Video Article

Preparation of Oligomeric β-amyloid$_{1-42}$ and Induction of Synaptic Plasticity Impairment on Hippocampal Slices

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Abstract

Impairment of synaptic connections is likely to underlie the subtle amnesic changes occurring at the early stages of Alzheimer s Disease (AD). β-amyloid (Aβ), a peptide produced in high amounts in AD, is known to reduce Long-Term Potentiation (LTP), a cellular correlate of learning and memory. Indeed, LTP impairment caused by Aβ is a useful experimental paradigm for studying synaptic dysfunctions in AD models and for screening drugs capable of mitigating or reverting such synaptic impairments. Studies have shown that Aβ produces the LTP disruption preferentially via its oligomeric form. Here we provide a detailed protocol for impairing LTP by perfusion of oligomerized synthetic Aβ1-42 peptide onto acute hippocampal slices. In this video, we outline a step-by-step procedure for the preparation of oligomeric Aβ$_{1-42}$. Then, we follow an individual experiment in which LTP is reduced in hippocampal slices exposed to oligomerized Aβ$_{1-42}$ compared to slices in a control experiment where no Aβ$_{1-42}$ exposure had occurred.

Video Link

The video component of this article can be found at https://www.jove.com/video/1884/

Protocol

Resuspending β-amyloid peptide

Before getting started have ready:

- Aβ$_{1-42}$ (lyophilized powder)
- 1,1,1,3,3,3-Hexafluoro-2-Propanol (HFIP)
- Low-binding polypropylene microcentrifuge tubes (1.5 ml)
- GasTight Hamilton syringe with Teflon plunge stop (250 µl)
- Dimethylsulfoxide (DMSO)

1. Allow lyophilized Aβ$_{1-42}$ to equilibrate at room temperature for 30 minutes to avoid condensation upon opening the peptide vial.
2. Under the fume hood, re-suspend Aβ$_{1-42}$ peptide in ice cold HFIP to obtain a 1 mM solution and vortex the solution for a few seconds.
3. Using a glass GasTight Hamilton syringe with Teflon plug, quickly divide the Aβ$_{1-42}$/HFIP solution equally into three polypropylene vials and seal the vials.
4. The vials are then incubated for 2 hours to allow for Aβ monomerization. Next, open the vials and concentrate the Aβ$_{1-42}$/HFIP solution under vacuum by using a SpeedVac centrifuge (800 g, room temperature) until a clear peptide film is observed at the bottom of the vials. Check carefully the temperature inside the SpeedVac centrifuge to avoid peptide degradation (max 25°C).
5. Seal the vials and store the aliquots at -80°C. Aβ film can be stored for 6 months.
6. Re-suspend one film by adding DMSO to obtain a concentration up to 5 mM Aβ$_{1-42}$. Sonicate in the water bath for 10 minutes to ensure complete re-suspension.
7. Aliquot this 5mM Aβ$_{1-42}$ solution into polypropylene vials. Seal all the vials and store them at -20°C. Aliquots should be thawed only once. Beware of water condensation inside the freezer. Peptides in solution degrade very easily.

Oligomerization

Before getting started have ready:

- Aβ$_{1-42}$/DMSO (5mM)
- Sterile phosphate buffer
- Low-binding polypropylene microcentrifuge tubes (1.5 ml)

- Low-binding polypropylene centrifuge tubes (50 ml)

1. Dilute the obtained 5mM $A\beta_{1-42}$/DMSO aliquot with sterile phosphate buffer (up to 100 µl).
2. Vortex for 30 seconds and then incubate for 12 hours at 4°C

Hippocampal slice treatment

When treating hippocampal slices, it is important to avoid non-specific peptide adhesion onto beakers, perfusion tubing surfaces, and the recording chamber. Use preferentially low-binding polypropylene tubing and containers. Avoid the use of glassware or generic plastic-ware. Perfusion media should be serum- or albumin-free.

Aβ perfusion

Before getting started have ready:

- Oligomerized $A\beta_{1-42}$
- Recording buffer (in mM: 124 NaCl, 4.4 KCl, 1 Na_2HPO_4, 25 $NaHCO_3$, 2 $CaCl_2$, 2 $MgCl_2$, and 10 glucose)
- Electrophysiological recording set-up with interface chamber
- Transverse hippocampal slices incubated in oxygenated recording buffer for at least 90 minutes post-dissection (T = 29° C; constant perfusion rate = 2 ml/minute)

1. Immediately prior to the experiment, dilute the oligomerized $A\beta_{1-42}$ aliquot to the appropriate working concentration (200 nM or higher) in a polypropylene tube with pre-oxygenated recording buffer.
2. Perfuse the $A\beta_{1-42}$ solution for 20 minutes to the slices before the induction of synaptic plasticity.

Induction of plasticity and follow-up

1. Typically, the most widely-used model of synaptic plasticity is the LTP. It can be induced through tetanic stimulation of a given synapse in the hippocampus. We record field responses from synapses between the Shaffer's collateral projections and CA1 pyramidal neurons in the stratum radiatum. Our tetanic stimulation consists of a theta burst stimulation which includes 3 trains separated by 15 second intervals. Each train consists of 10 bursts at 5 Hz where each burst entails 5 pulses at 100 Hz. Apply the tetanic stimulation immediately after the perfusion of oligomerized Aβ has been completed.
2. Immediately after the tetanic stimulation switch back to perfusion with normal recording buffer. Maintain a constant perfusion rate, recording chamber temperature and proper oxygenation throughout the duration of the experiment.

Representative Results and possible problems

The Aβ oligomerized according to the classical protocol of Stine et coll.[1], generates Aβ monomers and a variety of oligomers of different sizes (dimer, trimer, etc.) The perfusion of oligomerized $A\beta_{1-42}$ leads to decreased LTP in Aβ-treated slices compared to control slices. Under our experimental conditions, where 3-5 month old C57BL/6J male mice are used, LTP in 200 nM Aβ-treated slices is on average 150% of the baseline values at two hours after tetanic stimulation, while in control slices LTP values are on average 200-250% of baseline2. In some cases, treatment with Aβ may fail to reduce hippocampal LTP. Several critical errors that might occur include excessive film drying during Aβ concentration and incomplete oligomerization. Aβ perfusion in hippocampal slices might be another source of concern because of the physicochemical properties of the Aβ peptide in solution, which is prone to non-specific adhesion to plastics. Troubleshooting of these issues is discussed below.

Discussion

As described above, several problems in the preparation of oligomeric Aβ may result in unimpaired LTP. One way to evaluate whether Aβ degradation or the lack of Aβ oligomerization may have occurred is to evaluate the Aβ preparation using TRIS-Tricine PAGE/Western Blotting analysis and analytical ultracentrifugation (not described in this protocol). When Western Blotting samples are prepared under non-denaturing/non-reducing conditions, a successful preparation should generate a Western Blotting signature featuring bands corresponding to monomers and different oligomers[3]. An often occurring issue is the peptide instability during the drying step in the SpeedVac concentrator. In fact, high-temperature drying leads to protein degradation, noticeable by marked peptide coloration to brown. Concentration of the HFIP solution has to be carried out at room temperature and monitored carefully. Given the several procedural steps occurring in the protocol, errors in dilution may accidentally lead to poor oligomerization or misleading Aβ concentration. Interestingly, the perfusion with Aβ concentrations lower than suggested would result in increased LTP values[3]. Again, perfusion affects the possible outcome of Aβ treatment on slices. Indeed, maintaining a constant temperature of the bath solution in our electrophysiological experiments is critical as temperature is known to affect Aβ conformations when solved in phosphate buffer. Another source of concern is that oligomeric Aβ may non-specifically stick to plastic surfaces of containers and tubing used for preparation and perfusion thereby reducing the effective concentration exposed to hippocampal slices. Thus, it is critical to use low-protein binding plastic-ware to ensure reliable preparations and perfusions throughout different experiments. In this issue, we propose polypropylene plastic-ware which has been shown not to affect the ratio of different Aβ species[4]. Extensive literature suggests that Alzheimer's Disease originates as a synaptic disorder[5]. It is likely that the subtlety and variability of the earliest amnesic symptoms, occurring in the absence of any other clinical signs of brain injury, are due to discrete changes in the function of a single synapse, produced at least in part, by Aβ species (e.g. $A\beta_{42}$ and $A\beta_{40}$)[2,6,7,8]. The discovery that a preparation containing oligomerized synthetic $A\beta_{1-42}$ can impair LTP[9] *in vitro* has led to numerous investigations into mechanisms of synaptic dysfunction induced by Aβ elevation in brain. Using this protocol, we describe a procedure for preparing oligomeric Aβ which successfully impairs LTP at the Shaffer collateral-stratum radiatum connection in hippocampal slices. This

experimental paradigm has tremendous value for investigating mechanisms of Aβ-mediated pathogenesis as well as testing potential drugs, which may mitigate synaptic dysfunction[2,10,11].

Disclosures

Experiments on animals were performed in accordance with the guidelines and regulations set forth by the Columbia University IACUC.

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