubMed: 20140958]
 35. Olabarria M, Noristani HN, Verkhratsky A, Rodríguez JJ. Age-dependent decrease in glutamine synthetase expression in the hippocampal astroglia of the triple transgenic Alzheimer's disease mouse model: mechanism for deficient glutamatergic transmission? *Mol Neurodegener*. 2011; 6:55. [PubMed: 21801442]
 36. Yeh CY, Vadhwana B, Verkhratsky A, Rodríguez JJ. Early astrocytic atrophy in the entorhinal cortex of a triple transgenic animal model of Alzheimer's disease. *ASN Neuro*. 2011; 3:271–279. [PubMed: 22103264]
 37. Zorec R, Horvat A, Vardjan N, Verkhratsky A. Memory Formation Shaped by Astroglia. *Front Integr Neurosci*. 2015; 9:56. [PubMed: 26635551]
 38. Hansson E. Could chronic pain and spread of pain sensation be induced and maintained by glial activation? *Acta Physiol (Oxf)*. 2006; 187:321–327. [PubMed: 16734769]
 39. Zorec R, Parpura V, Verkhratsky A. Astroglial Vesicular Trafficking in Neurodegenerative Diseases. *Neurochem Res*. 2017; 42:905–917. [PubMed: 27628292]
 40. Zorec R, Parpura V, Verkhratsky A. Astroglial vesicular network: Evolutionary trends, physiology and pathophysiology. *Acta Physiol (Oxf)*. 2017
 41. Davletov B. A multiple sclerosis drug sheds light on astrocyte biology. *Acta Physiol (Oxf)*. 2014; 212:8–10. [PubMed: 24947320]
 42. Cedazo-Minguez A, Popescu BO, Ankarcona M, Nishimura T, Cowburn RF. The presenilin 1 deltaE9 mutation gives enhanced basal phospholipase C activity and a resultant increase in intracellular calcium concentrations. *J Biol Chem*. 2002; 277:36646–36655. [PubMed: 12121968]
 43. Pigino G, Pelsman A, Mori H, Busciglio J. Presenilin-1 mutations reduce cytoskeletal association, deregulate neurite growth, and potentiate neuronal dystrophy and tau phosphorylation. *J Neurosci*. 2001; 21:834–842. [PubMed: 11157069]
 44. Takashima A, Murayama M, Murayama O, Kohno T, Honda T, Yasutake K, Nihonmatsu N, Mercken M, Yamaguchi H, Sugihara S, Wolozin B. Presenilin 1 associates with glycogen synthase kinase-3 β and its substrate tau. *Proc Natl Acad Sci U S A*. 1998; 95:9637–9641. [PubMed: 9689133]
 45. Zhang W, Han SW, McKeel DW, Goate A, Wu JY. Interaction of presenilins with the filamin family of actin-binding proteins. *J Neurosci*. 1998; 18:914–922. [PubMed: 9437013]
 46. Potokar M, Kreft M, Li L, Daniel Andersson J, Pangrsic T, Chowdhury HH, Pekny M, Zorec R. Cytoskeleton and vesicle mobility in astrocytes. *Traffic*. 2007; 8:12–20. [PubMed: 17229312]
 47. Schliwa M, Euteneuer U, Bulinski JC, Izant JG. Calcium lability of cytoplasmic microtubules and its modulation by microtubule-associated proteins. *Proc Natl Acad Sci U S A*. 1981; 78:1037–1041. [PubMed: 7015328]
 48. Salter MW, Hicks JL. ATP-evoked increases in intracellular calcium in neurons and glia from the dorsal spinal cord. *J Neurosci*. 1994; 14:1563–1575. [PubMed: 8126555]

49. de Diego AM, Lorrio S, Calvo-Gallardo E, Garcia AG. Smaller quantal size and faster kinetics of single exocytotic events in chromaffin cells from the APP/PS1 mouse model of Alzheimer's disease. *Biochem Biophys Res Commun.* 2012; 428:482–486. [PubMed: 23123627]
50. Centemeri C, Bolego C, Abbracchio MP, Cattabeni F, Puglisi L, Burnstock G, Nicosia S. Characterization of the Ca^{2+} responses evoked by ATP and other nucleotides in mammalian brain astrocytes. *Br J Pharmacol.* 1997; 121:1700–1706. [PubMed: 9283706]
51. Milosevic M, Stenovec M, Kreft M, Petrusic V, Stevic Z, Trkov S, Andjus PR, Zorec R. Immunoglobulins G from patients with sporadic amyotrophic lateral sclerosis affects cytosolic Ca^{2+} homeostasis in cultured rat astrocytes. *Cell Calcium.* 2013; 54:17–25. [PubMed: 23623373]
52. Verkhratsky A, Burnstock G. Purinergic and glutamatergic receptors on astroglia. *Adv Neurobiol.* 2014; 11:55–79. [PubMed: 25236724]
53. de Wit H. Molecular mechanism of secretory vesicle docking. *Biochem Soc Trans.* 2010; 38:192–198. [PubMed: 20074058]
54. Verkhratsky A, Matteoli M, Parpura V, Mothet JP, Zorec R. Astrocytes as secretory cells of the central nervous system: idiosyncrasies of vesicular secretion. *EMBO J.* 2016; 35:239–257. [PubMed: 26758544]
55. Shang S, Wang C, Liu B, Wu Q, Zhang Q, Liu W, Zheng L, Xu H, Kang X, Zhang X, Wang Y, Zheng H, Wang S, Xiong W, Liu T, Zhou Z. Extracellular Ca^{2+} per se inhibits quantal size of catecholamine release in adrenal slice chromaffin cells. *Cell Calcium.* 2014; 56:202–207. [PubMed: 25103334]
56. Ales E, Fuentealba J, Garcia AG, Lopez MG. Depolarization evokes different patterns of calcium signals and exocytosis in bovine and mouse chromaffin cells: the role of mitochondria. *Eur J Neurosci.* 2005; 21:142–150. [PubMed: 15654851]
57. Zhang C, Wu B, Beglopoulos V, Wines-Samuelson M, Zhang D, Dragatsis I, Sudhof TC, Shen J. Presenilins are essential for regulating neurotransmitter release. *Nature.* 2009; 460:632–636. [PubMed: 19641596]
58. Schwartz JP, Wilson DJ. Preparation and characterization of type 1 astrocytes cultured from adult rat cortex, cerebellum, and striatum. *Glia.* 1992; 5:75–80. [PubMed: 1531812]
59. Han W, Li D, Stout AK, Takimoto K, Levitan ES. Ca^{2+} -induced deprotonation of peptide hormones inside secretory vesicles in preparation for release. *J Neurosci.* 1999; 19:900–905. [PubMed: 9920653]
60. Krzan M, Stenovec M, Kreft M, Pangrsic T, Grilc S, Haydon PG, Zorec R. Calcium-dependent exocytosis of atrial natriuretic peptide from astrocytes. *J Neurosci.* 2003; 23:1580–1583. [PubMed: 12629160]
61. Stenovec M, Kreft M, Poberaj I, Betz WJ, Zorec R. Slow spontaneous secretion from single large dense-core vesicles monitored in neuroendocrine cells. *FASEB J.* 2004; 18:1270–1272. [PubMed: 15180959]

Figure 1.

Spontaneous mobility of peptidergic secretory vesicles is attenuated in PS1 E9 astrocytes. (A) Confocal images of the live cultured control and (B) PS1 E9 astrocyte expressing ANP.emd stored in individual vesicles; scale bars, 10 μ m. (C) Peptidergic vesicle tracks (N=40) reconstructed in a 15-s epoch in the control (Con) and (D) PS1 E9 astrocyte, respectively. Note more elongated vesicle tracks in the control astrocyte. (E) Track length (TL), (F) maximal displacement (MD), (G) directionality index (DI) and (H) speed of peptidergic vesicles in the control, PS1wt and PS1 E9 astrocytes. Note diminished TL, MD, DI and vesicle speed in PS1 E9 astrocytes. The numbers above the top of the bars (mean $\pm$ SEM) indicate the number of vesicles analyzed; the numbers at the bottom of the bars indicate the number of cells analyzed. * P <0.05, $\ddagger$ P <0.001 (Mann-Whitney U test).

Figure 2.

Spontaneous mobility of glutamatergic secretory vesicles is attenuated in PS1 Δ E9 astrocytes. (A) Confocal images of the control and (B) PS1 Δ E9 astrocyte expressing VGLUT1-EGFP incorporated into the membrane of individual vesicles observed as fluorescent puncta of different size; scale bars, 10 μ m. (C) Reconstructed tracks of glutamatergic vesicles ($N=20$) in a 15-s epoch in the control (Con) and (D) PS1 Δ E9 astrocyte, respectively. (E) Track length (TL), (F) maximal displacement (MD), (G) directionality index (DI) and (H) speed of glutamatergic vesicles in the control and PS1 Δ E9 astrocytes. Note diminished TL, MD, DI and vesicle speed in PS1 Δ E9 astrocytes. The numbers above the top of the bars (mean $\pm$ SEM) indicate the number of vesicles analyzed; the numbers at the bottom of the bars indicate the number of cells analyzed. $\ddagger P < 0.001$ (Mann-Whitney *U* test).

Figure 3.

ATP-evoked reduction in directional mobility of peptidergic vesicles is larger in PS1 Δ E9 than in the control astrocytes. (A–D) Mobility of peptidergic vesicles (A, B, TL; C, D, MD; mean $\pm$ SEM) in the control (Con) and PS1 Δ E9 astrocytes before (–), and within the first (1') and the second (2') minute after stimulation with 100 μ M ATP. Note a lesser reduction of these trafficking parameters in PS1 Δ E9 astrocytes owing to a reduction in their initial values at rest; similar “flooring” is evident in the ATP-evoked reduction in both groups of cells. The numbers at the top indicate the number of vesicles and the numbers at the bottom of the bars indicate the number of cells analyzed (here and in Figure 5). ‡ P <0.001 vs. non-stimulated

vesicle mobility (Mann-Whitney U test). (E, F) The plots displaying the relationship between MD and TL (directionality index, DI) in peptidergic vesicles in the control ($N=5$) (black circles) and PS1 E9 ($N=5$) astrocytes (white circles) during the first (E) and the second minute (F) after ATP stimulation. A linear function (black and blue lines) of the form $[MD=MD_0+a \times (TL)]$ was fitted to the data; the slope ($a \pm SEM$) correspond to the DI (displayed in the top) was diminished significantly ($\dagger P < 0.001$; ANCOVA) in PS1 E9 astrocytes.

Figure 4.

Post-stimulatory recovery of peptidergic vesicle mobility is slower in PS1 E9 astrocytes.

(A–D) Vesicle mobility (TL (A), MD (B), DI (C) and speed (D)) measured in 15-s epochs (mean $\pm$ SEM) before (–15 s) and after (0–120 s) application of 100 μM ATP to astrocytes.

Note, distinct initial diminishment of vesicle mobility that coincided with an ATP-evoked increase in $[\text{Ca}^{2+}]_i$ (the onset of stimulation at 0 s); over time the vesicle mobility slowly recovered.

The tendency for recovery of vesicle mobility was more pronounced in the control (black diamonds) than PS1 E9 astrocytes (blue squares). A linear function of the form $[\text{MD} = \text{MD}_0 + a \times (\text{TL})]$ was fitted to the data. The slopes ($a \pm \text{SEM}$; displayed in the top)

indicate the speed of recovery of given parameter in the control (black) and PS1 E9 (blue) astrocytes. The slopes (a) of functions fitted to TL, MD and Speed were diminished significantly in PS1 E9 astrocytes (* $P<0.05$, † $P<0.01$; ANCOVA).

Figure 5.

ATP-evoked reduction in directional mobility of glutamatergic vesicles is larger in PS1 E9 than in the control astrocytes. (A–D) Mobility of glutamatergic vesicles (A, B, TL; C, D, MD; mean±SEM) in control (Con) and PS1 E9 astrocytes before (-), and within the first (1') and the second (2') minute after stimulation with 100 μM ATP. Note retarded initial trafficking values at rest, which with a similar “flooring” after ATP application lead to a lesser ATP-evoked reduction in MD and TL for glutamatergic vesicles in PS1 E9 astrocytes. [†] $P < 0.01$, [‡] $P < 0.001$ vs. non-stimulated vesicle mobility (Mann-Whitney U test). (E, F) The plots displaying the relationship between MD and TL (directionality index, DI) in

glutamatergic vesicles in control ($N=5$) (black diamonds) and PS1 E9 ($N=5$) astrocytes (blue squares) during the first (E) and during the second minute (F) after ATP stimulation. A linear function (black and blue lines) of the form $[MD=MD_0+a \times (TL)]$ was fitted to the data; the slope ($a \pm SEM$) (displayed in the top) diminished significantly ($\dagger P < 0.001$; ANCOVA) in PS1 E9 astrocytes during the second minute after ATP stimulation.

Figure 6.

Post-stimulatory recovery of glutamatergic vesicle mobility is slower in PS1 E9 astrocytes. (A–D) Vesicle mobility (TL (A), MD (B), DI (C) and speed (D)) measured in 15-s epochs (mean $\pm$ SEM) before (–15 s) and after (0–120 s) application of 100 μM ATP to astrocytes. Following initial diminishment, the vesicle mobility recovered slowly. The tendency for recovery of mobility was more pronounced in the control (black diamonds) than PS1 E9 astrocytes (blue squares). A linear function of the form $[\text{MD}=\text{MD}_0+a\times(\text{TL})]$ was fitted to the data. The slopes ($a\pm\text{SEM}$; displayed in the top) indicate the speed of recovery of given

parameter in the control (black) and PS1 E9 (blue) astrocytes. The slope (a) of function fitted to MD was diminished significantly in PS1 E9 astrocytes (* P <0.05; ANCOVA).

Figure 7.

Reduced peptide secretion from PS1 E9 astrocytes coincides with diminished mobility of ANP-laden vesicles. (A) Confocal images of peptidergic vesicles in the control (top), PS1wt (middle) and PS1 E9 (bottom) astrocytes discharging fluorescent cargo (ANP.emd) during cell stimulation with 100 μ M ATP. The rapid decrease in fluorescence ($\sim$ 0.5 s) indicates rapid cargo release. Time marks in the top right corners indicate the time in respect to the arbitrary starting point (0 s). Scale bar, 0.5 μ m. (B) Normalized time-dependent changes in vesicle fluorescence (from A). The horizontal dotted lines indicate the minimum and maximum fluorescence levels. The vertical dashed lines indicate the period where the

vesicle fluorescence was measured from the images displayed in (A). The addition of ATP (100 μ M) is indicated by the horizontal rectangle. (C) The number of ATP-evoked secretory events indicative of full-collapse vesicle fusion is diminished in PS1^{-/-} E9 astrocytes. * $P < 0.05$ vs. controls or PS1wt astrocytes (Mann-Whitney U test). (D–F) Frequency histograms and the cumulative frequency plot (Cum frequency; coloured solid lines) of ATP-evoked secretory events in controls (D), PS1wt (E) and PS1^{-/-} E9 astrocytes (F). Note similar time distribution of ATP-evoked release events; more events were observed in the first than in the second two-minute period after ATP stimulation.