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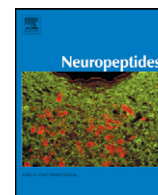

Kinin B2 receptor can play a neuroprotective role in Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is characterized by cognitive decline, presence of amyloid-beta peptide (Aβ) aggregates and neurofibrillary tangles. Kinins act through B1 and B2 G-protein coupled receptors (B1R and B2R). Chronic infusion of Aβ peptide leads to memory impairment and increases in densities of both kinin receptors in memory processing areas. Similar memory impairment was observed in C57BL/6 mice (WTAβ) but occurred earlier in mice lacking B2R (KOB2Aβ) and was absent in mice lacking B1R (KOB1Aβ). Thus, the aim of this study was to evaluate the participation of B1R and B2R in Aβ peptide induced cognitive deficits through the evaluation of densities of kinin receptors, synapses, cell bodies and number of Aβ deposits in brain of WTAβ, KOB1Aβ and KOB2Aβ mice. An increase in B2R density was observed in both WTAβ and KOB1Aβ in memory processing related areas. KOB1Aβ showed a decrease in neuronal density and an increase in synaptic density and, in addition, an increase in Aβ deposits in KOB2Aβ was observed. In conclusion, memory preservation in KOB1Aβ, could be due to the increase in densities of B2R, suggesting a neuroprotective role for B2R, reinforced by the increased number of Aβ plaques in KOB2Aβ. Our data point to B2R as a potential therapeutic target in AD.

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1. Introduction

The oligopeptides bradykinin (BK) and kallidin, also called Lys-BK, are released in the plasma or interstitial fluid after the cleavage of kininogens by kallikreins and their effects are the result of the activation of two G-protein coupled transmembrane receptors, called B1 and B2 (B1R and B2R, respectively) (Regoli and Barabe, 1980). The B2R is a constitutive receptor, its stimulation leading to a fast desensitization (Smith et al., 1995; Bascands et al., 1993; Mathis et al., 1996). It mediates most of kinin actions and has high affinity for BK and high sensitivity to low concentrations of the synthetic antagonist HOE 140 (Regoli et al., 1998). Conversely, B1R has higher affinity to des-Arg9BK and Lys-des-Arg9-BK (Regoli and Barabe, 1980). B1R is resistant to desensitization and is barely distributed in tissues under physiological conditions (Leeb-Lundberg et al., 2005), but shows increased densities under pathological conditions, such as chronic neurological diseases (Marceau et al., 1998; Prat et al., 1999; Prat et al., 2000).

When administered in the central nervous system (CNS), BK leads to behavioral effects as initial excitation followed by sedation (Okada et al., 1977), electroencephalographic desynchronization (Kariya and Yamauchi, 1981), increase in arterial blood pressure and reduction of diuresis (Hoffman and Schimid, 1978; Fior et al., 1993; Lindsey et al., 1997; Cloutier et al., 2002). In the rat hippocampus, a single dose of BK promotes hyperphosphorylation of the Tau protein eliciting memory and learning impairment (Wang and Wang, 2002). Kinin B2R is widely distributed in the CNS, being described in several nuclei of rats (Cholewinski et al., 1991; Couture and Lindsey, 2000; Viel et al., 2008), mice (Ma et al., 1994a), guinea pigs (Lopes et al., 1983; Sharif and Whiting, 1991; Murone et al., 1997; Fujiwara et al., 1988), bovine cattle (Kozlowski et al., 1998) and humans (Ma et al., 1994b; Raidoo et al., 1996) as assessed by RT-PCR, immunohistochemistry and autoradiography.

Alzheimer's disease (AD) is a progressive neurodegenerative disease with severe impact on learning and memory. This disease is neurohistopathologically characterized by ultra-structural lesions and the formation of senile plaques, composed of amyloid-β peptide (Aβ) deposits, and neurofibrillary tangles, formed by hyperphosphorylation of tau-protein filaments.

In vitro studies showed an increased production of inositol triphosphate (IP3) after stimulation of BK in skin fibroblasts obtained from patients with familial AD but not in fibroblasts obtained from sporadic AD patients (Huang et al., 1995). Furthermore, a pharmacodynamic modulation of BK receptors and an increase in the phosphorylation of

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various proteins were observed in a cellular model of AD, after stimulation with BK (Jong et al., 2002; Zhao et al., 2002).

Our group showed significant neuronal loss and amyloid deposit formation in the cortex and the hippocampus and an increase in kinin concentration in the cerebrospinal fluid in rats infused with human 1–40 Aβ peptide (Iores-Marçal et al., 2006), suggesting an enhanced activation of the kallilkrein–kinin-system (KKS) (Iores-Marçal et al., 2006). Moreover, we showed a disruption in memory consolidation of rats submitted to the same protocol, accompanied by an increase in B1R densities in the ventral hippocampal commissure, fimbria, CA1 and CA3 hippocampal areas, habenular nuclei and optical tract, when compared to the correlated areas in the control group. The most remarkable observation was a significant increase of B2R densities in brain nuclei related to cognitive process and the absence of labeling in the same areas from the control group (Viel et al., 2008). Recently we showed changes in memory processing of B1R or B2R knockout mice (KOB1, KOB2) after i.c.v. chronic infusion of human 1–40 Aβ peptide. KOB2 mice showed early memory disruption when compared to C57Bl/6 animals suggesting that B2R may play a neuroprotective role. Besides, KOB1 mice showed no memory deficits, suggesting that the absence of B1R apparently prevented the cognitive deficit normally observed at the end of the infusion period (Amaral et al., 2010; Viel and Buck, 2011). Considering that normal receptor regulation can be altered by the absence of B1R or B2R in knockout animals the aim of this work was to evaluate the expression of B1R in KOB2 mice and of B2R in KOB1 mice, as well as the neuronal plasticity in these animals after i.c.v. chronic infusion of human 1–40 Aβ peptide (treated group) in comparison to the vehicle group (control group).

2. Material and methods

2.1. Tissue preparation

Brain samples were obtained from C57Bl/6 (wild type, WT), KOB1 and KOB2 mice, 28–31 g of body weight, 12 weeks of age, provided from our own breeding colony. Animals were kept in controlled room temperature (22–24 °C) and humidity (55–65%), with food and water *ad libitum* in a 12 h light/dark cycle. All surgery and care procedures were performed according to the guidelines for animal experimentation as stipulated in the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication No. 86–23, Bethesda, MD) and The Ethics Committee on Experimental Research from Santa Casa de Sao Paulo Medical School. All efforts were made to minimize the number of animals used and their level of suffering. In order to follow that, samples used in this paper are from animals used in a previous study describing the memory alterations of kinin receptors knockout mice after Aβ peptide infusion (Amaral et al., 2010), as described below.

The following method was described in a previous work from our research team, where the behavioral analysis of these animals was reported (Amaral et al., 2010). Briefly, mice were infused with Aβ peptide or vehicle (V) for 35 days in the lateral cerebral ventricle. Knockouts (KOB1Aβ, KOB1V; KOB2Aβ, KOB2V) and WT (WTAβ and WTV) were submitted to surgery for the implantation of mini-osmotic pumps (Alzet, model 1004, Cupertino, CA, USA) filled up with either the vehicle (4 mMHEPES, pH 8.0 — control group) plus 0.22 nmol E-64 (cystein protease inhibitor) or human (1–40) Aβ peptide (0.46 nmol — Aβ group) plus 0.22 nmol E-64. At the end of the infusion period, the animals were introduced into a chamber saturated with CO_2 until they became slightly unconscious (about 2 s) and were immediately killed by decapitation. The brains were immediately removed and frozen in 2-methylbutane cooled at −45 to −55 °C in dry ice, then stored at −80 °C until use. Serial sections of the tissues (20 μm) were cut on a cryostat chamber (−18 to −20 °C, Microm HM 505N, Francheville, France), thaw mounted on gelatin coated slides, desiccated for 5 min at room temperature and kept at −80 °C until use.

Peptide iodination was performed following a method described elsewhere with some modifications (Hunter and Greenwood, 1962; de Sousa Buck et al., 2002). Briefly, 5 μg of B1 antagonist HPP-[desArg10]-HOE 140 or B2 antagonist HPP-HOE 140 was incubated in 0.05 M phosphate buffer for 30 s in the presence of 0.5 mCi (18.5 MBq) of $Na^{125}I$ and 220 nmol of chloramine T in a total volume of 55 μL at room temperature. The reaction was interrupted by 20 μL of 13 mM sodium metabisulfite. The monoiodinated peptide was immediately purified by gel filtration on a Sephadex-G25 column (13 mL) equilibrated in 0.5% bovine serum albumin in 0.1 M acetic acid. The specific activity of the iodinated peptides was calculated to be approximately 2000 cpm/fmol or 1212 Ci/mmol based on the purity of the monoiodinated compound (>95%), determined by reversed phase HPLC.

2.2. Autoradiography for B1 and B2 receptors

This method was based on a procedure described elsewhere (Ongali et al., 2003a; Ongali et al., 2003b; Viel et al., 2008; Caetano et al., 2010). The concentration of radioligands corresponds to maximal specific binding on the saturation curves (Bmax) and was determined on the basis of previous studies (Murone et al., 1997; Ongali et al., 2003a; Ongali et al., 2003b; Cloutier et al., 2004). Briefly, slides were incubated for 90 min at room temperature using 150 pM of iodinated kinin B1 antagonist for the identification of B1R or 200 pM of iodinate kinin B2 antagonist for the identification of B2R. The non-specific binding was determined by simultaneous incubation using 2 μM of the respective unlabelled peptides. After the incubation, sections were exposed against Hyperfilm-MP (double-coated,24 cm × 30 cm, Amersham Biosciences, GEHealthcare, Uppsala, Sweden) for 72 h to 96 h at room temperature, along with autoradiographic [^{125}I] microscales containing predetermined amounts of radioactivity in nCi/mg of tissue prepared as previously described (Nazarali et al., 1989). At the end of exposure time films were developed following manufacturer instruction and the autoradiograms quantified using the MCID image analysis system (Interfoccus Europe, UK). For each animal, 6–10 sections were analyzed. The specific binding was obtained by subtracting the non-specific binding (< 5%) from the total binding from similar adjacent sections. Results were expressed as fmol of iodinated antagonist per mg of tissue (fmol/mg of tissue).

2.3. Immunohistochemical procedure

Slides containing 20 μm frozen slices were warmed at room temperature until they became totally dry and then were fixed with acetone at −20 °C for 5 min at room temperature. After that, they were rapidly washed three times in 0.1 M phosphate buffer saline (PBS) and incubated for 20 min in 0.3% hydrogen peroxide in methanol (300 mL) to quench endogenous peroxidases. Slides were then washed three times in PBS and were incubated for 30 min in 10% normal goat serum (NGS) in PBS to prevent nonspecific staining. Following that, they were incubated with primary antibodies: anti-synaptophysin polyclonal antibody (1:1000; ab68851, Abcam, during 6 h) to evaluate the density of synaptic terminals and anti-NeuN antibody (1:2000, Millipore ABN78, incubated for 2 h) to evaluate neuronal density. After incubation with antibodies, tissues were processed with avidin–biotin complex (Vectastain Elite ABC Kit, Rabbit IgG, PK-6101, Vector Laboratories, Burlingame, CA, USA) according to the manufacturer's instruction. Following this, the immune complex was visualized with 0.05% 3,39-diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St. Louis, MO, USA) plus 0.015% H_2O_2 in 0.01 M PBS, pH 7.2, for 4 min and 30 s exactly. Careful washing in water twice stopped the reaction. All antibodies, the avidin–biotin complex and detection solutions were diluted in PBS with 10% NGS to reduce nonspecific binding. At the end, slices were dehydrated in an ethanol series (50, 75, 95, 100%) cleared in xylene, mounted in “Entellan” (Merck, Darmstadt) and covered with a coverslip. The specificity of synaptophysin and NeuN

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