Disruption of estrogen receptor beta in mice brain results in pathological alterations resembling Alzheimer disease[1]

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ABSTRACT

AIM: To study the pathological characteristics of the mice with estrogen receptor β (ERβ) disruption in brain. **METHODS:** Immunohistochemistry method was applied in the study. **RESULTS:** β-Amyloid peptide($A\beta_{42}$) and apolipoprotein E (ApoE) immunoreactive substances were accumulated notably in cortex and limbic structures such as the hippocampus and amygdala in brain, resembling the pathological changes of human Alzheimer disease (AD). Aβ formed cloudy-like deposits in parenchyma of brain, while apoE also deposited along or surrounding the blood vessels. **CONCLUSIONS:** ERβ is crucial to the development of neural degenerative disease, so modulation of Aβ metabolism via ERβ signal pathway might be beneficial for AD prevention or therapy.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease prevalent among the elderly people, involving a number of genetic components, risk factors, and other poorly defined elements[1]. The synapse loss and neuronal cell death, characteristics of AD, are believed to result in large part from the neurotoxic effects of beta-amyloid peptide (Aβ), a 40-42 amino acid peptide (s) derived proteolytically from beta-amyloid precursor protein (APP)[2,3]. The deposition of amyloid peptide resulting in the formation of extracellular neuritic plaques in the brain are also diagnostic neuropathological features of this neurodegenarative disorder[4].

Apolipoprotein E is an important constituent of plasma lipoproteins and a ligand for several lipoprotein receptors. Three major forms of apoE (E2, E3, and apoE4) have been described in the plasma where they take part in the transport and cellular uptake of lipids[5]. Next to the liver, the brain is the second richest in the content of apoE mRNA. Genetic-epidemiological studies have also identified a highly reproducible association between the three common apoE gene polymorphisms and the relative risk for development of AD[6]. Furthermore, amyloid deposition as well as neuritic plague formation are dependent on ApoE expression[7].

It has been long recognized that estrogen is a potent neurotrophic and neuroprotective factor during embryonic and neonatal development. Its role in adult brain is more appealing because of the potential therapeutic importance in neurodegenerative disease[8]. Evidence from epidemiological studies supports enhanced cognitive function in women with AD taking estrogen

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replacement therapy (ERT) as well as a reduced risk for developing AD in healthy women receiving ERT. Estrogen also reduces Aβ burden in animal models of AD and secretion of Aβ from neuronal cultures[9,10].

Despite an important role of estrogen in determing the risk for AD, little is known about the mechanism for estrogen deficiency that subserves in AD pathogenesis. It is increasingly accepted that physiological levels of estrogen requires the involvement of estrogen receptors, while pharmacological levels of estrogen appear to by-pass receptors via cross-talk with other second messenger signaling molecules[11]. So the traditional signaling via ER is more appealing if implement of long-term estrogen therapy to reduce the neurodegenerative disease.

Although estrogen receptor was discovered in the 1980s, the more recent finding of its subtype, ERβ, has added the complexity to the study of estrogen signaling in the brain. ERβ was diversely distributed in a different pattern with ERα in brain[12], implying a special role of ERβ in signaling the protective action of estrogen in brain. ERβ knockout mice (BERKO) provide us a reasonable and practical way to approach the purpose quickly. Previous study reported neuronal deficit in BERKO brain[13], speculating ERβ's important role in the development of neural degenerative disease. In the present study, we verified the hypothesis by providing strong evidence of diversely deposition of Aβ and ApoE in BERKO brain.

MATERIALS AND METHODS

Animals and brain preparation BERKO mice were provided by Department of Medical Nutrition of Karolinska Institute. Heterozygote mice were used for breeding. C57BL/6J strain mice from whom BERKO were created, were set as wild-type (WT) control. The mice were free to tap water and rodent row. Female BERKO (2-4 months, *n*=8) were ovariectomized 2 weeks before anesthetized with an over-dose of pentobarbital sodium, and perfused through the heart with phosphate buffer (0.1 mol/L PBS), pH 7.4, followed by ice cold 40 g/L paraformaldehyde fixation in 0.1 mol/L phosphate buffer. The brains were carefully removed and postfixed in the fixative on 4 ℃ overnight, then were transferred into 30 % sucrose in 0.1 mol/L PBS until it settled. Serial sections (30 μm thick) were cut with a cryostat.

Immunohistochemistry Floating sections were incubated in 50 % (v/v) methanol with 1 % (v/v) H_2O_2 for 30 min to quench the endogenous peroxidases, followed by 100 g/L normal horse serum, 0.5 % (v/v) Trition X-100, 10 g/L BSA in 0.01 mol/L PBS for 30 min to block nonspecific binding. Sections were then incubated with anti-human $A\beta_{42}$ (1:50, Transduction Laboratories, UK) or apoE (1:100, Transduction Laboratories), both with 100 g/L normal horse serum, 0.5 % Trition X-100, 30 g/L BSA in 0.01 mol/L PBS for 36 h at 4 ℃. Immunohistochemistry method with ABC kit (Vector Laboratories) was applied afterwards. In brief, biotinylated rabbit anti-goat IgG (1:200) and avidin-biotin-complex (1:200) incubated for 2 h respectively. Staining was developed with 3,3-diaminobenzidine (Sigma) catalyzed by H_2O_2 for 10 min. Then the sections were completely washed in 0.01 mol/L PBS and finally mounted. The slides were observed under a microscopy (Olympus Optical Co, Japan) and the pictures were taken at a magnificent of ×200 by an attached digital camera.

RESULTS

Deposits of $A\beta_{42}$ in BERKO brain Amyloid plaques were found in great numbers in BERKO brain and had a wide distribution, most notably in cortex and limbic structures such as the hippocampus and medial amygdala (Fig 1). All the BERKO mice developed extracellular $A\beta_{42}$ deposits as early as 2 months old. In contrast, the deposits never occurred in age- and sex-matched WT mice (Fig 2). This could be described as "non or all" phenomenon. Micrographs of BERKO brain sections showed $A\beta_{42}$-immunoreactive (ir) deposits in representative sections. The deposits were predominantly diffuse with minimal $A\beta_{42}$-ir cellular staining, forming cloudy-like senile plaques. The plaques differed in size and intensity. Some had solid cores with thread-like $A\beta_{42}$ in the periphery, the others with radical formation.

The cerebral cortex contained a substantial density of deposits. $A\beta_{42}$-ir plaques were distributed predominantly in association with cortical zones while with a lower density in paralimbic cortical areas. Detailed examination of the whole sections clearly showed that the deposits within vessels were most frequent seen in neocortex, where $A\beta_{42}$ was present in plaques.

Accumulation of ApoE in brain ApoE positive staining was found primarily in the parenchyma as well as peri/para blood vessels in cortex, hippocampus, and hypothamus (Fig 3). Some ApoE vascular deposits lined along the blood vessels so well that gave prominence to

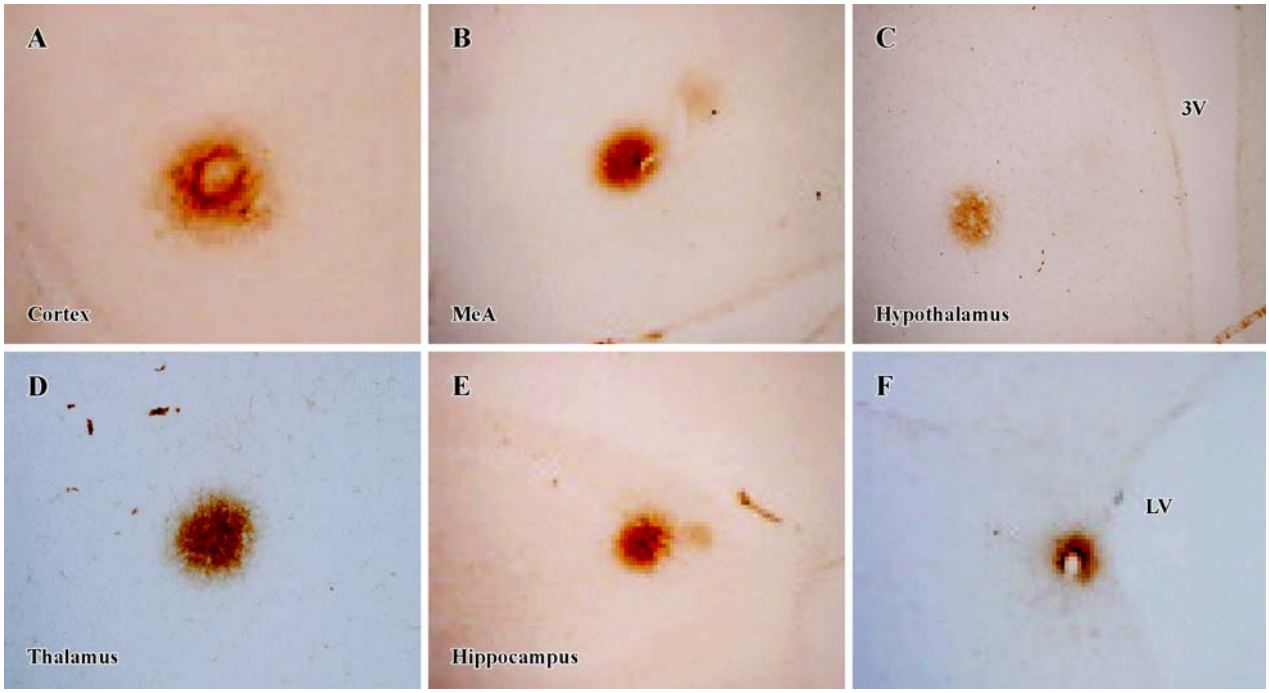

Fig 1. Aβ immunoreactive staining in cortex (A), medial amygdala (MeA,B), hypothalamus (C), thalamus (D), hippocampus (E), close to lateral ventricle (F).

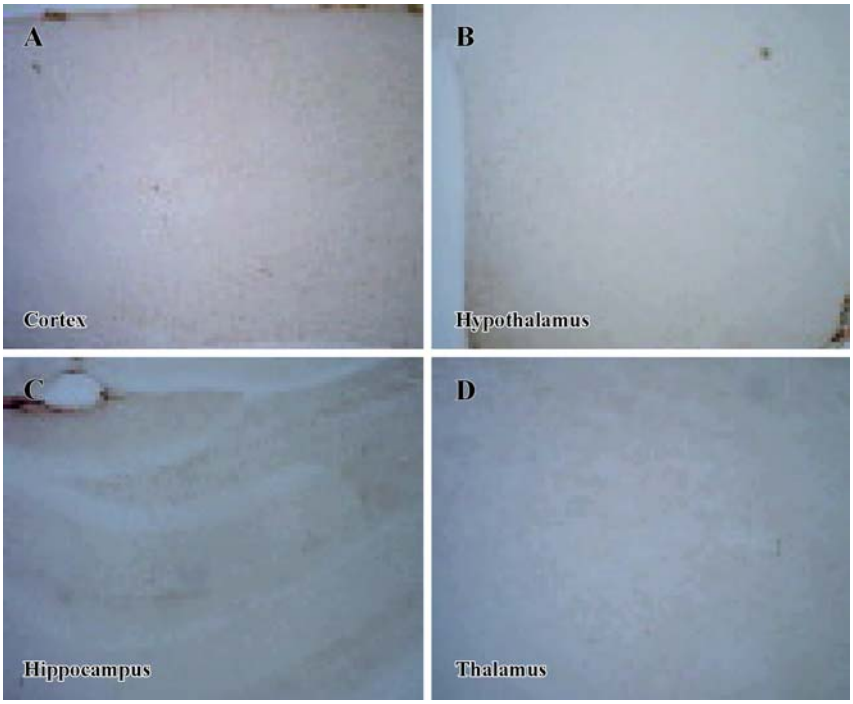

Fig 2. No Aβ-immunoreactive staining in cortex (A), hypothalamus (B), hippocampus (C), thalamus (D) in WT.

the vessels. The other positive staining were similar to $A\beta_{42}$ deposits, also forming the spots in the parenchyma. It was interesting to see some ApoE-ir substances surrounding the tiny vessels that were so close to the brain ventricles (Fig 3F, G), suggesting ApoE's role as the cholesterol transmitter between brain and cerebrospinal fluid. In every section of WT brain, no positive material could be seen in both the parenchyma or along

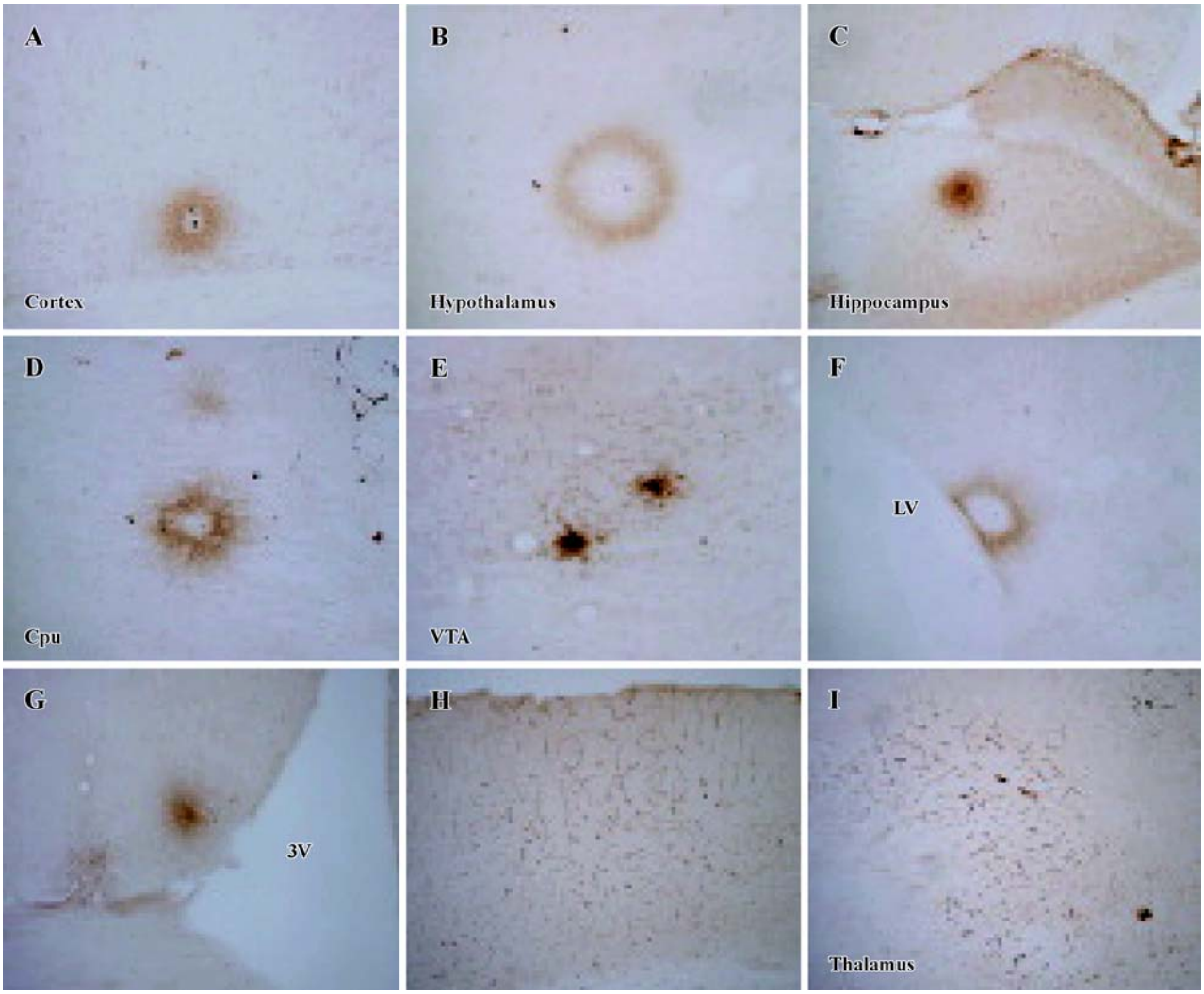

Fig 3. ApoE immunoreactive staining in parenchyma (A-G) and blood vessels or perivascular areas (H, I). Typical changes were represented in cortex (A), hypothalamus(B), hippocampus (C), caudate putamen (Cpu, D), ventral tegmentum area (VTA, E) and the area close to lateral (LV, F) or 3rd ventricle (3V, G).

the blood vessels (data not shown), confirming that ERβ disruption is the only explaination for $A\beta_{42}$ or apoE accumulation in brain. Obliterating the primary Aβ for $A\beta_{42}$ or apoE also yielded negative data, verifing the specificity of above results.

DISCUSSION

In this study, we found widespread β-amyloid deposits and apoE in the brain of BERKO mice, indicating that alteration in ERβ signaling pathway of functional neurons directly involved in neurodegenerative disease, supporting the hypothesis that ERβ plays a critical role in the neuroprotective effect of estrogen in brain.

Multiple levels of evidence implicate a central role of Aβ in the pathophysiology of AD. It is indicated that the neuritic dystrophy, neurofibrillary tangle formation, gliosis, microglial reactivity, and other degenerative changes seen in AD brains are results of altered metabolism of Aβ peptides[14].

Estrogen is identified as a potential modulator of Aβ precursor metabolism, it might also modify other factors contributing to Aβ deposition and fibril formation[15]. Our results implied the crucial point of ERβ in modulating Aβ metablism in brain. It is in line with previous report of neural loss and gliosis in BERKO which are also pathological characteristics of AD[13].

The cognitive function loss is the hallmark of AD patients, which has much to do with the malfunction of limbic system and cortex[16]. The distribution of Aβ deposits in these vulnerable areas such as hippocampus, amygdala, and cortex of BERKO brain contribute di-

rectly to the disfunction.

Neurodegeneration in AD is a pathological condition of neurons rather than an accelerated way of ageing[17]. So it was not surprised to see Aβ deposits in the brain of BERKO mice as young as 2 months old. It also suggests that disruption of ERβ signaling of estrogen in brain may lead to Aβ deposition directly.

Phytoestrogen such as kaempferol protects PC12 neuroblastoma and T47D human breast cancer cells from beta-amyloid-induced toxicity. The effects of the weak estrogen receptor agonists alpha-estradiol and kaempferol were comparable to that of the strong estrogen receptor agonist 17β-estradiol, suggesting a mode of action independent from the nuclear estrogen receptor[18]. It is a pity that the author ignored ERβ and drew a wrong conclusion. Phytoestrogen kaempferol is a potent agonist of ERβ stronger than ERα[19], so it may act via ERβ instead of ERa to exert the same effect as ERα agonist beta-estradiol. The seemed-negative data conversely supported the significant role for ERβ in protecting the PC12 cells from beta-amyloid-induced toxicity.

The accumulation of apoE in BERKO also suggested a consequent event resulted from ERβ disruption. ApoE is a plasma cholesterol and phospholipid transporter which plays a central role in lipoprotein metabolism in the brain. The apoE epsilon4 allele (ApoEepsilon4) is associated with a selective increase in deposition of the 40-amino acid form of Aβ (Aβ40) in endstage AD. As to the mechanism of apoE involvement in AD, several possibilities have been put forward that invoke the binding to tau protein affecting its hyperphosphorylation and therefore the microtubular stability, a differential rate of clearance of neurotoxic Aβ to which apoE binds[20]. Moreover, apoE forms stable complexes with recombinant beta-amyloid precursor protein, so Aβ can be conformationally modified by apoE *in vitro*.

Although we found some AD-related pathological alterations in the brain of BERKO mice, it deserves further investigation to determine the first event elicited by the disruption of ERβ pathway in brain. It was reported that ApoE may promote hypertension and contribute to AD pathogenesis by enhancement of vasoconstriction[21]. So it is reasonable to speculate a link between ERβ, hypertension, ApoE, cerebral amyloid angiopathy and AD. Anyhow, from the therapeutic point of view, modulation of Aβ metabolism via ERβ signal pathway might be beneficial in individuals with, or at risk for AD.

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