

RESEARCH PAPER

A β -amyloid oligomer directly modulates P/Q-type calcium currents in *Xenopus* oocytes

M Mezler, S Barghorn, H Schoemaker, G Gross and V Nimmrich

Neuroscience Research, GPRD, Abbott, Ludwigshafen, Germany

Correspondence

Mario Mezler, Abbott, D-67061 Ludwigshafen, Germany. E-mail: mario.mezler@abbott.com

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BACKGROUND AND PURPOSE

β -amyloid (A β) oligomers have been implicated in the early pathophysiology of Alzheimer's disease (AD). While the precise nature of the molecular target has not been fully revealed, a number of studies have indicated that A β oligomers modulate neuron-specific ion channels. We recently provided evidence that A β oligomers suppress isolated P/Q-type calcium currents in cultured nerve cells. Using a heterologous expression system, we aimed to prove a direct effect on the membrane channel mediating such current.

EXPERIMENTAL APPROACH

The effects of a synthetically generated A β oligomer, A β globulomer, were investigated on P/Q-type currents recorded from *Xenopus laevis* oocytes expressing the full P/Q-type calcium channel or the pore-forming subunit only. We also examined the effects of A β globulomer on recombinant NMDA receptor currents. Finally, we compared the modulation by A β globulomer with that induced by a synthetic monomeric A β .

KEY RESULTS

A β globulomer directly and dose-dependently modulated P/Q-type calcium channels. A leftward shift of the current-voltage curve indicated that the threshold for channel opening was reduced. The effect of A β globulomer was also present when only the α 1A subunit of the normally tripartite channel was expressed. In contrast, the monomeric A β had no effect on P/Q current. Also globulomer A β had no effect on glutamate-induced NMDA currents.

CONCLUSIONS AND IMPLICATIONS

The α 1A subunit of the P/Q-type calcium channel is directly modulated by oligomeric A β . Threshold reduction as well as an increase in current at synaptic terminals may facilitate vesicle release and could trigger excitotoxic events in the brains of patients with AD.

Abbreviations

A β , β -amyloid; AD, Alzheimer's disease; APP, amyloid precursor protein; I/V, current-voltage; LTP, long-term potentiation; NFR, normal frog Ringer solution

Introduction

Alzheimer's disease (AD) is a neurodegenerative illness accompanied by progressive accumulation of β -amyloid (A β) protein in brain. Such overload ultimately leads to the development of amyloid plaques that are a key histopathological feature of the disease. However, it is now generally accepted

that the soluble fraction of A β , rather than amyloid plaques, is detrimental to brain function (Haass and Selkoe, 2007). Within the soluble protein pool of A β species, aggregation to oligomeric states converts the peptide into pathological forms (Cleary *et al.*, 2005; Walsh *et al.*, 2005). Antibodies directed against the pathological conformation of the oligomeric aggregation state neutralize A β in amyloid precursor

protein (APP)-overexpressing mice and normalize cognitive deficits, underlining the relevance of oligomerization for toxicity (Hillen *et al.*, 2010).

Toxic effects of A β oligomers have been primarily studied using artificially generated forms. Application of such preparations suppresses synaptic plasticity *in vitro* (Lambert *et al.*, 1998; Wang *et al.*, 2004) and *in vivo* (Walsh *et al.*, 2002), and – upon prolonged exposure – induces synaptic decline (Lacor *et al.*, 2007). Cleary *et al.* (2005) showed that oligomers directly impair learning and memory after intraventricular injection into rat brain. It has therefore been suggested that oligomer-induced synaptic dysfunction may underlie cognitive deficits in AD (Haass and Selkoe, 2007). Although these studies highlight the pathophysiological consequences of oligomeric A β , the molecular mode of action remains largely unrevealed. Yet, there are some recent reports suggesting molecular targets that mediate the pathology of A β . For example, several studies indicate that oligomeric A β may disturb synaptic function via NMDA-related cascades (Kelly and Ferreira, 2006; De Felice *et al.*, 2007; Shankar *et al.*, 2007). Using a stable oligomer preparation – A β globulomer (Barghorn *et al.*, 2005) – we recently showed an oligomer-induced modulation of isolated P/Q-type calcium currents in cultured neurons. Synaptic vesicle release was altered, indicated by a diminished frequency of miniature postsynaptic currents (Nimmrich *et al.*, 2008).

These data clearly demonstrate a modulation of ion channel currents as part of the pathological amyloid cascade. However, it is unclear whether currents were directly or indirectly affected, leaving the question of the direct molecular target for A β oligomers unanswered. Based on the specificity of the effect for the P/Q-subtype (Nimmrich *et al.*, 2008), we assumed that a subunit of the P/Q-type voltage-gated calcium channel determining channel specificity was directly modulated by A β oligomers. The candidate target is the pore-forming subunit, which is the only part of the channel unique to the P/Q-type. To test this hypothesis we expressed the P/Q-type channel recombinantly in *Xenopus laevis* oocytes and studied voltage-gated calcium currents after application of an A β oligomer. Selective expression of the pore-forming α 1A subunit allowed us to narrow down possible effects to a single protein target.

Methods

Preparation of A β_{1-42} globulomer and monomer

A β_{1-42} globulomer was prepared as described previously (Barghorn *et al.*, 2005). In brief, lyophilized A β_{1-42} synthetic peptide (H-1368, Bachem, Bubendorf, Switzerland) was disaggregated by using 100% 1,1,1,3,3,3 hexafluoro-2-propanol. After evaporation, A β_{1-42} was resuspended at a concentration of 5 mM in dimethylsulphoxide, diluted to a final concentration of 400 μ M in PBS containing 0.2% SDS. After 6 h incubation at 37°C, the sample was diluted with three volumes of H₂O and incubated for another 18 h at 37°C. The sample was concentrated by ultrafiltration (30 kDa cut-off), dialysed against 5 mM NaH₂PO₄, 35 mM NaCl, pH 7.4, centrifuged at 10 000 $\times$ g for 10 min, and the supernatant containing the

38/48 kDa A β_{1-42} globulomer was withdrawn. Concentrations of the globulomer were determined by Bradford measurement, and the peptide preparations stored at –80°C and diluted in normal frog Ringer solution (NFR; 115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, 10 mM HEPES pH 7.2) to the required concentrations immediately before the electrophysiological experiments. The A β globulomer concentrations stated are based on the preparation protocol and it is assumed that the A β globulomer is composed of 12 monomers. Thus, the molar concentration corresponds to 1/12 of the monomer equivalent. Currents were measured before, 10 min after incubation with A β_{1-42} globulomer in the bath solution and after additional 10 min washout with NFR where appropriate (see later discussion).

For control experiments, synthetic monomeric A β_{1-42} peptide was dissolved in 0.1% NaOH, yielding a 1 mM stock solution, which was frozen at –80°C. Immediately before the experiment, this solution was diluted to the appropriate concentrations in NFR solution, which was added to the bath manually. The purity and monomeric state of this preparation was verified by means of a Coomassie-stained SDS-PAGE (data not shown).

Functional expression in *X. laevis* oocytes

Preparation of oocytes and the measurement of the membrane current on whole oocytes was carried out as described previously (Methfessel *et al.*, 1986; Mezler *et al.*, 2001; 2008). Female *X. laevis* animals (Nasco, Fort Atkinson, WI, USA) were anaesthetized in solution with 0.2% Tricain (Sigma, St. Louis, MO, USA) and 2 g·L^{–1} NaHCO₃ (Sigma), ovary lobes were removed, and oocytes were released from the follicle tissue for 2 h with collagenase solution [Type I, Roche Applied Science, Mannheim, Germany, 2 mg·mL^{–1}, in Barth medium (88 mM NaCl, 1 mM KCl, 0.82 mM MgSO₄, 0.33 mM Ca(NO₃)₂, 0.41 mM CaCl₂, 2.4 mM NaHCO₃, 5 mM Tris-HCl, pH 7.4)]. Alternatively, oocytes were acquired from Ecocyte Bioscience (Castrop-Rauxel, Germany). Stage V and VI oocytes were selected by hand, plated one cell per well into 96-well microtitre plates (Greiner Bio-One, Frickenhausen, Germany; V-shape) in Barth medium supplemented with gentamicin (50 mg·mL^{–1}, Sigma), and each oocyte was injected with RNA prepared from pGemHeJuel plasmids (Liman *et al.*, 1992) containing human calcium channel or NMDA receptor subunits (hNR1, hNR2A). For RNA preparation, the plasmids were linearized with *Spe*I (hCACN A1A.2), *Stu*I (hCACN B4.2), *Nhe*I (hCACN B1.1 and hCACN A2D1), *Not*I (hNR2A), or *Sal*I (hNR1 NMDA subunit, hCACN A1A.1 and hCACN B3), and capped (5'-7-methyl guanosine) mRNA was transcribed with T7 RNA polymerase using the mMES-SAGE mMACHINE kit (Ambion Inc., Austin, TX, USA).

Oocytes were injected in a Roboocyte system (Multichannel Systems, Reutlingen, Germany) with 15 nL (3 ng· μ L^{–1}) RNA solution with a 1:1:1 ratio of one α , one β and one α 2 δ 1 subunit of the calcium channel. Furthermore, cells were injected with 15 nL (420 ng· μ L^{–1}) of the α 1A (CACNA1A.2) subunit only, when the studies were aimed at the identification of the interaction partner of the globulomer preparations. For the functional studies with the NMDA receptor, cells were injected with 15 nL (2 ng· μ L^{–1}) of hNR1 and hNR2A in a 1:1 ratio. The oocytes were then incubated for 2–5 days at 18°C.

Electrophysiological measurements in *X. laevis* oocytes

Oocytes plated in 96-well plates were measured with the Roboocyte system (Multichannel Systems). The measurement chamber was continuously perfused with NFR solution. For the measurements, either barium solution (90 mM NaCl, 2.5 mM KCl, 10 mM BaCl₂, 5 mM HEPES, pH 7.4) or NFR was applied as indicated. All compounds were diluted in barium solution or NFR, and measurements carried out at room temperature. To administer compounds, the software-driven perfusion was switched between reservoirs with the appropriate solutions. The flow rate of 5 mL min⁻¹ resulted in an exchange of solutions at the oocyte within 1–2 s. All A β preparations were directly pipetted into the well containing the oocytes to avoid adsorption to the tubing. NMDA receptors were stimulated with 10 μ M glycine and 10 μ M glutamate in NFR through the perfusion system and currents measured at a holding potential of –60 mV. The plateau current of the channel after control or treatment conditions was compared.

A β preparations were diluted immediately before the experiment and 100 μ L added fresh directly to the measurement chamber containing 100 μ L buffer to reach the final concentration. The oocytes were then incubated for 10 min with A β and measured. To avoid activation of the calcium-dependent chloride channel during the voltage steps, the oocytes were injected with 15 nL of a 10 mM 1,2-bis(*o*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (BAPTA) solution in water at least 30 min before measurement. w-Agatoxin IVA was used at 100 nM in barium solution, cadmium was used as a 300 μ M solution in barium Ringer. If not stated otherwise, the current–voltage relationships were normalized to the maximal current of the first (non-stimulated) *I/V* curve.

Data acquisition and analysis were performed with the internal Roboocyte software and statistical analysis carried out with GraphPad Prism5 (GraphPad Software Inc., La Jolla, CA, USA). Data are presented as mean $\pm$ SEM. Currents were compared statistically through the one-way ANOVA with Dunnett's multiple comparison test or *t*-test. The voltage-dependence of steady-state activation current was described by a Boltzmann function with normalized current (I/I_{max}).

Results

Different P/Q-type channel compositions are functionally expressed in *X. laevis* oocytes

In this study, we aimed to examine the effect of A β oligomers on currents elicited at the recombinantly expressed P/Q-type calcium channel and to compare them with effects elicited at the recombinantly expressed NMDA receptor. We expressed the proteins of both channels in oocytes from *X. laevis*. The *Xenopus* oocyte system is particularly suited for the expression of voltage-gated and ligand-gated ion channels (for review see, e.g. Buckingham *et al.*, 2006) and several expression studies with P/Q channels and NMDA receptors have been published (e.g. Verdoorn *et al.*, 1987; Sather *et al.*, 1993). To validate our expression system, we initially performed studies to characterize the P/Q current and the glutamate-induced NMDA receptor current. For the analysis of the P/Q channel,

we employed a voltage step protocol with -P/4 subtraction for leak correction (Figure 1A). Functional currents were obtained as shown in Figure 1C and D. The voltage steps of 10 mV and a length of 500 ms were separated by 2 s of –60 mV holding potential and the P/4 protocol performed from the software. Oocytes not expressing the P/Q channel do not respond to this stimulation (Figure 1B). Cells expressing the P/Q α 1A.2 subunit alone (Figure 1C) or the tripartite channel, consisting of α 1A.2, β 1.1 and α 4 δ 1 (Figure 1D) respond with typical calcium channel currents (Sather *et al.*, 1993; Stea *et al.*, 1994). Differences in channel kinetics that we observed when the accessory subunits were missing are in line with previous observations of recombinant P/Q currents in *Xenopus* oocytes (Sather *et al.*, 1993; Stea *et al.*, 1994) or HEK293 cells (Moreno *et al.*, 1997). In the case of the α 1A.2-expressing cells, repeated *I/V* curves did not change the current profile even after four consecutive stimulations of single cells, while a slight rundown was measured for the tripartite channel under these conditions (8.6 $\pm$ 8% increase of current after 10 min at the second *I/V* curve, and 22 $\pm$ 9% after additional 10 min in the third *I/V* curve, *n* = 7, comparison with the first *I/V* curve in both cases; data not shown). Furthermore, control oocytes were stable under the same conditions and did not show any response to the voltage protocol, equally after four consecutive stimulations (data not shown). To further prove that the currents obtained resemble P/Q channel currents previously described, currents were recorded after exposure to 100 nM w-agatoxin IVA. While the current of the cell was completely blocked in the oocytes expressing the α 1A subunit (Figure 1E and F), the same concentration inhibited the channel current to approximately 50% in the tripartite channel (Figure 1G and H). A modulation of agatoxin sensitivity has been described previously (Moreno *et al.*, 1997). In both cases, the inhibition by agatoxin was reversible after washout. Under similar conditions, 300 μ M cadmium inhibited the α 1A calcium current completely (data not shown).

A β oligomers dose-dependently increase recombinant P/Q-type calcium currents

We were intrigued by the previous findings of a modulation of P/Q-type calcium currents in hippocampal neurons and chose to use the recombinant expression system of *X. laevis* oocytes for further in-depth analysis. As in our previous publication, we used a synthetically prepared oligomer – A β globulomer – as representative for A β oligomers. A β globulomer is not readily soluble in Mg²⁺-containing buffers and also shows low solubility in Ba²⁺-containing media (data not shown). We therefore decided to use a pure Ca²⁺-containing Ringer solution (NFR), with the caveat of lower current sizes and recording quality. Yet, current recordings were robust, and we found a highly reproducible increase of P/Q channel current after the application of 200 nM A β globulomer, corresponding to 2.4 μ M monomer equivalent. Such current modulation was observed both in cells expressing the tripartite channel (Figure 2A and B) and those that only expressed the pore-forming unit (Figure 2C and D). At –10 mV the current was increased by 102% in the tripartite channel (thus 202 $\pm$ 30% total current, *n* = 9). Furthermore, a shift of the *I/V* dependence of channel activation to lower membrane potentials was apparent, indicating a higher voltage sensitivity of the channel opening. Similarly, the current was increased by

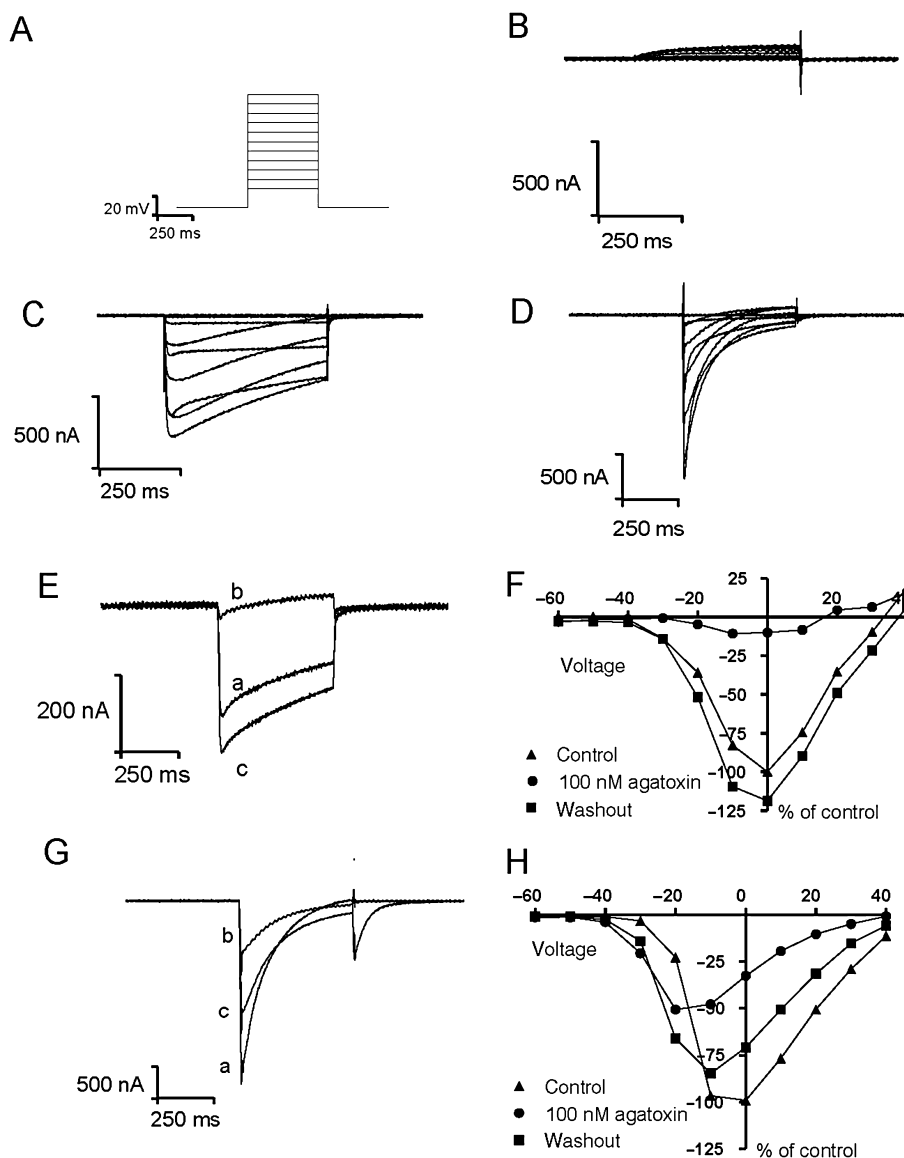


Figure 1

Initial characterization of the P/Q channels expressed in *Xenopus* oocytes. (A) I/V protocol used in the present study to evaluate the effects of substances on the P/Q calcium channel. (B) Superimposed records of current traces and I/V dependence of a control oocyte not expressing P/Q channel. (C) Superimposed records of current traces and I/V dependence of a cell expressing the P/Q $\alpha 1A$ subunit. (D) Superimposed records of current traces and I/V dependence of a cell expressing the tripartite channel ($\alpha 1A.2$, $\beta 1.1$, $\alpha 2\delta 1$). (E) Superimposed records of current traces at -10 mV of a cell expressing the $\alpha 1A$ subunit treated with agatoxin; a, control current; b, current after 10 min incubation with 100 nM agatoxin; c, current after an additional 10 min washout period. (F) Current-voltage relationship of an exemplary cell treated as in (E). (G) Superimposed records of current traces at -10 mV of a cell expressing the tripartite channel; a, control current; b, after 10 min incubation with 100 nM agatoxin; c, current after an additional 10 min washout period. (H) Current-voltage relationship of an exemplary cell treated as in (G). Five hundred millisecond voltage steps of 10 mV increments with 3 s intervals were performed from -60 mV (holding potential -80 mV) to +40 mV. The linear capacity currents were subtracted. Also, the leak current was subtracted at each voltage step through the -P/4 method employing the Roboocyte software. Buffer contains 10 mM Ba $^{2+}$ as charge carrier and to avoid activation of Ca $^{2+}$ -dependent chloride current, cells were injected with 15 nL of 10 mM BAPTA at least 30 min before measurement. Sampling frequency was 10 kHz; data were filtered at 3 kHz.

43% when cells only expressed the $\alpha 1A$ subunit (with a total current of $143 \pm 6.6\%$ of control, $n = 7$) at 0 mV, and the I/V curve was equally shifted to more negative potentials. In contrast, the replicate addition of the buffer control does not change the I/V response of the cells (Figure 2E and F, $n = 9$). As already indicated in Figure 2B and D, A β globulomer treat-

ment appears to change the steady-state activation kinetics of the tripartite channel, as well as of the channel missing the accessory subunits. Indeed, the calculated steady-state kinetics demonstrate a leftward shift of the half-maximal activation from -9.2 ± 0.7 mV ($n = 9$) of the cells stimulated with control buffer to -15 ± 1.4 mV ($n = 7$) for the A β globulomer-

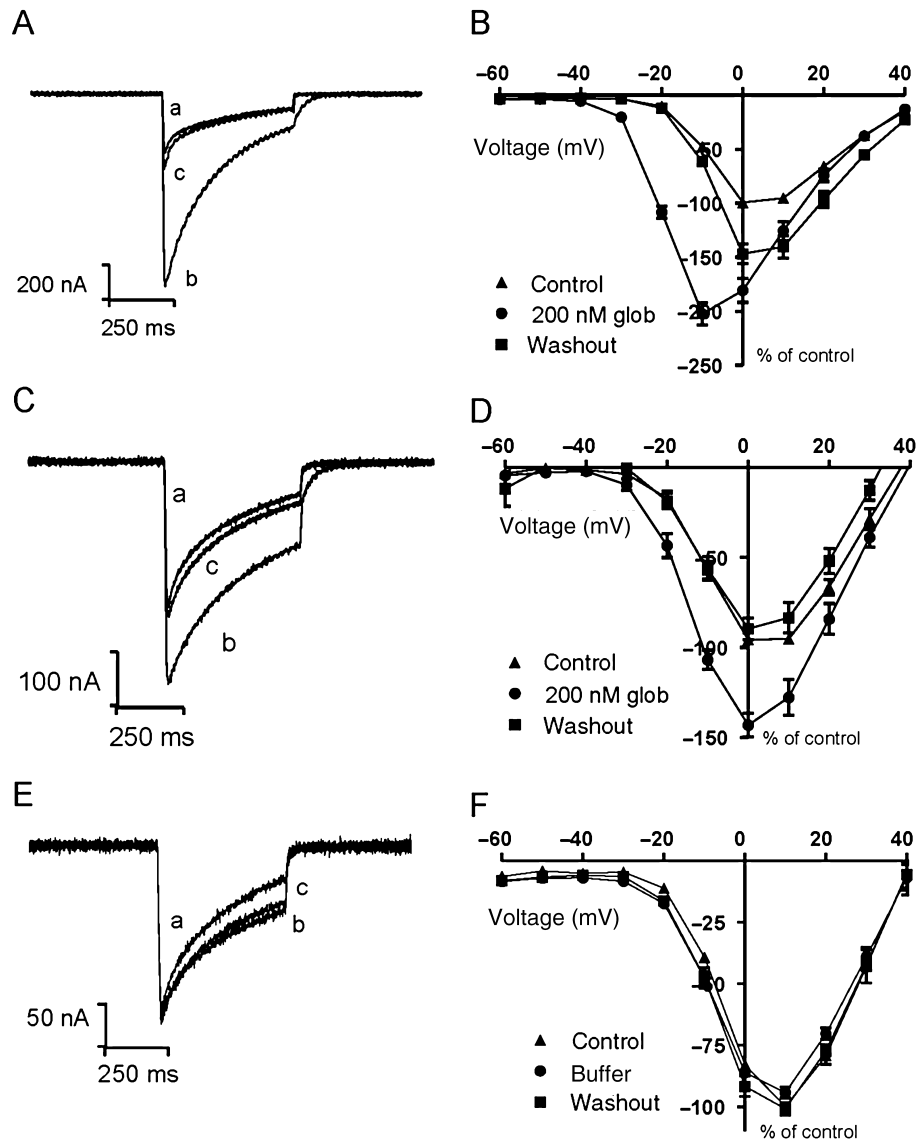


Figure 2

A β globulomer (glob) potentiates current through the P/Q calcium channel. (A) Superimposed exemplary traces of a cell expressing the tripartite channel treated with 200 nM A β globulomer at -10 mV (A β globulomer concentrations stated are based on its A β monomer peptide concentration). (B) Current–voltage relationship (*I/V* curves) of oocytes under the same conditions. $n = 9$ for all conditions. (C) Superimposed exemplary traces of a cell expressing the $\alpha 1A$ subunit of the P/Q channel treated with 200 nM A β globulomer at 0 mV. (D) *I/V* curves of oocytes under the same conditions. $n = 7$ for all conditions. (E) Superimposed traces of a cell expressing the $\alpha 1A$ subunit of the P/Q channel treated with control buffer (NFR) at 0 mV. (F) *I/V* curves of oocytes under the same conditions, $n = 9$. (A, C, E) a, initial current without treatment; b, current after 10 min incubation with A β globulomer; c, washout after 10 min; (B, D, F) For the current–voltage relationships, the curves represent the first, second and third measurements of the oocytes. NFR was used to dissolve the A β preparations; Ca^{2+} is the charge carrier in these studies.

stimulated $\alpha 1A$ P/Q channel. In the tripartite channel, a leftward shift from -14.4 ± 1.7 mV ($n = 5$) to -19.8 ± 0.5 mV ($n = 9$) was equally observed by A β globulomer stimulation. Both shifts were significantly different from their respective controls (Figure 3, $P = 0.0014$ for the $\alpha 1A$ channel and $P = 0.0022$ for the tripartite channel; *t*-test). When comparing the slope of the Boltzmann curves, control $\alpha 1A$ (4.78 ± 0.15 mV) and A β globulomer-stimulated $\alpha 1A$ (5.0 ± 0.16 mV) or control tripartite channel (3.9 ± 0.3 mV) and A β globulomer-stimulated tripartite channel (4.1 ± 0.1 mV) did not differ.

However, the slope significantly differed between the $\alpha 1A$ channel and the tripartite channel, both, comparing non-stimulated ($P = 0.013$) or A β globulomer-stimulated conditions ($P < 0.0001$). This indicates that the change of the slope is not dependent on A β globulomer stimulation but is influenced by the composition of the channel subunits.

In further experiments, we evaluated if the A β globulomer stimulation resulted in a concentration-dependent change of the current. As shown in Figure 4A, the maximal current at 0 mV mediated by the $\alpha 1A$ subunit increased in a

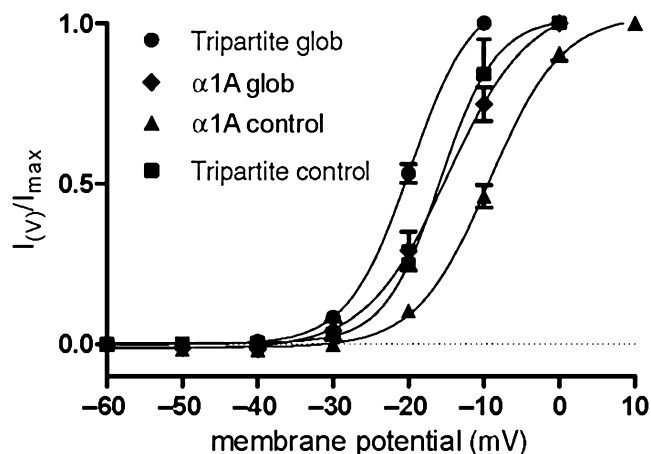


Figure 3

Steady-state activation curves are changed by A β globulomer (glob) treatment. Oocytes expressing the tripartite channel were treated with buffer (control) or the A β globulomer as were oocytes expressing the $\alpha 1A$ subunit. Second stimulations were analysed. Both V50 and slope changed significantly (see text). While the shift in the voltage-dependence seems to be A β globulomer-mediated, the slope change appears to be dependent on the channel subunit composition.

concentration-dependent manner from $86 \pm 2\%$ (buffer control, $n = 9$), through $85 \pm 6\%$ (2 nM, $n = 5$), $116 \pm 2.4\%$ (20 nM, $n = 4$) to $143 \pm 6.6\%$ (200 nM, $n = 7$). Both, the increases at 20 nM ($P < 0.01$) and 200 nM ($P < 0.001$), were statistically significant. These results are reflected in the previously described changes in the I/V relationship (Figure 4B). Equally, the current at -10 mV increased concentration-dependently in the stimulated tripartite channel (Figure 4C). Control current ($75.5 \pm 10.8\%$, $n = 5$) was non-significantly increased by stimulation with 2 nM A β globulomer ($99.6 \pm 13.6\%$, $n = 6$), and significantly changed with 200 nM A β stimulation ($162.1 \pm 9.8\%$, $n = 5$, $P < 0.001$). Again, the I/V curve indicates that the increase in channel current is paralleled by a leftward shift of the half-maximal activation, representing a higher voltage sensitivity of the channel opening by interaction with A β globulomer (Figure 4D).

A β monomers do not affect recombinant P/Q-type calcium currents

While these data unequivocally demonstrate that A β globulomer modulates the P/Q calcium channel and further shows that the $\alpha 1A$ subunit is necessary for this modulation, we needed to explore whether this interaction is indeed specific. To this end, we stimulated oocytes expressing the $\alpha 1A$ subunit with equimolar concentrations of A β monomer (2.4 μ M). The overall voltage-dependence of the channel current is not changed by A β monomer stimulation when comparing the three consecutive I/V curves of the first I/V protocol (Figure 5A1), the second protocol with stimulation of 2.4 μ M monomer (Figure 5A2) or the third protocol of washout (Figure 5A3). The derived I/V curves for all three protocols are identical (Figure 5B). Also, the current at 0 mV is identical in all three conditions (Figure 5C). The comparison of the mean values of the second (stimulated) I/V

protocol at 0 mV of control (86 ± 2 , $n = 9$), A β monomer (99.8 ± 8 , $n = 5$) and A β globulomer (143 ± 6.6 , $n = 7$) validates that the monomer does not influence the peak current of the $\alpha 1A$ subunit.

A β oligomers do not affect NMDA receptor currents

Next, we wanted to know if the A β globulomer also affects the current of an unrelated ion channel. To test this, we chose the NMDA receptor, which has been proposed as possible target for A β oligomers (Kelly and Ferreira, 2006; Shankar *et al.*, 2007; Texido *et al.*, 2011). In cells expressing the GluN1/GluN2A subunits of the NMDA receptor, we initially performed a concentration-response curve of glutamate in the presence of 10 μ M glycine (Figure 6A). The EC_{50} value of 1.2 μ M in buffer not containing Mg^{2+} corresponds to published results. Oocytes not injected with the NMDA receptor subunits did not respond to stimulation. Cells expressing the GluN1 subunit only responded with small currents and were not further analysed. For the following studies, currents were elicited by 10 μ M glycine and 10 μ M glutamate in GluN1/GluN2A expressing oocytes. Protocols were identical to the studies on the P/Q channel; initial measurement of current was followed by addition of A β preparation or MK-801, 10 min incubation and subsequent measurement of a second current in the presence of the substance. The dimeric receptor was inhibited by MK-801 in a concentration-dependent manner (Figure 6B). Incubation of 1, 5 or 10 μ M MK-801 for 10 min resulted in significant block of the receptor current from $108 \pm 4\%$ ($n = 7$) in NFR control to $48 \pm 6\%$ (1 μ M; $n = 3$), $8 \pm 1\%$ (5 μ M; $n = 3$) and $4 \pm 1\%$ (10 μ M; $n = 3$). We concluded that the NMDA receptor is functionally expressed in the oocytes and is appropriately inhibited by a selective ligand. Typical currents to study the effect of buffer (Figure 6C; trace a: control conditions, trace b: second current following addition of buffer) and A β globulomer (Figure 6D; trace a: control conditions, trace b: second current following A β globulomer stimulation) demonstrate that in contrast to our findings on the P/Q channel, A β globulomer did not influence the current of the NMDA receptor under our experimental conditions. Both, the second stimulations with buffer ($107 \pm 9\%$, $n = 5$) and with A β globulomer ($107 \pm 1\%$, $n = 4$) were identical (Figure 6E). In addition we tested a potential effect of equimolar A β monomer concentrations on the NMDA current. As demonstrated in Figure 6E, 2.4 μ M A β monomer did not change the NMDA current ($105 \pm 4\%$, $n = 3$).

Discussion and conclusions

This study is the first demonstration of a direct modulation of a recombinant voltage-gated ion channel by A β oligomers. Although previous publications indicated an involvement of ion channels in the pathology of A β (Kelly and Ferreira, 2006; Shankar *et al.*, 2007; Nimmrich *et al.*, 2008), here we provide evidence that A β oligomers directly modulate the pore-forming subunit of a specific voltage-gated calcium channel, the P/Q-type calcium channel.

Oligomeric forms of A β are thought to cause synaptic impairment, and this has been suggested to underlie cogni-

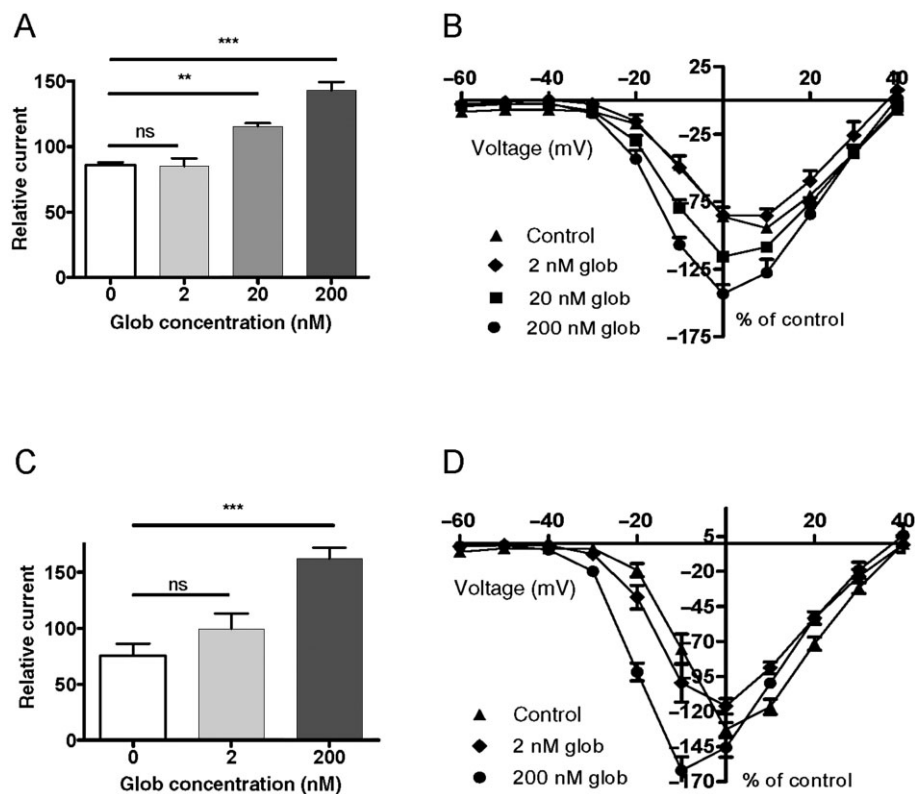


Figure 4

The A β -globulomer-induced change of current through the calcium channel is dose-dependent. (A) Relative current at 0 mV, normalized to the maximal current of the first I/V curve of each oocyte expressing the α 1A subunit. Columns represent the normalized current of the second I/V curve from cells stimulated with control buffer or increasing concentrations of A β globulomer (glob). (B) Current-voltage relationship of the same oocytes shown in (A); $n = 9, 5, 4$ and 7 for the different treatment options. (C) Relative current at -10 mV, normalized to the maximal current of the first I/V curve for each oocyte expressing the tripartite channel. Columns represent the normalized current of the second I/V curve from cells stimulated with control buffer or increasing concentrations of A β globulomer. (D) Current-voltage relationship of the same oocytes; $n = 5, 6$ and 5 for the different treatment options. $**P < 0.01$; $***P < 0.001$ (one-way ANOVA with Dunnett's multiple comparison test).

tive deficits in AD (Haass and Selkoe, 2007). For example, A β oligomers interfere with long-term potentiation (LTP), which has ultimately been linked to cognitive deficits (Walsh and Selkoe, 2004). Moreover, prolonged exposure to A β oligomers may lead to retraction of synaptic processes and possibly neuronal cell death via excitotoxic pathways (Harkany *et al.*, 2000; Molnár *et al.*, 2004). As such, neurodegeneration in AD may resemble a two-step process in which oligomer-induced modulations of synapse physiology precede morphological and perhaps irreversible cellular modifications (Walsh and Selkoe, 2004). Since it became apparent that soluble oligomeric forms of A β are associated with dementia, the pathophysiological consequences of A β oligomer exposure to neurons have been thoroughly investigated. Yet, detailed analyses of the molecular cascade initiated by oligomeric A β are sparse, and the exact mode of action remains largely undefined. However, the precise understanding of the molecular mechanism of A β toxicity is pressing, as only knowledge about the nature of the molecular target will open novel avenues for the development of new therapeutics.

Here, we provide evidence that the α 1A subunit of the P/Q-type calcium channel is directly modulated by A β oligomers. For this study, we used an oligomeric form, which on

one hand has been validated by its neuropathological features, and on the other hand, is stable in a buffer that is otherwise suitable for patch clamp experiments. A β globulomer is a synthetic A β dodecapeptide, which has been validated by several studies; it binds to primary hippocampal neurons and blocks LTP in hippocampal slices (Barghorn *et al.*, 2005). Using monoclonal antibodies directed against A β globulomer, the epitope has also been found in brain of AD patients and APP-overexpressing mice (Barghorn *et al.*, 2005). We recently reported that treatment of APP-overexpressing mice with an antibody directed against the globulomer epitope reverses A β pathology and normalizes cognitive deficits (Hillen *et al.*, 2010). We therefore believe that the synthetically generated A β oligomer used in the study presented here – A β globulomer – is a good surrogate for toxic A β oligomer species occurring in AD brain.

A β oligomers specifically modulate the α 1A subunit of the recombinant P/Q-type calcium channel

Recently, there were several independent observations that oligomers modulate the presynaptic vesicle release machinery. Kelly and Ferreira (2007), for example, reported depletion

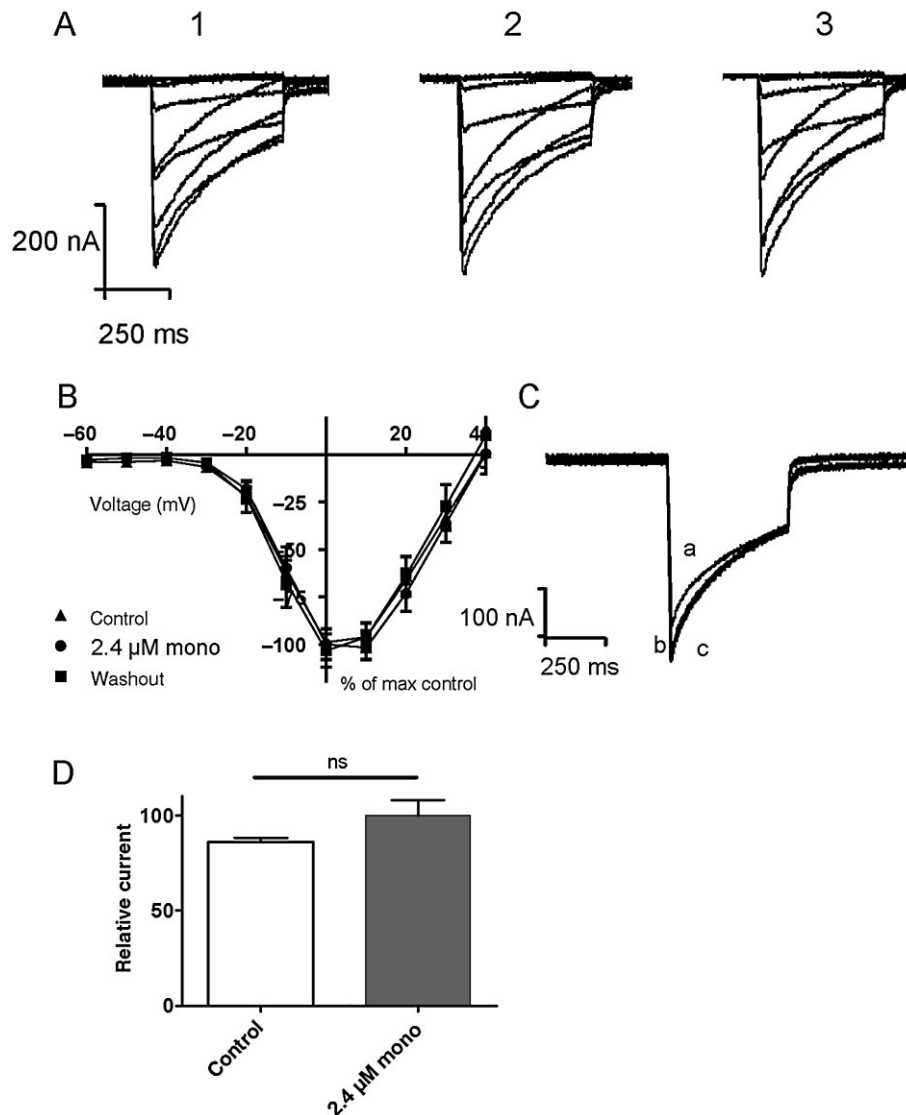


Figure 5

A β monomer does not influence the current-voltage relationship of the P/Q channel. (A) An example of superimposed current recordings of a cell expressing the $\alpha 1A$ subunit of the P/Q channel. 1, first measurement without stimulation; 2, measurement after 10 min stimulation with 2.4 μM monomer solution; 3, measurement after an additional 10 min washout period. (B) Current-voltage relationship of the cells treated as in (A); $n = 5$ for all conditions. (C) An example of superimposed currents at 0 mV; a, initial current without treatment; b, current after 10 min incubation with A β globulomer; c, washout after 10 min. (D) Relative current at 0 mV, normalized to the maximal current of the first I/V curve of each oocyte expressing the $\alpha 1A$ subunit. Columns represent the normalized current of the second I/V curve from cells stimulated with control buffer or A β monomer. The equimolar monomer preparation (compared with previous A β globulomer) did not significantly influence the peak channel current (t -test).

of the readily releasable pool of synaptic vesicles after application of A β oligomers. We recently suggested that A β oligomers modulate P/Q-type calcium currents in cultured hippocampal neurons. As a result, spontaneous synaptic vesicle release was impaired at both inhibitory and excitatory synapses (Nimmrich *et al.*, 2008). Encouraged by this finding, we now analysed this effect in more detail using a recombinantly expressed P/Q-type channel as a model and prove a direct effect of A β oligomers on the $\alpha 1A$ pore-forming subunit of the channel. Accessory subunits do not appear to be involved. This may explain the specificity of the effect for the

P/Q-type channel, as voltage-gated calcium channels only differ in their amino acid sequence of the pore-forming subunit. In contrast, molecules binding to one of the accessory subunits – like gabapentin – are therefore not specific for a particular voltage-gated ion channel (Sutton *et al.*, 2002; Sills, 2006).

Interestingly, in the present study we demonstrated an A β oligomer-induced increase of the channel current and a leftward shift of the activation kinetics of the channel. This is in contrast to our previous finding in hippocampal neurons, which indicated a current reduction under treatment with A β

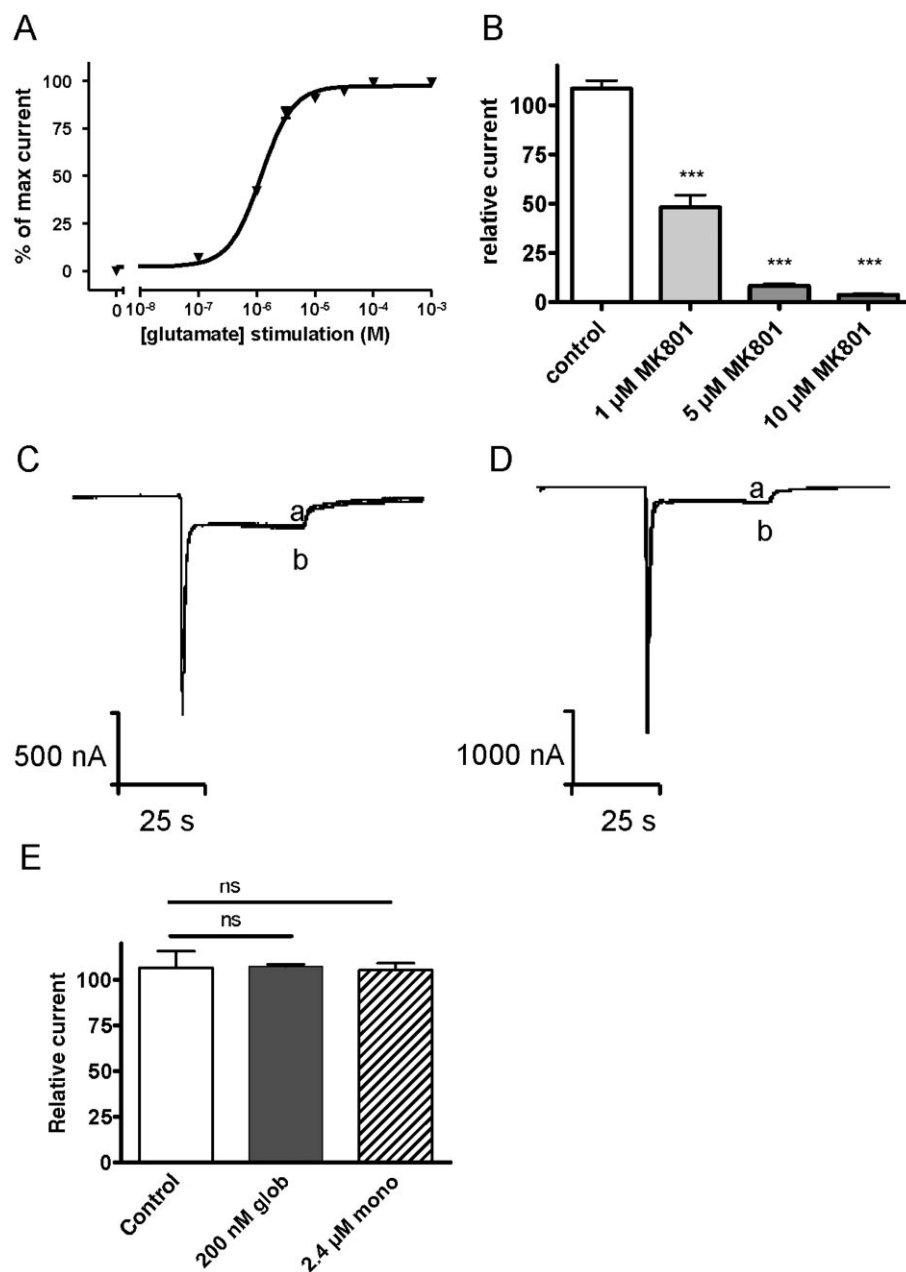


Figure 6

Characterization of NMDA receptors (GluN1/GluN2A) expressed in *Xenopus* oocytes. (A) Concentration-response curve of glutamate in the presence of 10 μ M glycine. (B) Inhibition of the NMDA-mediated current with MK-801. Columns represent averaged plateau currents normalized to the first (control) current after incubation with control buffer ($n = 7$) or increasing concentrations of MK-801 (1, 5 and 10 μ M; $n = 3$ /group). Significant inhibition was observed at all concentrations tested ($P < 0.0001$; one-way ANOVA with Dunnett's multiple comparison test). (C) Examples of currents of the NMDA receptor induced by glutamate and glycine (10 μ M each); a: initial current, b: current after 10 min incubation with control buffer. (D) Examples of currents of the NMDA receptor induced by glutamate and glycine (10 μ M each); a: initial current, b: current after 10 min incubation with 200 nM A β globulomer. (E) Neither A β monomer nor A β globulomer (glob) influenced the glutamate-induced plateau current of NMDA receptors. Columns represent plateau currents normalized to the first (control) current (a in C and D). No statistical differences were found among the groups (one-way ANOVA).

oligomers (Nimmrich *et al.*, 2008). We do not have a full explanation for this contrasting observation. Yet, a bidirectional regulation of ion channels by modulators is well known. Koch and colleagues, for example, showed that the potassium channel blocker κ -conotoxin PVIIA enhances and

reduces the potassium current of the *shaker* K⁺ channel depending on its activation state (Koch *et al.*, 2004). The P/Q-type channel modulator R-roscovitine exhibits both agonist and antagonist effects on P/Q-type currents, which is determined by concentration and molecular conformation

(Buraei and Elmslie, 2008). The L-type calcium channel agonist Bay K 8644 enhances or suppresses cardiac calcium currents depending on the holding potential (Kass, 1987; Schreibmayer *et al.*, 1992). Such paradoxical regulation has also been shown for other dihydropyridines (Wei *et al.*, 1986). These studies indicate that subtle differences in compound-channel interaction may lead to opposite effects on current flow. As ion channels are movable targets that exist in different conformations depending on the activation state, slight differences in holding potential may effect the interaction with molecules that bind to the channel. Although our experiments allowed proper voltage control, we are unaware whether the channel requires the exact same membrane potential for a given activation state in different cellular systems. A bidirectional regulation by A β oligomers has indeed been observed in previous studies, which show both an oligomer-induced increase and decrease of the frequency of mEPSCs, indicating contrary regulation in vesicle release (Shankar *et al.*, 2007; Abramov *et al.*, 2009). This could be a direct consequence of a P/Q channel modulation, as shown by Nimmrich *et al.* 2008. On a systems level, an A β oligomer-induced bidirectional regulation of LTP has been observed by Puzzo *et al.*, 2008.

It is thought that fine tuning of ion channel kinetics is mediated by numerous factors in the microenvironment of the channel (Evans and Zamponi, 2006; Tedford and Zamponi, 2006), which may also lead to varying channel kinetics in different systems. Furthermore, differences in modulation may be brought about by genetic variations between the channels expressed in different systems. So far, the cDNA for hippocampal P/Q channels has not been identified. At least two splice variants for the P/Q-type channel exist (Tsunemi *et al.*, 2002), and there are numerous loci of variation identified by transcript scanning (Soong *et al.*, 2002). The exact composition of the P/Q-type channel variants in different brain cells has not been fully examined, and to date, it is not clear which exact splice variants and accessory subunits exist in hippocampal neurons.

While the effect described here differs from the one observed in pharmacologically isolated currents in cultured neurons, we provide further evidence that the P/Q channel is a direct target of an AD-relevant toxic β -amyloid species. Furthermore, we identified the pore-forming α 1A subunit as the affected part of the channel. The current is increased over a wide range of concentrations, excluding the possibility that the mode of modulation is dependent on the concentration. It will be an exciting topic for future studies to examine the kinetics of A β oligomer-induced calcium channel modulation in intact brain tissue.

A β oligomers do not modulate glutamate-induced NMDA currents

Several studies have indicated that the NMDA receptor is affected by oligomeric A β preparations (Kelly and Ferreira, 2006; Shankar *et al.*, 2007). Texido *et al.* (2011) recently showed a direct effect of A β oligomers on NMDA currents generated by activation of the heterodimeric GluN1/GluN2A complex, but found less of an effect of oligomers when applied to receptors composed of GluN1/GluN2B subunits. We therefore analysed whether the globulomer would also modulate glycine-induced NMDA currents generated by

GluN1/GluN2A. Our data do not support a direct modulation of NMDA receptor currents by A β oligomers. At least for the experiments using longer incubation times, it is possible that NMDA receptor currents are modified further downstream in an A β -induced signalling cascade. Also, an oligomer-induced increase of glutamate release may well be neutralized by a concurrent NMDA receptor block, thereby preventing postsynaptic pathological cascades.

Ittner *et al.* (2010) recently suggested that the NMDA receptor containing the GluN2B subunit is involved in A β -induced excitotoxicity. Here, we cannot exclude the possibility of an effect of A β globulomer on other NMDA receptor subtypes.

A β monomer does not affect P/Q-type calcium currents

It is now generally accepted that oligomerization is necessary for conversion of A β peptides into neuropathogenic forms. Monomeric A β does not impair synapse physiology in a number of *in vitro* (Trommer *et al.*, 2005; Walsh *et al.*, 2005; Shankar *et al.*, 2007) and *in vivo* (Walsh *et al.*, 2002) studies, although oligomers are highly detrimental under those conditions. Cognitive function is also completely inert to A β monomers when injected into rat brains, whereas oligomers specifically impair cognitive function (Cleary *et al.*, 2005). In line with those reports, we also do not find an effect of A β monomers on P/Q-type calcium currents, indicating that oligomerization is a requirement for toxicity.

Conclusions

We conclude that A β oligomers directly and specifically modulate the pore-forming unit of recombinant P/Q-type calcium channels. An increase of P/Q channel activity ultimately leads to a larger proportion of released neurotransmitter, which offers a possible explanation for the observed excitotoxicity in AD brain. Besides the discovery of this specific regulation, we introduce a heterologous system that may serve for electrophysiological characterization of new therapeutics. Future studies need to address whether modulation of P/Q-type calcium currents or the prevention of the oligomer-channel interaction can ameliorate synaptic deficits *in vitro* and possibly improve cognitive impairment *in vivo*.

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Conflict of interest

The authors state no conflict of interest.

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