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Hormetic effect of amyloid-beta peptide in synaptic plasticity and memory

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Abstract

One of the hot topics in Alzheimer's disease research field is the "amyloid hypothesis" postulating that the increase and deposition of beta-amyloid peptides (Aβ) is the main pathogenetic factor. However, antiamyloid-based therapies have so far been a failure and, most importantly, growing evidences suggest that Aβ has important physiologic functions. Based on our previous findings demonstrating that low concentrations of Aβ enhanced both synaptic plasticity and memory, whereas high concentrations induced the well-known impairment of cognition, here we show that Aβ acts on hippocampal long-term potentiation and reference memory drawing biphasic dose-response curves. This phenomenon, characterized by low-dose stimulation and high-dose inhibition and represented by a U-shaped or inverted-U-shaped curve, resembles the characteristics of hormesis. The Aβ double role raises important issues on the use of Aβ level reducing agents in Alzheimer's disease.

Keywords: Amyloid-beta; Hormesis; Alzheimer's disease; Synaptic plasticity; Memory; Hippocampus; Low doses

1. Introduction

The term hormesis refers to a biphasic dose-response phenomenon characterized by low-dose stimulation and high-dose inhibition represented by a bell-shaped, J-shaped, U-shaped or inverted-U-shaped curve, depending on the parameter measured (Calabrese and Baldwin, 2001, 2002). This phenomenon was shown for the first time by Hugo Schultz, over a century ago (Kendig et al., 2010), who noticed that some chemicals stimulated or inhibited yeast growth and respiration depending upon the applied doses. The general concept of low-dose-stimulation versus high-dose-inhibition was then known as the Arndt-Schulz' law but, given the poor knowledge of its scientific bases, the growing fame of classical toxicology, and the association with homeopathy, hormesis fell into disuse (Calabrese, 2009). Recently, however, hormesis has gained a renewed success and several studies have been published (for reviews see Calabrese, 2008a, 2008b, 2010). As pointed out by these studies, the definition of hormesis is not limited to the biphasic character of the response, but implies a specific adaptative biological effect of a substance on a variable acting by uni- or polymodal mechanisms and characterized by specific qualitative and quantitative features (Calabrese and Baldwin, 2002). Indeed, according to hormesis principles, biological systems are damaged by high doses of a stressor whereas the same substance, at low doses, is able to positively stimulate several physiologic functions from cell growth to cognition. Several, if not all, physiological molecules are likely to present a hormetic effect, exhibiting opposite effects at high or low concentrations. Few examples include: (1) glutamate, the principal excitatory neurotransmitter, which stimulates synaptic plasticity and memory at physiological low doses (Rezvani, 2006), whereas at higher doses becomes toxic and is involved in pathologies such as stroke (Wieloch, 1985; Choi, 1988) and Alzheimer's disease (AD) (Mattson, 2004, 2008a); (2) glucocorticoids, whose effect on memory might be described as

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an inverted-U shape curve (Lupien, 2005); (3) nitric oxide, the par excellence "Janus" molecule, which might be neurotoxic or neuroprotective (Calabrese, 2001b; Duncan and Heales 2005; Lowenstein et al. 1994; Mattson 2008b; Puzzo et al., 2006), and many others (Calabrese and Baldwin, 1998). Here, we focused on amyloid-beta (Aβ), a peptide widely known because it is produced in high amounts during AD. Aβ derives from the cleavage of amyloid-precursor protein (APP) that undergoes a complex proteolytic processing catalyzed by α-β- and γ-secretases generating several fragments (Chow et al., 2010; Mattson, 1997). The normal function of APP remains poorly understood, although some fragments might have physiological properties (Chow et al., 2010; Randall et al., 2010). Aβ, instead, is considered a toxic fragment (Hardy and Selkoe, 2002) causing synaptic dysfunction and memory impairment (Selkoe, 2002). However, the peptide is normally produced in the healthy brain and growing evidence indicates that it might have a physiologic function. Indeed, APP, and β- and γ-secretases have been shown to be involved in synaptic plasticity and memory (Dawson et al., 1999; Laird et al., 2005; Ma et al. 2007; Phinney et al., 1999; Saura et al., 2004; Seabrook et al., 1999; Wang et al., 2008) and Aβ itself, at picomolar concentrations, is likely to play a neurotrophic and neuroprotective role (Giuffrida et al., 2010; López-Toledano and Shelanski, 2004; Plant et al., 2003; Yankner et al., 1990). Interestingly, Cirrito et al. (2008) have demonstrated that synaptic transmission induces an increase of Aβ generation and release, and Kamenetz et al. (2003) suggested that endogenous Aβ might regulate synaptic plasticity with a feedback mechanism. Recently, we have demonstrated that low picomolar amounts of exogenously applied Aβ42 enhance synaptic plasticity in vitro and memory in vivo (Puzzo et al., 2008) and that endogenous Aβ is necessary for synaptic plasticity and memory (Puzzo et al., 2011). Here, we show that dose-response curves for the effect of Aβ on hippocampal synaptic plasticity and memory resemble the hormetic characteristics, raising several issues when designing effective and safe approaches to AD therapy.

2. Methods

2.1. Animals

We used 3–4 month-old wild type (WT) mice (C57BL/6), obtained from a breeding colony kept in the animal facility of the Department of Bio-Medical Sciences, Section of Physiology (University of Catania). The animals were maintained on a 12-hour light/dark cycle (with light onset at 6:00 A.M.) in temperature and humidity-controlled rooms, and food and water were available ad libitum.

2.2. Aβ preparation

Aβ42 was prepared as previously described (Puzzo et al., 2008). A peptide film was obtained suspending the peptide (American Peptide, Sunnyvale, CA, USA) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP; Sigma, St. Louis, MO, USA). Dimethylsulfoxide (DMSO; Sigma) was added and the compound was sonicated for 10 minutes, aliquoted, and stored at −20 °C. Twenty-four hours before the experiment, an aliquote of dimethylsulfoxide-Aβ was added, phosphate-buffered saline (PBS; 5 mM), vortexed for 30 seconds, and incubated at 4 °C. This peptide contained both monomers and oligomers, as demonstrated by routinely characterization using Western blot analysis (Puzzo et al., 2008). The following doses of peptides were prepared in artificial cerebrospinal fluid (ACSF) prior to the administration: 2 pM, 20 pM, 200 pM, 2 nM, 20 nM, 200 nM, 2 μM, and 20 μM. The final amounts of exogenous peptide injected in vivo into each hippocampus ranged from $9 \times 10^{(-15)}$ to $9 \times 10^{(-8)}$ grams.

2.3. Electrophysiological measurements

Electrophysiological recordings were performed as previously described (Puzzo et al., 2008). Briefly, transverse hippocampal slices (400 μm) were cut and maintained in a recording chamber at 29 °C, continuously bubbled with 95% O_2 and 5% CO_2 and perfused with artificial cerebrospinal fluid (composition in mM: 124.0 NaCl, 4.4 KCl, 1.0 Na2HPO4, 25.0 $NaHCO_3$, 2.0 $CaCl_2$, 2.0 $MgSO_4$, 10.0 glucose). Field excitatory postsynaptic potentials (fEPSP) were recorded by placing the stimulating electrode at the level of the Schaeffer collateral fibers and the recording electrode in the CA1 stratum radiatum. Basal synaptic transmission (BST) was assayed by plotting the stimulus voltages against slopes of fEPSP. For long-term potentiation (LTP) experiments, a 15-minute baseline was recorded every minutes at an intensity that evokes a response approximately 35% of the maximum evoked response. LTP was induced using θ-burst stimulation (4 pulses at 100 Hz, with the bursts repeated at 5 Hz and each tetanus including 3 ten-burst trains separated by 15 seconds). Responses were recorded for 2 hours after tetanus and measured as fEPSP slope expressed as percentage of baseline. Data were analyzed as residual potentiation given by the average among the last 5 recording points.

2.4. Infusion technique

Mice were implanted with a 26-gauge guide cannula into the dorsal part of the hippocampi (coordinates: Posterior = 2.46 mm, Lateral = 1.50 mm to a depth of 1.30 mm) (Paxinos, 1998). Cannulas were fixed to the skull with acrylic dental cement (3M ESPE AG, Seefeld, Germany). After a 6–8-day recovery, mice were handled once a day for 3 days before behavioral experiments. Then, 20 minutes before each test, they were bilaterally injected with different doses of Aβ42 or vehicle in a final volume of 1 μL over 1 minute through infusion cannulas that were connected to a microsyringe by a polyethylene tube. During infusion, animals were handled gently to minimize stress. After infusion,