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Prazosin, an α_1-adrenoceptor antagonist, prevents memory deterioration in the APP23 transgenic mouse model of Alzheimer's disease

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ABSTRACT

Noradrenergic deficits have been described in the hippocampus and the frontal cortex of Alzheimer's disease brains, which are secondary to locus coeruleus degeneration. Locus coeruleus is the brain stem nucleus responsible for synthesis of noradrenaline and from where all noradrenergic neurons project. In addition, it has been suggested that noradrenaline might play a role in modulating inflammatory responses in Alzheimer's disease. In this study we aimed to investigate the effect of various agonists and antagonists for adrenergic receptors on amyloid precursor protein processing. Among them, we found that prazosin, an α_1-adrenoceptor antagonist, was able to reduce the generation of amyloid β in N2a cells. Treatment of transgenic APP23 mice with prazosin prevented memory deficits over time. Although prazosin did not influence amyloid plaque load, it induced astrocytic proliferation and increased the release of apolipoprotein E and anti-inflammatory cytokines. These findings suggest that chronic treatment with prazosin leads to an anti-inflammatory response with potential beneficial effects on cognitive performance.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder in the aged population. It is characterized by certain neuropathologic features, including the presence of amyloid beta (Aβ) plaques, neurofibrillary tangles, synaptic loss, and widespread neuronal death (Mucke, 2009). It is believed that in AD, degeneration of the cholinergic basal forebrain neurons leads to cortical cholinergic hypofunction and therefore to cognitive decline and profound dementia (Palmer, 1996; Whitehouse et al., 1981, 1982). Data obtained from postmortem AD brains and cerebrospinal fluid from AD patients indicated deficits both in the noradrenergic and the serotonergic systems (Palmer and DeKosky, 1993). Loss of noradrenergic neurons is extremely pronounced in locus coeruleus (LC) at early stages of the disease, probably contributing to the worsening of cognitive impairments (Marien et al., 2004). The neurons from LC, which is located on the lateral aspect of the fourth ventricle, constitute the primary source of noradrenaline (NA) in the central nervous system and project laterally to all areas of the brain (Aston-Jones et al., 1996, 2000).

NA is known to modulate inflammatory gene expression, alter cytokine production and response, and modify lymphocyte and antibody production (Feinstein et al., 2002). LC lesions by the selective neurotoxin N-(2-chloroethyl)-N-ethyl-2 bromobenzylamine (DSP4) in rats treated with synthetic Aβ revealed increased neuroinflammation through inhibition of glial activation and Aβ clearance mechanisms (Heneka et al., 2002, 2010; Weinshenker, 2008). In addition, it was recently demonstrated that treatment with DSP4 in AD mouse models led to increased Aβ deposition, elevated neuroinflammation, increased neurodegeneration, and memory loss (Heneka et al., 2006; Kalinin et al., 2007). It can therefore be concluded that functional noradrenergic signaling and innervation from the LC is required for the maintenance of a balanced Aβ-free environment and that LC degeneration in AD aggravates neurodegeneration and cell death. In accordance with those findings, experiments in NA-depleted mice have highlighted the requirement of NA in memory retrieval and social memory (Weinshenker, 2008).

Drugs targeting adrenergic receptors (ARs) have shown remarkable success in protecting cultured cells from Aβ toxicity and

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even improving some AD symptoms, such as memory loss, in rats and monkeys (Arnsten et al., 1988; Ramos et al., 2008). Considering this success, our question was whether alteration of AR activation was likely to have an effect on amyloid precursor protein (APP) processing. Therefore, the aim of this study was to determine whether different AR agonists and/or antagonists might affect APP processing using both in vivo and in vitro approaches. We found that prazosin, an α_1-adrenergic antagonist might affect APP processing in vitro and it has potential beneficial effects in preventing memory loss in vivo in an animal model of AD.

2. Methods

2.1. Materials and antibodies

Antibodies used for detection of proteins of interest were 6E10 (against Aβ1–16) and 4G8 (against Aβ17–24) from Covance; Aβ[N] from IBL (against the N-terminus of Aβ); R1(57) against the carboxy-terminus of APP was a kind gift from Dr P. Mehta (New York State Institute for Basic Research in Developmental Disabilities); anti-apolipoprotein E (APOE) from Santa Cruz; anti-inducible nitric oxide synthase (anti-iNOS) from Cayman Chemical; anti-ionized calcium binding adaptor molecule 1 (anti-IBA1) from Wako; anti-glial fibrillary acidic protein (anti-GFAP) (clone 2.2B10) was a kind gift from Dr Douglas Feinstein (University of Illinois at Chicago); anti-neuronal nuclei (anti-NeuN) from Millipore UK, and anti-β-actin from Abcam. All the agonists and antagonists were obtained from Tocris. Tissue culture reagents were purchased from Invitrogen, and all other reagents were purchased from Sigma, UK, unless stated otherwise.

2.2. In vitro methods

2.2.1. Cell lines and treatment

Mouse neuroblastoma N2a cells stably transfected with APP695 containing the Swedish mutation (K595N/M596L), so-called N2asw, were obtained from G. Thinakaran (University of Chicago, Chicago, USA). Naive Chinese hamster ovary (CHO) cells, CHO cells stably overexpressing wild type human presenilin-1 (PS1) and wild type APP (PS70) were a generous gift from Dr Selkoe (Brigham and Women's Hospital, Boston). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 5% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin sulphate, and 0.2 mg/mL G418 at 37 °C in a 5% CO_2 atmosphere.

Cell treatment was carried out for 18 hours before harvesting. Drugs were dissolved in either dimethyl sulfoxide or H_2O. N2asw cells were treated with 50 μM of the following drugs: cirazoline, a nonselective α_1-AR agonist and α_2-AR antagonist; UK-14304, an α_2-AR agonist; isoprenaline, a nonselective β-AR agonist; prazosin and naftopidil, both α_1-AR antagonists; RX-821002, an α_2-AR antagonist; propranolol, a nonselective β-AR antagonist; noradrenaline, a nonselective endogenous AR agonist; or 5-hydroxytryptamine, a non-selective endogenous 5-hydroxytryptamine receptor agonist. The prazosin dose-response curve was carried out from 1 μM–10 μM by enzyme-linked immunosorbent assay (ELISA) and from 10 μM–100 μM by Western blot analysis. At least 3 replicate samples were run for each treatment group and each experiment was reproduced at least 3 times.

2.2.2. Western blot analysis

Cell lysates and brain homogenates were extracted with 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 150 mM NaCl, and 50 mM Tris-HCl, pH 7.2, supplemented with Roche Complete protease inhibitor cocktail, and equal amounts of protein (20–100 μg) samples were separated in SDS-polyacrylamide gel electrophoresis gels, followed by immunoblot analysis with primary antibodies and detected with horseradish peroxidase-conjugated secondary in 5% nonfat dried milk in Tris-buffered saline with Tween 20 (TBST). Membranes were developed using ECL (GE Amersham, UK) reagents and using Hyperfilm ECL audioradiography film in an automated developer from Konica, SRX 101A. The intensity of the bands was quantified by densitometry using the Image J software (National Institutes of Health) and normalized to β-actin.

2.2.3. Determination of secreted Aβ and sAPPα

Aβ and soluble APPα (sAPPα) in the medium were measured by Western blot analysis using 4%–12% NuPAGE gels (Invitrogen) followed by transfer to nitrocellulose membranes and immunodetection with antibody 6E10 as described previously (Sastre et al., 2003). The volume of medium used was adjusted to protein concentrations measured in cell lysates. Aβ subtypes were determined by ELISA using sensitive ELISA kits (Wako, Osaka, Japan and IBL, Hamburg, Germany, respectively) (Guardia-Laguarta et al., 2009).

2.2.4. Subcellular fractionation

Cultured N2asw cells were mechanically broken in hypotonic buffer (10 mM Tris, 1 mM ethylenediaminetetraacetic acid [EDTA], 1 mM ethylene glycol tetraacetic acid [EGTA]) with Roche complete mini cocktail protease inhibitor. Samples were then centrifuged at 5000 rpm for 5 minutes. The supernatant containing the membrane and cytosol fractions was then centrifuged for 45 minutes at 13,000 rpm at 4 °C.

2.2.5. Analysis of APP C-terminal domains

Carboxy-terminal domains (CTFs) were analyzed by immunoblot analysis with antibody against the carboxy terminus of APP of membrane extracts from APPsw stably transfected N2a cells. For CTFγ, membranes were pelleted by centrifugation for 20 minues at 16,000 rpm at 4 °C, washed, and resuspended in assay buffer (150 mM sodium citrate, pH 6.4). To allow generation of CTFγ, samples were incubated at 37 °C for 1 hour. After termination of the assay, samples were separated by centrifugation at 16,000 rpm at 4 °C. Supernatants were separated by electrophoresis using 4%–12% NuPage gels, and the CTFγ was analyzed by immunoblot analysis with the R1(57) antibody (Sastre et al., 2001).

2.3. In vivo methods

2.3.1. Animals

In the present study 19 APP23 mice, 9 months old (Novartis), and 21 wild type mice (males and females) were used for the experiments. The APP23 mice express the human APP751 isoform carrying the Swedish double mutation (K670N-M671L) under the control of the murine Thy1.2 expression cassette (Sturchler-Pierrat et al., 1997). The animals were hemizygous or littermate control mice and had been backcrossed with C57BL/6J mice (Charles River, UK) for at least 8 generations. All the animals were kept in individually ventilated cages in a 12/12 hour light/dark cycle with controlled temperature and humidity and had food and water ad libitum. During the treatment period, the animals received 1 intraperitoneal injection of prazosin daily for 2 weeks (1 mg/kg dissolved in saline). The drug dose was chosen according to previous publications from other groups, which indicate that it crosses the blood brain barrier (Liu et al., 2012; Maheux et al., 2012; Milane et al., 2010). In this particular strain 1 mg/kg/d was the highest dose we could deliver, because preliminary experiments showed increased mortality with higher doses (data not shown). After the completion of the treatment and the behavioral tests, animals were anesthetized and transcardially perfused with