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Research article

5-HT1B and other related serotonergic proteins are altered in APPswe mutation

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HIGHLIGHTS

- Both *in vitro* and *ex vivo* models with APPswe mutation are used.
- 5-HT1B and SERT expressions are reduced in APPswe models.
- Previous report of reduced released 5-HT in AD models is confirmed.

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ABSTRACT

Serotonergic dysfunction is implicated in Alzheimer's disease (AD). In addition, reductions in brain of both monoamine synthesis and release have been reported. Serotonin 1B receptors (5-HT1B), along with serotonin transporter (SERT) are among the regulators of extracellular 5-HT levels. We investigated the effect of the familial AD APP (Amyloid precursor protein) K670N/M671L double mutation, APP Swedish mutation (APPswe), on the expression of 5-HT1B, SERT, MAOA, p11 and 5-HT and its metabolite 5-HIAA in SH-SY5Y human neuroblastoma cell line stably transfected with APPswe mutation. In addition, hippocampal expressions of 5-HT1B and SERT were assessed in wild type and transgenic mice expressing APPswe mutation (Tg2576) at different age groups. We found a reduction of 5-HT1B as well as SERT in both APPswe *in vitro* and *ex vivo*. P11 and 5HT were also reduced, whereas 5HT turnover and MAOA were increased. Our results indicate that APPswe induced decreased 5-HT1B expression and 5-HT release, as well as increased MAOA activity and 5-HT breakdown. Further studies to explore the detailed mechanism behind reduced 5-HT1B and SERT in AD and their clinical implications are needed.

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1. Introduction

The neurodegeneration in Alzheimer's disease (AD) involves molecular and cellular events that eventually lead to dysfunction of many neural systems, including the serotonergic pathway [17]. The degeneration of serotoninergic neurons in AD affects the dorsal and median part of raphe nucleus, projecting to hippocampus, neocortex and other brain areas [17], and is associated with a rapid progression of symptoms and cognitive impairment [18], depression, psychosis and aggressive behavior [6,13].

Abbreviations: AD, Alzheimer's disease; 5-HT, 5-hydroxytryptamine; SERT, serotonin transporter; PS, presenilin; SLC6A4, salute family 6A4; PET, positron emission tomography; GAPDH, glyceraldehydes 3-phoshate dehydrogenase; IHC, immunohistochemistry; APPswe, APP Swedish mutation; Tg2576, transgenic mice for APP (K670N/M671L)gene; WT, wild type; ANOVA, analysis of variance.

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Excessive Aβ deposition and enhanced plaque formation in APPswe/PS1ΔE9 mouse model are associated with degeneration of serotonin (5-HT) releasing neurons [15]. The levels of 5-HT and its metabolite 5-hydroxyindolacetoacetic acid (5-HIAA) are also shown to be reduced in post mortem AD brain [17].

Modulation of 5-HT receptor activity can affect APP processing, for example an increase of the non-amyloidogenic pathway is associated with increased 5-HT4 activity [9]. Several other 5HT-receptor changes have been reported in AD, including decreases in densities of 5HT1A and 5HT2A receptors [17], and a loss of 5-HT1A in AD has been demonstrated using binding studies in post-mortem brain [2]. However, very little data describing the status of 5-HT1B receptors in AD is available. One postmortem binding study showed that 5-HT1B densities in the frontal and temporal cortices are reduced and correlated to cognitive and behavioral changes in AD [7]. 5-HT1B regulates 5-HT release and works as an autoreceptor by means of a negative feedback mechanism, that ultimately leads to decreased 5-HT release from the raphe nucleus [8]. 5-HT1B is widely expressed in the brain having presynaptic auto- or heteroreceptor functions in both serotonergic and non-serotonergic synapses [19].

Our aim here was to assess the effect of APPswe mutation in the expression 5-HT1B and related proteins that are involved in the regulation of 5-HT. We examined the expression of p11, SERT, and MAOA together with the released 5-HT and 5-HIAA in cell and animal models of AD-related amyloid pathology. We show that amyloid pathology produced by the overexpression of the APPswe mutation results in reduced expression of the serotonergic 5HT1B receptor *in vivo* and *ex vivo* as well as other significant alterations of the system.

2. Methods

2.1. Materials

The 5-HT1B antibody and the S100A10 (p11) antibody were purchased from (Abcam, Cambridge, UK). The SLC6A4 antibody was purchased from (Lifespan Bioscience, USA). Taqman gene expression assay for 5-HT1B, SERT, MAOA and GAPDH were purchased from (Life Technologies, Sweden) and MAO- Glo™ kit was purchased from (Promega, USA).

2.2. Transgenic mice

Female Tg2576 mice, overexpressing the human gene encoding the amyloid precursor protein (APP) with the Swedish double mutation (K670N/M671L), were used. The Tg2576 mice develop soluble Aβ aggregates and cognitive dysfunction at 3 months and amyloid plaques after 12 months [9]. Mice were sacrificed by decapitation and brain samples collected at 6, 9, 12 and 24 months of age. The hippocampi were dissected out on ice, weighed and fresh-frozen on a piece of foil on dry ice and stored in −70 °C. Experiments with mice brain samples were approved by the Stockholm Södra Animal Research Ethical Committee, S157/08.

2.3. Cell culture

SH-SY5Y cells were obtained from American Type Culture Collection (ATCC, USA). Stable transfection of APP with the Swedish KM670/671NL double mutation (APPswe) and a cytomegalovirus promoter was performed as described previously [25]. Empty vector (pcDNA3.1) transfected cells were used as control. APP gene expression for both type of cells are shown in (Fig. 1E).

2.4. Western blotting

Immuoblotting was performed as previously described [16]. Primary antibodies were diluted in TBS-Tween buffer in different working concentrations 1:1000. Each experiment was performed 3 times, with cell passage numbers between 5 and 20.

2.5. RNA purification, cDNA synthesis and relative real time- PCR by relative standard curve method

Total RNA from SH-SY5Y cell cultures and hippocampus tissue was isolated using RNeasy mini kit (Qiagen) and DNAse treatment (RNase-Free DNase Set Qiagen). Extracted RNA samples were then reverse transcribed using High Capacity cDNA Reverse Transcription kit (Applied Biosystems). For real-time PCR amplification assays, Relative Standard Method supplied by Applied Biosystem was used [14].

2.6. Laser scanning confocal microscopy and immunocytochemistry

Immunofluorescence staining for SH-SY5Y cells was performed as described before [25] and inspected with a Nikon Eclipse E600 W.

2.7. Measurement of 5-HT and 5-HIAA by high performance liquid chromatography with electrochemical detection (HPLC–ECD)

For 5-HT and 5-HIAA measurements from cell media, cells were plated in 6 well plates at constant density of 1.25×10^5 cells per well as described before [24]. Cells were incubated over night with serum free media. Concentrations of 5-HT and 5-HIAA in cell culture media were determined using HPLC with electrochemical detection previously described [23,11].

2.8. MAOA enzyme activity

MAO-A enzyme activity in the protein lysate was determined using MAO-Glo™ assay from Promega, USA as described before [20]. The values of luminescence signals are adjusted to their sample's protein concentration and expressed measured in relative light units (RLU) per μg of protein.

2.9. Statistical analysis

Unpaired one way *T*-test, Mann–Whitney for two groups' comparison, or ANOVA and Kruskall Wallis test if more than two groups were used to compare groups according to data normality (SPSS version 16). A p value of ≤ 0.05 was set as a level of significance. Data was expressed as mean ± SEM.

3. Results

3.1. 5-HT1B, p11 and SERT protein levels are reduced in cells overexpressing the APPswe mutation

To investigate the effect of APPswe mutation on 5-HT1B, p11 and SERT protein expression, 30 μl of the protein lysate, from cells ($n = 6$), was separated in 10% SDS gel and then immunoblotted with specific antibodies (Fig. 1A). APPswe transfected SH-SY5Y, (Fig. 1E), showed significantly lower levels of 5-HT1B than controls (cells transfected with an empty vector) ($p < 0.0001$) (Fig. 1B). The adapter protein p11 was also reduced in APPswe (p value <0.0001) as shown in (Fig. 1C). Finally, there was a non-significant trend toward reduced SERT in APPswe transfected cells compared to con-

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