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The apoE receptor apoER2 is involved in the maintenance of efficient synaptic plasticity

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Abstract

ApoER2 is one of the major receptors for ApoE in the brain, and has been shown to be involved not only in lipoprotein endocytosis, as other members of the LDL receptor family of receptors, but also in various cellular functions such as signalling and cellular guidance. By using a model of synaptic plasticity in mice lacking none, one or two alleles of the apoER2 gene, we investigated the implication of such a receptor deficiency on the remodelling process. Our results indicate that animals lacking apoER2 express higher levels of brain APP, as well as both key amyloid peptides, while apoE levels are slightly lower. Following entorhinal cortex lesioning, apoE levels increase in the deafferented hippocampus, while a delay in the increase of APP was observed. Hippocampal amyloid levels are also increased in response to the lesion, and highly potentiated by the complete absence of apoER2 gene. The results suggest a significant role for apoER2 in signalling various proteins in response to massive deafferentation and may participate in maintaining efficient synaptic plasticity and dendritic remodelling.

Keywords: Apolipoprotein E receptor; apoER2; Synaptic plasticity

1. Introduction

Recent work has shown that apolipoprotein E (apoE) has more than one possible receptor in the brain, thus enabling diverse lipoprotein transport to cells requiring a lipid input. Six receptors for apoE have been described in the brain (reviewed in [2,8]): the low density lipoprotein (LDL) receptor, the low density lipoprotein related protein (LRP), the very low density lipoprotein (VLDL) receptor, the apoE receptor type 2 (apoER2/LRP8), the LR11 receptor ([10,16,29], and the gp330/megalin receptor [12]). ApoE is a crucial protein moiety of lipoproteins, allowing them to bring various lipidic components to cells, via receptor-mediated endocytosis (reviewed in [13]). In the brain, the main beneficiaries of this mechanism are neurons, particularly due to their inability to divide, making repair and remodelling processes essential to their survival and proper function [6,19]. Neurons require cholesterol and other lipids to remodel their membranes and grow new terminals, either during synaptic plasticity or in response to a neurodegenerative insult. They can either produce these lipids by *de novo* cholesterol and phospholipid synthesis or acquire them by increasing their lipid intake, made possible through the cell surface receptors they express, and thereby relying on lipoproteins entry via apolipoprotein binding.

During the earliest stages of Alzheimer's disease (AD), the main cerebral structures affected are layer II of the entorhinal cortex, the subiculum and the CA1 region of the hippocampus [1]. The perforant pathway links the entorhinal cortex and the hippocampus by a large number of neuronal fibers relaying information associated with memory between these two regions. By selectively disrupting the perforant pathway with an electrolytic lesion, thus severing the connection between the entorhinal cortex and the hippocampus, we are able to produce an animal model with which to study reactive synaptic plasticity. This disruption of the perforant path will cause the surviving neurons to reorganize themselves in order to compensate for the loss, thereby trig-

Abbreviations: AD, Alzheimer's disease; apoE, apolipoprotein E; apoER2, apoE receptor type 2; ECL, entorhinal cortex lesioning; APP, amyloid precursor protein; Aβ, amyloid-beta peptide

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gering an increased need for many molecules and proteins, as well as lipids. We have previously shown that following entorhinal cortex lesioning (ECL) in the rat, the expression of several proteins, including apoE, increases to participate in or support the regenerative process [20,21].

The presence of six known receptors allowing apoE internalization in the brain suggests functional redundancy, but also indicates there may be an underlying regulation mechanism between these various receptors. It has not yet been shown how the various proteins involved in apoE metabolism (namely the various receptors, apoE and the components carried by the lipoprotein) in the brain are responsive when the expression of one of the receptors is disrupted. We have selected apoER2 as a target, due to its expression pattern. This receptor is most highly expressed in the brain [11] and it is very homologous to the LDLr and VLDLr [11]. Interestingly, apoER2 was detected in rat sympathetic neuron cultures that displayed no LRP expression [22]. It has been hypothesized that PNS and CNS neurons would express distinct receptor populations, offering a possible explanation for the apparent redundancy of receptors in the brain. However, other studies have shown that each receptor may be able to respond to different trigger signals binding to their cytoplasmic tail [14], indicating that each may have a unique role, as well as share common activities. Within their cytoplasmic tails, members of the LDL receptor family share a common NPXY motif [18,30] which is a consensus sequence involved in triggering various cellular internalization signals.

Our objective in this study is two-fold: first to characterize the expression of selected proteins involved in Alzheimer's disease pathophysiology in intact apoER2-knockout mice, and second, to determine the impact of a lack of apoER2 during the degeneration–regeneration events initiated following the experimental disruption of the perforant pathway.

2. Materials and methods

2.1. ApoER2-knockout mice

ApoER2-knockout mice were prepared as previously described [29] without any modifications. Animals expressing none, one or two alleles of apoER2 were generated.

2.2. Entorhinal cortex lesioning

Mice of 12 weeks of age were anesthetized by intramuscular injection of a solution containing Xylazine (50 mg), Acepromazine (10 mg), Ketamine (200 mg) prepared in a sterile saline solution. Once anesthetized, animals were secured in a stereotaxic apparatus, allowing for stable coordinate location. Electrolytic disruption of the perforant pathway was produced by consecutive 10 s pulses of 1 mA, in four different coordinates (2 depths) encompassing the connections between the entorhinal cortex and the hippocampus, as described previously [11]. Following suturing, the animals were given an intramuscular injection of anti-inflammatory Flunixin Meglumine (25 mg) and a subcutaneous injection of 0.5 cc of lactated Ringer solution. Animals were nursed for 24–36 h, including booster injections of Ringer solution when needed.

At different time points following surgery, animals were sacrificed in order to recover the brain. These were snap-frozen in iso-pentane and conserved at −80 °C until further use. Time points chosen were 0, 10, 21 and 35 days post-lesion (DPL).

2.3. Tissue preparation

Samples were separated within time points, to be used either for immunohistochemical measurements on tissue slices or as tissue homogenates for protein quantification.

Cryostat sectioning was performed on frozen tissue, producing slices of 16 μm thickness on poly-L-lysine coated glass slides. Slides were then dehydrated overnight and kept at −80 °C until further use. These were used for immunohistochemical stainings.

Following dissection of brains, region-specific tissue homogenisation was performed by sonicating samples for 2–3 bursts of 20 s, on ice, using a minimal volume of phosphate-buffered saline (PBS). Protein content was determined by using the BCA assay (Pierce).

2.4. Immunohistochemistry

Tissue was fixed for 30 min by a 4% paraformaldehyde solution. Following every step, slides are washed twice with PBS. Slides were incubated in a 3% peroxyde solution (in PBS) for 15 min, followed by a permeabilization step using TritonX-100 (0.4%) for 30 min. This was followed by a 1 h blocking step using a solution of 1% horse serum in PBS, as indicated by the Vectastain ABC detection kit used (Vector Laboratories, Burlingame California, USA). Antibody solution was prepared in PBS (mouse monoclonal anti-APPA4, Boehringer Manheim, Laval, Canada) and slides were incubated overnight at 4 °C. After washing, the slides were incubated with a secondary antibody (mouse IgG) for 2 h at room temperature, followed by incubation with the detection reagent for 2 h. Slides were then washed with PBS, followed by three washes in a Tris 50 mM pH 7.6 solution. Slides were allowed to react with the DAB solution (in Tris 50 mM) for 5–20 min until staining was satisfactory. Reaction is stopped by addition of PBS, slides are then dehydrated and mounted with DPX and glass coverslips. Microscopic evaluation was performed using a Nikon ER600 microscope, mounted with a 35 mm camera. Photographs were taken using Kodak Royal Gold 400 film, and images were acquired using a Canon FB320P scanner, using the CanoCraft CS-P software.