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APOLIPOPROTEIN E RECEPTORS AND AMYLOID EXPRESSION ARE MODULATED IN AN APOLIPOPROTEIN E–DEPENDENT FASHION IN RESPONSE TO HIPPOCAMPAL DEAFFERENTATION IN RODENT

C. PETIT-TURCOTTE,[a,b] N. AUMONT,[a,b] J.-F. BLAIN[a,b] AND J. POIRIER[a,b]*

[a]*McGill Center for Studies in Aging, 6825 LaSalle Boulevard, Verdun, Quebec, Canada H4H 1R3*

[b]*Douglas Hospital Research Center, 6875 LaSalle Boulevard, Verdun, Quebec, Canada H4H 1R3*

Abstract—The entorhinal cortex lesion paradigm is a widely accepted and efficient method to provoke reactive synaptogenesis and terminal remodeling in the adult CNS. This approach has been used successfully to contrast the profile of reactivity from various proteins associated with Alzheimer's disease pathophysiology in wild-type and apolipoprotein E (apoE)-deficient (APOE ko) mice. Results indicate that the production of the beta-amyloid 1–40 peptide (Aβ_{40}) is increased in response to neuronal injury, with a timing that is different between wild-type and APOE ko animals. Moreover, we report that baseline levels of the Aβ_{40} peptide are significantly higher in the APOE ko mice. The expression of the apolipoprotein E receptor type 2 (apoER2) is also modulated by the deafferentation process in the hippocampus, but only in APOE ko mice. These results provide novel insights as to the molecular mechanisms responsible for the poor plastic response reported in apoE4-expressing and apoE deficient mice in response to hippocampal injury.

Key words: Alzheimer's disease, lipoprotein receptors, amyloid, synapses, entorhinal cortex lesion.

Apolipoprotein E (apoE) is a crucial protein in the brain, involved in maintaining the integrity of the synapto-dendritic network, as well as upholding lipid homeostasis in response to damage (reviewed in (Poirier et al., 1995b)). By recruiting and redirecting free lipids to neighboring cells requiring a lipid input, apoE is recognized as the main lipid scavenger and transporter in the brain. In humans, the ε4 allele of apoE represents the strongest genetic risk factor ever identified for sporadic Alzheimer's disease (AD) (Strittmatter et al., 1993; Poirier et al., 1993b).

In light of these facts, models have been developed to further study the neurobiology of apoE in normal and pathophysiological contexts. An experimental model of electrolytic entorhinal cortex lesion (ECL), consisting in the disruption of neuronal fibers connecting the entorhinal cortex to the hippocampus (Lynch et al., 1972; Geddes et al., 1985), creates a two-part reaction by the surviving cells. There is an initial period of degeneration, where membranes are degraded and lipids are recycled, followed by a period of reinnervation, where surviving cells use the recycled molecules and grow new processes to compensate for the losses incurred by the lesion (Poirier, 1994). Apolipoprotein E-deficient (APOE ko) mice have been reported to exhibit region- and age-dependent synaptic losses (Masliah et al., 1995, 1996; Chapman and Michaelson, 1998; Chapman et al., 2000). When ECL is performed on APOE ko animals, the absence of apoE hinders severely both, the deafferentation and the reinnervation phases (Fagan et al., 1998; Champagne et al., 2005). These steps resemble some of those observed in the hippocampus of early stages of AD, where initial damage occurs in the entorhinal cortex, spreading to the hippocampus via the perforant pathway (Hyman et al., 1984; Braak and Braak, 1991). Furthermore, it was shown that AD subjects who are carriers of the apoE4 allele display marked allele copy dependent losses of synapses (Poirier et al., 1995a; Arendt et al., 1997) and apoE protein levels in the hippocampus and cortical areas (Bertrand et al., 1995; Beffert et al., 1999b).

In order for apoE to carry out its lipid transporting activities, it is essential that it gain access to the cells requiring lipids. This is ensured by the lipoprotein receptors expressed at the cell surface. Several apoE receptors in the brain have been identified, which all belong to the LDL receptor family (reviewed in Beffert et al., 1998b; Herz and Beffert, 2000). Due to their expression patterns and affinity for apoE, low density lipoprotein-related protein (LRP) (Rebeck et al., 1993; Narita et al., 1997), LDLr (Poirier et al., 1993b) and apolipoprotein E receptor type 2 (apoER2) (Kim et al., 1996; Posse De Chaves et al., 2000) are now recognized as the three main apoE receptors expressed in the brain.

Both LRP and apoER2 have been included in the hypothesis that members of the LDLr family hold cellular signaling functions involved in many events, including the regulation of amyloid precursor protein (APP) processing (D'Arcangelo et al., 1999; Willnow et al., 1999; Herz et al., 2000), neuronal migration (Hiesberger et al., 1999; Trommsdorff et al., 1999; D'Arcangelo et al., 1999; Dulabon et al., 2000), calcium influx through NMDA receptor (Bacskai et al., 2000; Qiu et al., 2002), neurite outgrowth (Holtzman et al., 1995; Qiu et al., 2004) as well as synaptic plasticity and learning (Weeber et al., 2002; Beffert et al., 2005).

*Corresponding author. Tel: +1-514-766-2010; fax: +1-514-888-4094.
E-mail address: judes.poirier@mcgill.ca (J. Poirier).

Abbreviations: AD, Alzheimer's disease; apoE, apolipoprotein E; APOE ko, apolipoprotein E-deficient; apoER2, apolipoprotein E receptor type 2; APP, amyloid precursor protein; Aβ_{40}, beta-amyloid 1–40 peptide; DPL, days post-lesion; ECL, entorhinal cortex lesion; LRP, low density lipoprotein-related protein; TBS, tris-buffered saline.

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In this study, we examined the impact of the lack of apoE during reactive synaptogenesis on the expression of various molecular markers known to interact with apoE or involved in AD pathophysiology. Our results indicate that the absence of apoE affects the time course of beta-amyloid 1–40 peptide ($A\beta_{40}$) alterations as well as induces a modulation of apoE receptors in response to the lesion which is not observed in wild type animals.

EXPERIMENTAL PROCEDURES

Animals

Animals used in this study were males purchased from Jackson Laboratories (Bar Harbor, ME, USA) and received at 8 weeks of age. The animals were housed in our facility until they reached 12 weeks of age. Control mice used were C57BL/6J to compare with APOE ko mice with the same genetic background.

ECL

Unilateral electrolytic ECL was performed as described previously (Blain et al., 2004). Briefly, the lambda 0 was taken by aligning the electrode with the suture lines. The skull was drilled to allow the electrode to pass through the four different coordinates. The electrode was inserted at a 6° angle in the right side of the brain following these coordinates: RC (+0, +0, +0.5, +1.0); L (−3.0, −3.5, −4.0, −4.0); DV (−3.0, −4.0 at each point). A 1 mA current was then applied for 10 s at each coordinate. The left side of the brain remained unlesioned and served as an internal control. Following suturing, the animals were given an i.m. injection of anti-inflammatory flunixin meglumine (25 mg) and an s.c. injection of 0.5 ml of lactated Ringer solution. Animals were nursed for 24–36 h, including booster injections of Ringer solution when needed. All procedures were carried out in accordance with the Canadian Guidelines for Use and Care of Laboratory Animals and were approved by the Animal Care Committee of McGill University. The number of animals was kept to a minimum as per university guidlines. Care was taken to minimize suffering. At different time points following surgery, animals were killed 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 (n=10), 10 days post-lesion (DPL; maximal deafferentation: n=15), DPL 21 (early reinnervation: n=15) and DPL 35 (near-complete synaptic turnover; n=15). The lesioning protocol has been associated with variable loss of animals at the different time points, the most pronounced death rate was observed at 35 DPL. However, we succeeded in obtaining a minimum of 5 healthy animals per time point, up the 14 animals at the shortest post-lesion time point (10 days).

Protein assays

Frozen brain tissue was allowed to slightly thaw while on a bed of crushed ice, sufficiently to allow dissection of brains, following which region-specific tissue homogenization 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). APP (Boehringer Ingelheim, Montreal, Quebec, Canada), apoER2 and LRP levels were determined by Western blot analysis. Briefly, 20 μg of total protein were run on a 4–12% gradient Bis–Tris gel electrophoresis (Invitrogen, NuPage), then transferred to a nitrocellulose membrane (Hybond, Amersham Pharmacia, UK). After blocking with 1% non-fat milk in tris-buffered saline (TBS), membranes were incubated with primary antibodies (both generous gifts form Drs. U. Beffert and J. Herz from U.T. Southwestern) as described elsewhere for apoER2 (Trommsdorff et al., 1999) and LRP (Herz et al., 1990). Detection was made using the ECL chemiluminescence kit (NEN Life Sciences Woodbrides, Ontario, Canada). Tubulin detection was used as a loading control. Between each primary antibody detection, membranes were stripped of bound antibodies by a Tris-buffered SDS (10%) solution at 70 °C for 30 min, followed by two washes in TBS buffer. Membranes were blocked overnight in a solution of 1% nonfat milk in TBS before being used again. Chemiluminescent signal was quantified using the Kodak Digital Science, Image Station 440cf Kodak 1D Image Analysis Software version 3.5 (Perkin Elmer Life Science).

Beta-amyloid ELISA

Amyloid peptide (Aβ1–40 and 1–42) content was determined by an ELISA assay, using a biotinylated monoclonal antibody (4G8, Senetek, St. Louis, MO, USA). This antibody specifically recognizes the first 16 amino acids of the amyloid peptide (Kim et al., 1990). Capture antibodies R163 (2 μg/ml) and R165 (2 μg/ml) (generous gift of Dr. P. D. Mehta) which recognize Aβ1–40 and 1–42 respectively, were used to coat 96 well microtiter plates, in 10 mM sodium carbonate (pH 9.6) by incubating overnight at 4 °C. Non-specific binding was blocked by a 2 h incubation of 0.1% bovine serum albumin. Following washing, 50 μl of either sample or standard peptide (Bachem, Torrance, CA, USA) was added to plates for 2 h at room temperature under gentle shaking. Following washing, the biotinylated detection antibody was added for 1 h, followed by a 1 h incubation with an alkaline-phosphatase-conjugated streptavidin (Zymed, San Francisco, CA, USA) to detect the biotinylated antibody. Bound antibody was quantified using the Attophos substrate (JBL Scientific, San Luis Obispo, CA, USA) following manufacturer's indications. Fluorescence was measured by using the FL600 Microplate reader (Bio-Tek Instruments) at ex=450 nm/20 nm and em=560 nm/20 nm. Sensitivity of both assays is approximately 100 pg/ml. Additional experimental details regarding this assay can be found in (Vaucher et al., 2001).

Statistical analyses

Comparison of strain and experimental conditions across days for each of the experimental conditions was analyzed using the analysis of variance statistical test. Significant interactions were decomposed by simple main effects tests, and t-tests were used for all pairwise comparisons. Graphs were prepared using GraphPad Prism software (version 3.0) and data analysis was performed using Datasim software.

RESULTS

Amyloid protein expression is responsive to reactive synaptogenesis

Following ECL, we determined the hippocampal levels of APP, $A\beta_{40}$ and $A\beta_{42}$ in wild type and APOE ko mice. APP expression, represented by the ratio of ipsi/contralateral side to the lesion, did not vary across time and between strains in response to the lesion (Fig. 1). Basal levels of Aβ peptides display significant strain differences for $A\beta_{40}$ (wt: 21.35±0.95 ng/ml vs. APOE ko: 26.43±1.62 ng/ml; P=0.012, two-tailed t-test) but not for $A\beta_{42}$ (P=0.09, two-tailed t-test). Time course analysis of hippocampal $A\beta_{40}$ levels (Fig. 2A) reveals clear differences between the lesioned and unlesioned hippocampi with peaks at 21 DPL in wt animals and at 35 DPL in APOE ko animals. In contrast, $A\beta_{42}$ levels are not affected by the lesion paradigm at any the time points examined in this study (Fig. 2B).