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β-Amyloid peptide$_{25–35}$ depresses excitatory synaptic transmission in the rat basolateral amygdala "in vitro"

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Abstract

The effects of β-amyloid peptide$_{25–35}$ on resting membrane potential, spontaneous and evoked action potential and synaptic activity have been studied in basolateral amygdaloid complex on slices obtained from adult rats. Intracellular recordings reveal that perfusion with β-amyloid peptide$_{25–35}$ at concentrations of 400 nM and less did not generate any effect on resting membrane potential. However, concentrations in the range of 800–1200 nM produced an unpredictable effect, depolarization and/or hyperpolarization, which were blocked by tetrodotoxin or 6-cyano-7-nitroquinoxaline-2,3-dione + D-(−)-2-amino-5-phosphonopentanoic acid together with bicuculline. Excitatory and inhibitory evoked responses mediated by glutamic acid or γ-aminobutyric acid decreased in amplitude after β-amyloid peptide$_{25–35}$ perfusion. Additionally, results obtained using the paired-pulse protocol offer support for a presynaptic mode of action.

To determine which type of receptors and/or channels are involved in the presynaptic mechanism of action, a specific blocker of alpha-7 nicotinic receptors (methyllycaconitine citrate) or L-type calcium channel blockers (calcicludine or nifedipine) were used. β-amyloid petide$_{25–35}$ decreased excitatory postsynaptic potentials amplitude in control conditions and also in slices permanently perfused with methyllycaconitine citrate. However, this effect was blocked in slices perfused with calcicludine or nifedipine suggesting the involvement of the L-type calcium channels. On the whole, these experiments provide evidence that β-amyloid peptide$_{25–35}$ affects neurotransmission in basolateral amygdala and its action is mediated through L-type calcium channels.

Keywords: β-Amyloid peptide$_{25–35}$; Amygdala; Basolateral amygdaloid complex; Brain slices; Intracellular recordings; Rat

1. Introduction

According to Swanson [40], the basolateral amygdaloid complex is formed by the lateral, basolateral and basomedial nuclei. This complex receives afferents from cortex across the external capsule (EC) [9]. When stimulated, these connections generate an excitatory postsynaptic potential, which is blocked by D-AP5 and CNQX, specific blocking agents of the NMDA and non-NMDA receptors, respectively [26]. In this complex, GABA-ergic terminations originating from amygdaloid interneurons that terminate on pyramidal cells have also been described [6,23,25]. Moreover, the magnocellular division of the basal nucleus presents a high density of fibres positive to acetylcholinesterase [2]. The activation of these fibres brings about, in the pyramidal cells, depolarizations (EPSP) of long duration, which are blocked by atropine [44]. The muscarinic agonist carbachol mimicked this type of response in 90% of the amygdaloid pyramidal neurons. This effect was mediated by the closure of potassium channels [45] and/or opening of non-specific cation channels [47]. Also, other evidences advocate variable roles for some other neurotransmitters like serotonin [39], dopamine [3,30] and noradrenaline [11]. Consequently, the basolateral amyg-

Abbreviations: ABC, avidin–biotin–peroxidase complex; ACSF, artificial cerebrospinal fluid; AD, Alzheimer's disease; AMPA, α-amino-3-hydroxy-5-methyl-isoxazolepropionic acid; CNQX, 6-cyano-7-nitroquinoxalline-2,3-dione; D-AP5, D-(−)-2-amino-5-phosphonopentanoic acid; EC, external capsule; EPSP, excitatory postsynaptic potential; GABA, γ-aminobutyric acid; IPSP, inhibitory postsynaptic potential; NMDA, *N*-methyl-D-aspartic acid; PB, phosphate buffer; PPS, paired pulse stimulation; PPF, paired pulse facilitation; RMP, resting membrane potential; SEM, standard error of the mean; TTX, tetrodotoxin

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daloid complex is an important nucleus of neurochemical interaction.

There is abundant evidence, which points towards the fact that the accumulation of beta amyloid is the primary event in the pathogenesis of Alzheimer's disease (AD) [13,35]. The main pathological changes in AD take place in regions of the temporal lobe, including the hippocampus, enthorinal cortex, and parahippocampical gyrus. Among these brain regions, the number of senile plaques has been reported to be the highest in amygdala [34]. The harmful effects of this substance would be produced at a cellular level and, fundamentally, at a synaptic level; the loss of synapses is one of the first structural changes detected in Alzheimer's disease [4,8]. The special sensitivity of the nerve terminals and/or of the subsynaptic membrane to the β-amyloid peptide$_{25–35}$ peptide would be generated either by its union to the alpha-7 nicotinic receptors [41], in this case generating a decrease in the influx of Ca^{2+} and thus an inactivation of the presynaptic membrane [29,41], or by the direct action of the peptide on the metabolism of the calcium, mobilizing the intracellular calcium [38] or activating L-type calcium channels [32,36].

Although the neurotoxic effect of β-amyloid peptide$_{25–35}$ is widely documented [28], there are few data regarding its modulating effects in short term neurotransmission [5,17]. For this reason, the objective of the present work has been to study the effects of β-amyloid peptide$_{25–35}$ on the electrophysiological properties of amygdaloid cells and on synaptic transmission mediated through AMPA-Kainate and GABA receptors in basolateral amygdaloid complex.

2. Materials and methods

2.1. Preparation of slices

Amygdala slices were prepared for intracellular recording as [46]. Briefly, male or female Wistar rats (80–100 g), raised in the animal colony of the University of Salamanca, were deeply anesthetized with halothane gas and then decapitated. The brains were quickly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF) in which the NaCl (117 mM) was replaced by sucrose (234 mM) to maintain osmolarity. Coronal slices (400 μm thick) were cut using a vibratome, transferred to an incubation chamber, and incubated for at least 2 h at room temperature. A single slice containing the amygdaloid complex was transferred to an interface recording chamber (Medical Systems Corp.) and perfused continuously with ACSF composed of 117 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4 and 11 mM glucose. The ACSF was bubbled with carbogen gas (95% O_2; 5% CO_2) and maintained at $30 \pm 2\,^{\circ}C$.

2.2. Recording

Monopolar stainless steel electrodes (effective resistance 5 MΩ WPI Inc.) were used to electrically stimulate the EC, usually inserted dorsally to the recording site to elicit orthodromic responses. Square-wave pulses of 200 μs duration and 100–500 μA intensities were adjusted to just sub-threshold for orthodromic spike generation. Intracellular recordings were obtained from the lateral and basolateral amygdaloid nuclei using borosilicate glass microelectrodes (140–180 MΩ) filled with a 3 M potassium acetate solution and connected to the headstage of an intracellular recording amplifier (Bio-Logic VF 180). The microelectrode tips were inserted into the lateral or basolateral subdivision of the amygdala, 1–2 mm from the EC. Synaptic potentials were elicited orthodromically by stimulating the EC, which contains amygdala afferents from higher-order sensory cortices [9]. EPSPs and IPSP were characterized according to their amplitude, as a function of the resting potential and latency. Postsynaptic potentials were averaged (usually 10) and, if the membrane potential changed during drug applications, the membrane was manually clamped to its original resting value before taking measurements. Data were acquired and stored as analogue signals on videocassettes using a modified video recorder (Cibertec Physiorec-3). Data were transferred to a microcomputer using an analog-to-digital converter interface (CED 1401) for off-line analysis using customized software.

Recordings of neurons were characterized by studying their response to depolarizing and hyperpolarizing current pulses [46]. Only neurons that exhibited stable resting membrane potentials below −60 mV in the absence of dc holding current and generated overshooting action potentials were included for analysis.

2.3. Morphological characterization of recorded cells

At the end of electrophysiological recordings, selected neurons were stained by the intracellular injection of biocytin [46], using an electrode filled with a solution of 2% biocytin in 2 M potassium acetate. Positive current pulses of 0.2 nA intensity and a frequency of 3–5 Hz were applied for a period of 6 min. After injection, slices were transferred to an incubation chamber for 30 min and then fixed by immersion using a solution of 1.25% glutaraldehyde in 0.1 M phosphate buffer (PB) for 35 min. Fixed slices were embedded in a 2% solution of agar, cryoprotected with 30% sucrose in PB and sectioned at 45 μm on a freezing microtome (HM400R, Microm, Heidelberg, Germany). Sections were collected in PB, rinsed three times in the same buffer and then incubated with avidin–biotin–peroxidase complex (ABC) (Vector Labs, Burlingame, CA) for 3 h at room temperature. For visualization of the biocytin complex, 3,3′-diaminobenzidine was used as chromogen. The reaction was intensified with nickel ammonium sulphate. The sections were then washed several times in PB, mounted on slides, dehydrated and coverslipped. Every second section was counterstained with cresyl violet to determine the position of the filled neurons with respect to the cytoarquitectural subdivisions of the amygdala.