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Pentapeptides derived from Aβ1–42 protect neurons from the modulatory effect of Aβ fibrils—an in vitro and in vivo electrophysiological study

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Short fragments and fragment analogues of beta-amyloid 1–42 peptide (Aβ1–42) display a protective effect against Aβ-mediated neurotoxicity. After consideration of our earlier results with in vitro bioassay of synthetic Aβ-recognition peptides and toxic fibrillar amyloids, five pentapeptides were selected as putative neuroprotective agents: Phe-Arg-His-Asp-Ser amide (Aβ4–8) and Gly-Arg-His-Asp-Ser amide (an analogue of Aβ4–8), Leu-Pro-Tyr-Phe-Asp amide (an analogue of Aβ17–21), Arg-Ile-Ile-Gly-Leu amide (an analogue of Aβ30–34), and Arg-Val-Val-Ile-Ala amide (an analogue of Aβ38–42). In vitro electrophysiological experiments on rat brain slices demonstrated that four of these peptides counteracted with the field excitatory postsynaptic potential-attenuating effect of Aβ1–42; only Arg-Val-Val-Ile-Ala amide proved inactive. In in vivo experiments using extracellular single-unit recordings combined with iontophoresis, all these pentapeptides except Arg-Val-Val-Ile-Ala amide protected neurons from the NMDA response-enhancing effect of Aβ1–42 in the hippocampal CA1 region. These results suggest that Aβ recognition sequences may serve as leads for the design of novel neuroprotective compounds.

Keywords: Alzheimer's disease; β-amyloid 1–42; Neuroprotective pentapeptides; Electrophysiology; Microiontophoresis; Single-unit activity; Hippocampus; Transmission electron microscopy; EPSP; Primary motor cortex

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, which is characterized neuropathologically by amyloid plaques formed mainly by the β-amyloid peptides Aβ1–40 and Aβ1–42, neurofibrillary tangles (abnormally twisted forms of the tau protein), a basal forebrain cholinergic deficit, and extensive neuronal loss together with synaptic changes in the cortex and hippocampus (Selkoe, 2001). Aβ1–42 has neurotoxic effects (Bayer et al., 2001; Dahlgren et al., 2002; Datki et al., 2003; Lambert et al., 1994; Wie et al., 2000) and induces axonopathy (Higuchi et al., 2002; Terwel et al., 2002). Aggregated Aβ1–42 has been shown to exert modulatory effects on ion channel currents in neurons by disrupting the intracellular ion homeostasis (Bores et al., 1998; Yu et al., 1998) and enhancing the NMDA receptor-mediated response (Molnar et al., 2004), which seems to play a central role in the early steps of neurodegeneration in AD (Cowburn et al., 1997). Additionally, hippocampal excitatory postsynaptic potentials (EPSPs) and long-term potentiation (LTP) are modified both in vivo and in vitro (Chen et al., 2000; Klyubin et al., 2004; Larson et al., 1999; Saleshando and O'Connor, 2000) by Aβ1–42.

The toxicity of the Aβ peptides proved to correlate well with their aggregation properties (Pike et al., 1993), and it is the fibrillar form of the Aβ peptides that interacts with cell membrane receptors (reviewed by Verdier and Penke, 2004; Verdier et al., 2004). In several studies (Soto, 1999; Tjernberg et al., 1996), Aβ1–42 has been used as a therapeutic target for drug design. One of these approaches led to the discovery of peptide-like inhibitors of Aβ1–42 aggregation (beta-sheet breakers, BSBs; Soto, 1999; Soto et al., 1996, 1998; Tjernberg et al., 1996; reviewed by Findeis, 2000). Inhibition and/or reversion of Aβ self-aggregation may provide a good treatment of the underlying cause of AD (Talaga, 2001). The affinity-based inhibition of Aβ aggregation and toxicity was recently applied in the design of Aβ-recognition sequences (Cairo et al., 2002; Pallitto et al., 1999).

Our present aim was to find novel peptide fragments and analogues that inhibit the neuromodulatory effects of fibrillar Aβ1–42. For the identification of peptide recognition sites, we have

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designed different pentapeptides spanning the entire Aβ1–42 sequence. Peptide design was supported by theoretical considerations and our former results. We earlier found that Aβ31–35 (Ile-Ile-Gly-Leu-Met; IIGLM) is neurotoxic (Penke et al., 1994), however, the tetrapeptide propionyl-Ile-Ile-Gly-Leu amide (Pr-IIGLa) protects glial and neuronal cells from the toxic effect of Aβ1–42 (Laskay et al., 1997). Similarly, the C-terminal pentapeptide of Aβ1–42 (Gly-Val-Val-Ile-Ala amide, GVVIAa), which is critical for Aβ aggregation (Jarrett et al., 1993), interferes with Aβ toxicity. In a neuronal viability test, this pentapeptide proved to be protective (Hetenyi et al., 2002). Aβ4-8 (Phe-Arg-Gly-Asp-Ser, FRGDS) is a possible ligand for integrin-like receptors (Lorenzo et al., 2000; Sabo et al., 1995) and we presume that this Aβ fragment and its analogues might serve as inhibitors for the Aβ-integrin interactions.

On the basis of previous results, novel compounds of four families of pentapeptides (analogues of Aβ4–8, 17–21, 30–34, and 38–42) were synthetized. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test, the most common and simple cell viability assay (Schiff et al., 1985) was used in our experiments for screening of the neuroprotective effect of several hundreds of new compounds. Five pentapeptides were selected as putative neuroprotective agents against fibrillar Aβ neurotoxicity. Two of them (Phe/Gly-Arg-His-Asp-Ser amide, FRHDSa and GRHDSa) are integrin ligand analogues; Leu-Pro-Tyr-Phe-Asp amide (LPYFDa; Datki et al., 2004) is a slightly modified analogue of Soto's Leu-Pro-Phe-Phe-Asp (LPFFD). Arg-Ile-Ile-Gly-Leu amide (RIIGLa) and Arg-Val-Val-Ile-Ala amide (RVVIAa) are water-soluble analogues of the peptides that we earlier prepared and studied (propionyl-Ile-Ile-Gly-Leu amide and Gly-Val-Val-Ile-Ala amide). The fibrillar structure of the applied Aβ1–42 was verified by transmission electron microscopy. The selected pentapeptides were tested against the Aβ1–42 mediated neuromodulation by using in vitro and in vivo electrophysiological methods.

Materials and methods

Peptide synthesis

Aβ1–42 and the pentapeptides were synthetized in our laboratory by a solid-phase procedure involving the use of Wang-resin and Fmoc chemistry. The details of the synthesis process will be published elsewhere. The synthetic peptides were purified on a C-4 RP-HPLC column with an acetonitrile gradient; pure fractions were pooled and lyophilized. Aβ1–42 was repeatedly lyophilized from aqueous solution resulting in protofibrils and fibrils. Purity control and proof of structure were achieved by amino acid analysis and mass spectrometry (ESI MS, FinniganMat TSQ 7000).

Transmission electron microscopy (TEM)

A 0.5 mg/ml solution of Aβ1–42 was prepared by dissolving the peptide in distilled water with constant pipetting for 2 min, followed by a sonication for 10 min. Electron micrographs were taken after incubation for 20 h at 37°C. A 10 μl volume of sample was adsorbed on 400-mesh carbon-coated copper grids (Electron Microscopy Sciences, Washington, PA) and fixed with 0.5% (v/v) glutaraldehyde solution. Specimens were stained with 2% (w/v) uranyl acetate and studied by using a Philips CM 10 transmission electron microscope at 100 kV routinely at magnifications of ×46,000 and ×64,000.

MTT assay for cell viability

The standard method of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay modified by Datki et al. (2003) was used to measure the protective effect of the pentapeptides, using differentiated SH-SY5Y cells. The amount of formazan formed during the reduction of MTT was measured by spectrophotometry.

Animals

Male Wistar rats were housed individually and had free access to food and water. All efforts were made to minimize animal suffering. The principles of laboratory animal care (NIH publication No. 85-23) and the protocol for animal care approved by the Hungarian Health Committee (1998) and the European Communities Council Directive of 24 November 1986 (86/609/EEC) were followed.

In vitro electrophysiology

Sixty-four young (postnatal days 20–30) rats were decapitated, and coronal slices (400 μm) were prepared from their primary motor cortices with a vibratome (Campden Instruments) in a solution composed of (in mM): 130 NaCl, 3.5 KCl, 1 NaH_2PO_4, 24 $NaHCO_3$, 1 $CaCl_2$, 3 $MgSO_4$, and 10 D-glucose (all from Sigma, Germany), saturated with 95% O_2 and 5% CO_2. The slices were then transferred to a Haas recording chamber and incubated at room temperature for 1 h in the solution used for recording (differing only in that it contained 3 mM $CaCl_2$ and 1.5 mM $MgSO_4$). The flow rate was 1 ml/min. To stimulate the layer II/III horizontal pathways (constant current, 0.2 ms pulses delivered at 0.033 Hz), a bipolar stainless-steel microelectrode (FHC, USA) was positioned approximately 350 μm below the surface and 2 mm from the midline. The stimulus intensity was adjusted between 20 and 70 μA to evoke the half-maximum response. Glass micropipettes used for recordings were filled with artificial cerebrospinal fluid (ACSF) and were randomly broken off under microscopic control so that the impedance was between 1.0 and 1.5 MΩ. The recordings were carried out at the same depth, 300–500 μm from the stimulation at ~34°C (Hess and Donoghue, 1999).

Application of Aβ1–42 and the putative neuroprotective pentapeptides

For each pentapeptide, three different solutions were made in ACSF: (a) Aβ1–42 (10^{-5} M), (b) a freshly prepared mixture of Aβ1–42 (10^{-5} M) plus one of the following pentapeptides (Leu-Pro-Tyr-Phe-Asp amide, Arg-Val-Val-Ile-Ala amide, Arg-Ile-Ile-Gly-Leu amide, or Gly-Arg-His-Asp-Ser amide, 10^{-4} M), and (c) the pentapeptide by itself (10^{-4} M). 15 μl solution was applied in the close vicinity of the recording electrode with a micromanipulator equipped with a Hamilton syringe. In control experiments, the same amount of ACSF was administered (which never altered the electrophysiological activity). The data were amplified (SEC-LX05, npi, Germany), filtered (1 Hz–3 kHz), acquired at a 10 kHz sampling rate on a pClamp8 system and Digidata 1320 A/D board (Axon Instruments, USA), and