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Pharmacological Modulations of the Serotonergic System in a Cell-Model of Familial Alzheimer's Disease

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Abstract. Serotonin (5-HT) plays a central role in the integrity of different brain functions. The 5-HT homeostasis is regulated by many factors, including serotonin transporter (SERT), monoamine oxidase enzyme (MAO), and several 5-HT receptors, including the 5-HT1B. There is little knowledge how the dynamics of this system is affected by the amyloid-β (Aβ) burden of Alzheimer's disease (AD) pathology. SH-SY5Y neuroblastoma cells transfected with the amyloid precursor protein (APP) gene containing the Swedish mutations causing familial AD (APPswe), were used as a model to explore the effect of Aβ pathology on 5-HT1B and related molecules including the receptor adaptor protein (p11), SERT and MAOA gene expression, and MAOA activity after treatment with selective serotonin reuptake inhibitor (SSRI) (sertraline), and a 5-HT1B receptor antagonist. Sertraline led more than 70 fold increase of 5-HT1B gene expression ($p < 0.001$), an increased serotonin turnover in both APPswe and control cells and reduced intracellular serotonin levels by 75% in APPswe cells but not in controls ($p > 0.05$). Treatment with the 5-HT1B receptor antagonist increased SERT gene-expression in control cells but not in the APPswe cells. 5-HT and 5-HT1B antagonist treatment resulted in different p11 expression patterns in APPswe cells compared to controls. Although MAOA gene expression was not changed by APPswe overexpression, adding 5-HT lead to a significant increase in MAOA gene expression in APPswe but not control cells. These findings suggest that the sensitivity of the 5-HT1B receptor and related systems is affected by APPswe overexpression, with potential relevance for pharmacologic intervention in AD. This may at least partly explain the lack of effect of SSRIs in patients with AD and depression.

Keywords: 5-HT1B receptor, Alzheimer's disease, APPswe, MAOA, p11, serotonin, SERT

INTRODUCTION

The neurotransmitter serotonin (5-hydroxytryptamine) or 5-HT plays a major role in a variety of biological functions including memory, mood, sleep, and cognition [1]. This monoamine is derived from

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the essential amino acid tryptophan and degraded by the enzyme monoamine oxidase A (MAOA) into 5-hydroxyindolacetoacetic acid (5-HIAA). A total of 14 different subtypes of 5-HT receptors facilitate the function of 5-HT. The large numbers of receptors subtypes, transporters, enzymes and intermediate metabolites contribute to both integrity and complexity of 5-HT pathway [2].

The serotonin transporter (SERT) regulates 5-HT transmission in synaptic space by facilitating uptake from the synaptic cleft into the presynaptic neuron. 5-HT also affects SERT, as 5-HT has been shown to increase SERT density *in vivo* in the limbic system and neocortex [3]. A variety of mechanisms, including pre- and post-synaptic receptors, regulate the production and release of 5-HT.

The 5-HT1B receptor is an important player in 5-HT synaptic homeostasis functioning as a presynaptic autoregulatory receptor to decrease 5-HT synthesis and release [4] in key brain areas such as hippocampus and entorhinal and frontal cortex. The 5-HT1B receptor also stimulates SERT [4, 5]. Of note, the 5-HT1B receptor also serves as a heteroreceptor and activation inhibits cholinergic output [6]. 5-HT1B expression in the cell membrane surface is regulated by the p11 (S100A10) protein [7, 8], which has been linked to depression [9].

Together with the 5-HT1A receptor, 5-HT1B receptor opposes the extracellular increase of 5-HT produced by antidepressant drugs such as the selective serotonin reuptake inhibitors (SSRI) through its negative autoregulation mechanism [10]. The 5-HT1B receptor gene expression is altered by chronic anti depressant treatment in animal models of depression [11]. The discrepancy between the rapid molecular and late clinical effect of SSRIs is attributed to the time needed for SSRI to desensitize both 5-H1A and 5-HT1B receptor and subsequent postsynaptic signaling changes [10]. 5-HT1B, together with other 5-HT receptors, has been shown to couple to the downstream protein mitogen-activated protein-kinases (MAPK), which might mediate long-term changes, such as synthesis of new proteins, a likely key event in the long-term effect of 5-HT [12, 13].

The 5-HT1B receptor is involved in mood [7] and cognition [14]. For example, in animal studies, stimulation of the hippocampal 5-HT1B receptor impairs reference memory and performance in spatial memory tasks [15], and cognition is improved after 5-HT1B receptor knock-out [16, 17] or after administration of a 5-HT1B receptor antagonis [14].

Many neurodegenerative disorders affect 5-HT synthesis, trafficking, transmission, reuptake and downstream signal transduction [18]. Alzheimer's disease (AD) is the most common neurodegenerative disease. In addition to the defining cognitive changes, neuropsychiatric symptoms, including depression and anxiety are common and have a significant effect on the quality of life of patients and their caretakers [19]. The pathological characteristics are neuronal loss, amyloid plaques and neurofibrillary tangles [20] where the deposition of amyloid-β (Aβ) peptides into plaques is believed to be the earliest pathological event, driving the disease [21]. AD is associated with major serotoninergic alterations due to involvement of the raphe nucleus and related projections [2, 22]. In addition, both soluble and insoluble Aβ species are associated with impaired synaptic plasticity and dysfunctional neurotransmission in serotoninergic neurons [23]. Reductions in 5-HT and its metabolite levels have been reported in brain tissue [24] and cerebrospinal fluid in AD [25]. Several 5-HT receptors have been studied in AD, but few studies of the 5-HT1B receptor exist. One report showed that 5-HT1B binding pattern is reduced in postmortem frontal and temporal cortices in sporadic AD and this reduction correlates with the cognitive decline [6]. The serotonergic changes in AD may contribute to both cognitive and neuropsychiatric symptoms in AD and also represent treatment targets [26]. Of note, whereas serotonergic drugs have antidepressant effect in the elderly, they seem to be less effective in AD [27] suggesting that the AD related serotonergic changes influence the response to serotonergic agents.

Given its key role in depression and cognition, a better understanding of the 5-HT1B receptor function and its interaction with SERT, p11, and 5-HT in AD will increase the understanding about pathophysiology of depression and cognitive decline in AD, and provide treatment-relevant information. We recently studied the 5-HT1B receptor and related molecules in cells transfected with the amyloid precursor protein (APP) containing the Swedish double mutation (APPswe), located next to the cleavage site of the β-secretase and leading to increased production of the Aβ peptides [28], and found that the 5-HT1B receptor was significantly reduced in APPswe cells [29]. Similar reductions were found in SERT and p11 whereas MAOA activity and 5-HIAA/5-HT turnover index were increased. To further understand how 5-HT1B receptor and related systems in AD react to pharmacological manipulations, we studied the effects of an

SSRI (sertraline), 5-HT and a specific 5-HT1B antagonist (SB224289) on the gene expression of these molecules, as well as elements of the mitogen activated protein kinase pathway (MAPK) and 5-HT and the degradation product 5-HIAA. We compared the outcomes of pharmacological modulations between APPswe cells and the empty vector control cells. We hypothesized that there would be significant differences between APPswe and control cells, which might have relevance for the occurrence of depression in AD and for the lack of efficacy to SSRI and other serotonergic antidepressants.

METHODS

Materials

5-HT, serotonin creatinine monophosphate was purchased from Sigma, USA. The 5-HT1B antagonist (SB224289) and sertraline Hydrochloride (sertraline HCl) were purchased from Tocris, UK. The human untagged 5-HT1B cDNA inserted into pCMV6-XL4 vector for the receptor overexpression was purchased from Origene, USA. MAO-Glo^{TM} kit for measuring MAOA enzyme activity was purchased from Promega, USA. The primary antibodies for the p70S6, phospho-p70S6 (Thr389), phospho-p70S6-(Thr421, Ser424), p70S6 and pMAPK (42–44), total MAPK and β-Actin were purchased from Cell signaling, USA. Taqman gene expression assay for the human 5-HT1B-receptor, SERT, MAOA, p11, RPLP0 and GAPDH were purchased from Thermo Fisher Scientific, USA. Proteome Profiler^{TM} Array, Human phospho-MAPK array kit was purchased from R&D systems, UK.

Cell culture and pharmacological modulation

Human neuroblastoma cell line, SH-SY5Y, was obtained from American Type Culture Collection (ATCC, USA). The cDNA sequence for APP with the Swedish KM670/671NL double mutation (APPswe) was cloned into a pcDNA3.1 vector, carrying the gentamycin resistance gene. The vector contained a cytomegalovirus promoter, and was stably transfected into SH-SY5Y cells, cell transfected with the empty vector are used as control group in this study. We used the same APPswe cell model showing increased levels of APP, $A\beta_{42}$, and $A\beta_{40}$ [30].

Modification of the serotonergic system was performed by treatment with the selective 5-HT1B antagonist (SB224289) and, 5-HT and the SSRI sertraline hydrochloride. An overnight starvation of cells in serum-free medium preceded treatments. The experimental conditions for these experiments are shown in (Supplementary Table 1).

5-HT1B receptor transient overexpression

To further evaluate changes in 5-HT metabolites after modification of the 5-HT1B receptor, a 5-HT1B transient overexpression was performed. A total of 4×10^5 of SH-SY5Y cells were seeded on a 6-multiwell plate to reach 80–90% confluence at the time of transfection. Before transfection, cells were washed twice with PBS and 800 μl of Opti-Mem (Thermo Fisher Scientific, USA) was added. A total of 1.2 μg of human 5-HT1B cDNA (Origene, USA) and 3 μl of Lipofectamine (Life Technologies, Sweden) were mixed separately and incubated for 5 min with 100 μl of Opti-Mem. Empty vector was used as a control. The 5-HT1B cDNA was then combined with the Lipofectamine, incubated for 15 min and added to the cells. Five hours later, the medium was discarded and fresh complete medium was added to the cells. After 24 h, medium was discarded; cells washed twice in PBS and 1 ml of medium without serum was added to the cells. Twenty-four hours later, medium and cells were collected and stored at –80°C for further analysis. Medium was centrifuged at 800 g for 5 min to remove possible floating cells or debris before freezing.

Western blot

Western blot for protein quantification was performed as previously described [29, 31]. Each experiment was performed 3 times with cell passages between 4 and 16 and (n = 6–9). All primary antibodies were diluted in TBS-Tween 1:1000 dilution. Secondary anti-mouse or anti-rabbit antibodies were diluted 1:5000.

Relative real-time RT-PCR (rtRT-PCR) using Relative Standard Curve Method

Total RNA was isolated using RNeasy mini and DNase treatment (RNase-Free DNase Set, Qiagen, USA). Quality and concentration of extracted RNA was determined with agarose gels and Nanodrop. A total of 20 0 ng of RNA was then reversely transcribed using High Capacity cDNA Reverse Transcription kit (Applied Biosystems, USA). We used the relative standard method as shown before

[29, 32]. The stability of the endogenous control, both for Glyeceraldehyde-3-Phosphate (GAPDH) and the large ribosomal protein (RPLP0) were assessed using the normalization factor (NF) for both endogenous genes which was calculated using GeNorm 3.3 visual basic application for Microsoft Excel [33]. Results are expressed as mRNA copy numbers of all target transcripts adjusted by a NF and the values calculated were compared to controls (set at 100%) and reported as the mean ± SEM in all experiments.

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

For 5-HT and 5-HIAA measurements in cell media (extracellular) and cell lysate (intracellular) HPLC-ECD was used, as described before [34]. The measurements of these metabolites were expressed as concentration units for 5-HT and 5-HIAA separately or a 5-HIAA/5-HT ratio was calculated as an index for 5-HT turnover. Briefly, the HPLC system consisted of a HTEC500 (Eicom, Kyoto, Japan), and a CMA/200 Refrigerated Microsampler (CMA Microdialysis, Stockholm, Sweden) equipped with 20 μl loop and operating at +4°C. The potential of the glassy carbon working electrode was +450 mV versus the Ag/AgCl reference electrode. The separation was achieved on a 200 × 2.0 mm Eicompak CAX column (Eicom, Kyoto, Japan). The mobile phase was a mixture of methanol and 0.1M phosphate buffer (pH6.0) (30 : 70, v/v) containing 40 mM potassium chloride and 0.13 mM EDTA-2Na. Concentrations of 5-HIAA were determined by a separate HPLC system with electrochemical detection (HTEC500). The potential of the glassy carbon working electrode was +750 mV versus the Ag/AgCl reference electrode. The separation was achieved on a 150 × 3.0 mm Eicompak SC-5ODS column (Eicom, Japan). The mobile phase was a mixture of methanol and 0.1 M citrate – 0.1 M sodium acetate buffer solution (pH3.5) (16 : 84, v/v) containing 210 mg/L Octanesulfonic acid sodium salt and 5 mg/L EDTA-2Na. The chromatograms were recorded and integrated by use of the computerized data acquisition system Clarity (DataApex, The Czech Republic).

MAPK protein arrays

The relative phosphorylation of the human MAP kinases were determined using Proteome Profiler™ Array, Human phospho-MAPK array kit (R&D systems, UK) [35]. Membranes were blocked with Array buffer 5 for 1 h at room temperature. A total amount of 150 μg of protein, from control cells or APPswe cells, was incubated together with the antibody detection cocktail and membranes overnight at 4°C on a rocking shaker. The membranes were then washed 3 times for 10 min each and incubated in streptavidin HRP, diluted in buffer 5, for 30 min on a rocking shaker at room temperature. Membranes were washed again with washing buffer 3 times for 10 min each and then incubated for one minute using 1 ml of substrate reagent per membrane. Finally, multiple exposures using CCD camera were performed.

In a previous study, the p70S6 kinase was shown to couple with 5-HT1B receptor [36], therefore we explored the effects on the three specific phosphorylation for the p70S6 kinase; phospho-p70S6 kinase (Thr389), phospho-p70S6 kinase (Thr421, Ser424) or phospho-p44/42 MAPK (p-ERK1/2) (Thr202/ Tyr204), using western blotting.

MAOA enzyme activity

The enzyme activity of MAOA was assessed using MAO-Glo™ kit (Promega, USA) as described before [37]. Briefly, 12.5 μl of 4X MAO substrate preparations was combined with 12.5 μl of 4Xtest preparation to create methyl ester luciferase. Twenty-five μl of MAOA enzyme was added to each well to initiate the MAOA reaction. For negative controls, either 25 μl of MAOA enzyme or 12.5 μl of test preparation were added and the plate was incubated at room temperature for 1 h. To generate stabilized luminescence signals, 50 μl/well of luciferase detection solution was added and incubated again at room temperature for 20 min. A plate reader (Tecan Safire II) was used to measure luminescence at an integration time of 1 s per well. Relative light units (RLU) were calculated by subtracting values from negative controls without MAOA enzyme from test preparations.

Statistical analysis

Depending on data normality assessed by Kolmogorov-Smirnov test (SPSS version 22), unpaired T-test or Mann-Whitney test was used for comparison of two groups, whereas one way ANOVA or Kruskall Wallis test followed by Tukey's *post hoc* or Dunn's tests were used depending on normality and number of groups in the analysis. A p-value of ≤ 0.05 was set as a level of significance. Data are either expressed as mean ± SEM when normally distributed

or as median ± interquartile range (IQR) otherwise, in addition to plots showing the distribution. Each experiment is repeated 3–6 times independently.

RESULTS

SERT, p11, and 5-HT1B gene expression

A large and significant increase of 5-HT1B mRNA by 70,4 fold was observed after sertraline treatment in APPswe cells ($p < 0.001$) but not in control cells as shown in (Fig. 1A). Sertraline treatment did not affect SERT gene expression in any of the groups (Fig. 1B).

Treatment with the 5-HT1B-receptor antagonist led to a 51% increase SERT gene expression in control cells, both with ($p < 0.001$) and 22.7% increase in without ($p < 0.05$) adding 5-HT, which did not occur in APPswe cells (Fig. 1C). No effects of addition of 5-HT were observed on 5-HT1B gene expression in either group (Fig. 1D).

The basal gene expression of p11 is markedly increased by 12.7 fold in APPswe compared to control cells ($p < 0.0001$) (Fig. 1E). A further non-significant increase was observed after the 5-HT1B antagonist treatment in APPswe cells, but not in control cells. Combination of 5-HT1B antagonist and 5-HT treatment resulted in reduced expression of p11 in both controls and APPswe cells.

5-HT and 5-HIAA measurements after sertraline and 5-HT1B antagonist

No significant difference was observed between the intracellular 5-HT at the basal levels or after treatment with the antagonist (SB224289) (Fig. 2A). Sertraline treatment led to a significant reduction in intracellular 5-HT levels in APPswe cells by around 75% ($p < 0.05$) (Fig. 2B). There was no effect of the antagonist (Fig. 2C), but sertraline increases intracellular 5-HIAA in both APPswe and control cells by 1.6 and 1.8 fold, respectively ($p < 0.05$ and 0.01 respectively) (Fig. 2D). Although sertraline produced no change in intracellular 5-HT or 5-HIAA/5-HT ratio in control cells (Fig. 2E), it significantly increased 5-HIAA/5-HT ratio in APPswe cells ($p < 0.05$) (Fig. 2F). As previously reported [29] 5-HT levels were reduced in media of untreated APPswe cells by 59.2% compared to control cells ($p < 0.05$) (Fig. 2G). No effect on the extracellular cell medium levels of 5-HT, 5-HIAA, or the 5-HIAA/5-HT ratio was detected after sertraline treatment (Fig. 2H, J, and L).

Neither 5-HT1B receptor antagonist (Fig. 2I) nor 5-HT1B transient overexpression, (Supplementary Figure 1), produced an effect on 5-HT or 5-HIAA in either cell group.

MAOA activity and gene expression

As shown in (Fig. 3A) and consistent with our and other previous reports [29, 38, 39], which showed increased MAOA basal activity in APPswe. APPswe cells revealed a higher MAOA activity compared to control cell (2.8 fold, $p < 0.05$). However, we observed a significant reduction of MAOA activity in APPswe cells treated with sertraline ($p < 0.001$) (Fig. 3A). Baseline MAOA gene expression did not differ between APPswe and control cells, but when adding 5-HT a significant increase in MAOA gene expression, a total of 2.2 fold increase was observed in APPswe cells ($p < 0.05$), but not in the control cells (Fig. 3B). No effect was observed on MAOA gene expression in either group after treatment with the 5-HT1B receptor antagonist.

Changes in MAOA gene expression after 5-HT treatment, could be related to the increased folds of 5-HIAA/5-HT ratio observed after adding 5-HT. Pre-treatment with 5-HT led to increased 5-HIAA productions in cell lysate and the cell media in both types of cells ($p < 0.05$ and <0.001 respectively), fold of changes are shown in (Fig. 3C, D), which seemed to be more pronounced in the APPswe than control cells.

MAP kinase phosphorylation

At baseline, the relative phosphorylation status of MAP kinases was reduced in the APPswe cells compared to the control as demonstrated by lower densities on dot blots (Fig. 4). Addition of 5-HT1B receptor antagonist reduced the MAPK phosphorylation in both groups. When adding 5-HT to the antagonist, the inhibition was lost in APPswe cells, whereas densities remained low or decreased even further in the control group. However, the Western blot analysis of cell homogenates did not indicate that the any of the drugs had a significant effect on the phosphorylation degree of phospho-70S6 kinase, in either group (Fig. 5).

DISCUSSION

Here, we report the effects of inhibition of SERT and the 5-HT1B receptor on a number of sero-

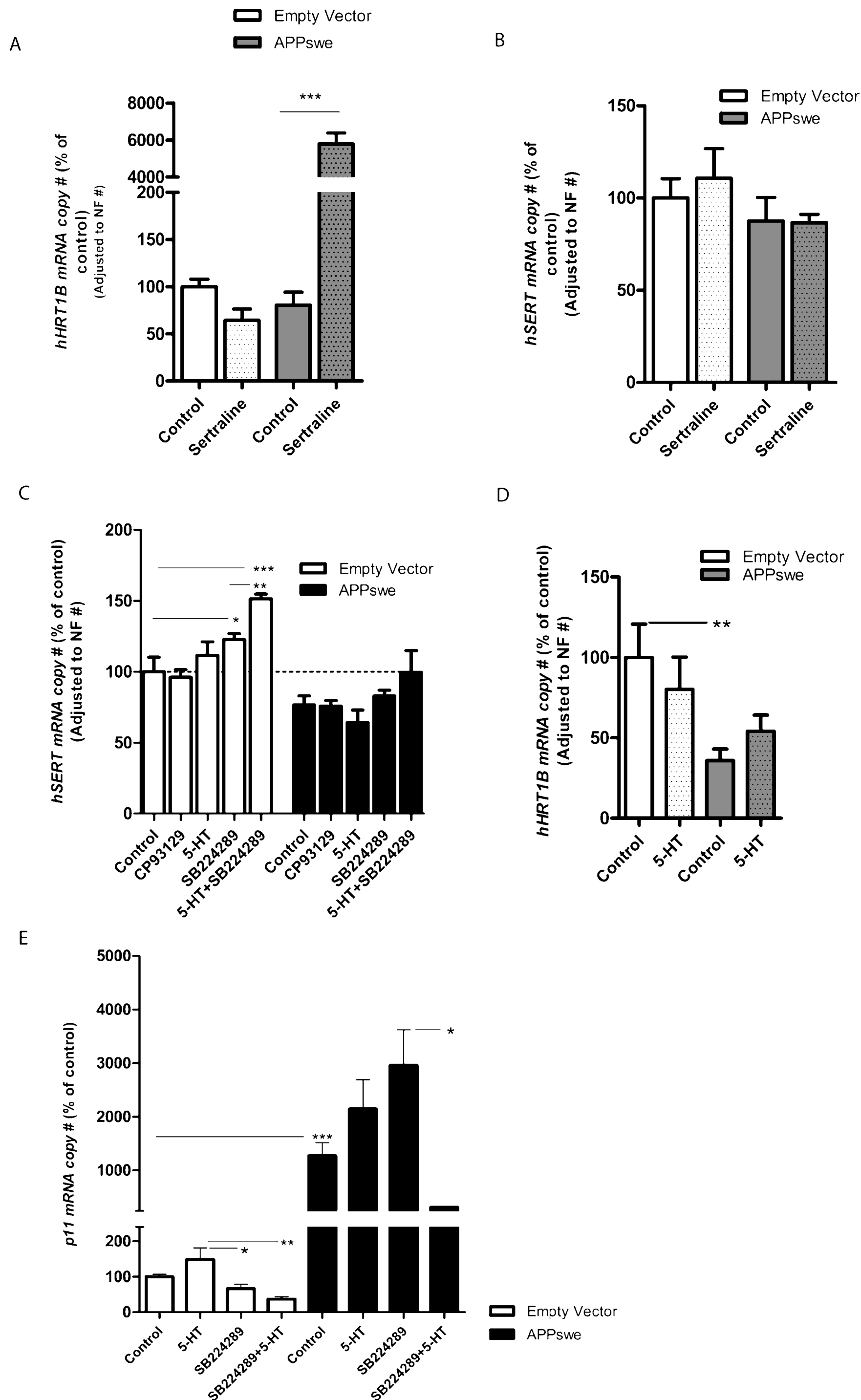

Fig. 1. Effect of modulations on SERT, 5-HT1B receptor, and p11 gene expression. A) Sertraline treatment effect on the 5-HT1B receptor and (B) SERT gene expression in APPswe (darker bars) and control cells. C) SERT gene expression and 5-HT1B modulations (D) effect of 5-HT on 5-HT1B gene expression. E) 5-HT1B gene expression is measured after 5-HT treatment. E S100A10 (p11) mRNA is measured after 5-HT1B pharmacological modifications. Data is represented as mean ± Standard error of mean (SEM). $^{*}p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$. Difference was assessed by T test within the same group.

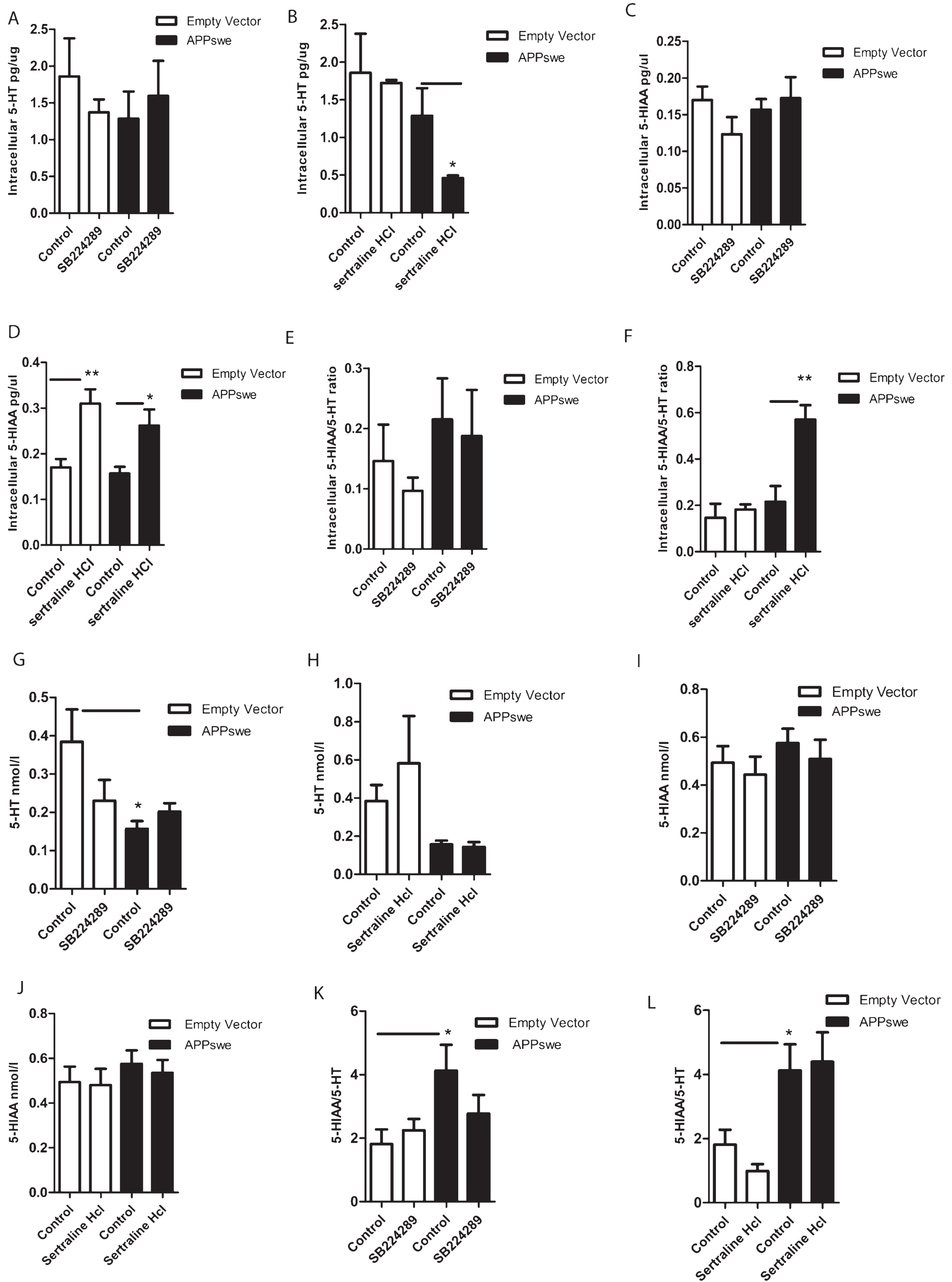

Fig. 2. Effect on sertraline and 5-HT1B antagonisms on 5-HT metabolites and 5-HIAA/5-HT index. A-F) The effect on intracellular metabolites and their 5-HIAA/5-HT index. G-L) The effect on extracellular metabolites and their 5-HIAA/5-HT index are measured in cell medium. Data is represented as mean ± Standard error of mean (SEM). $^{*}p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$. T test was used to compare APPswe and control cells groups.

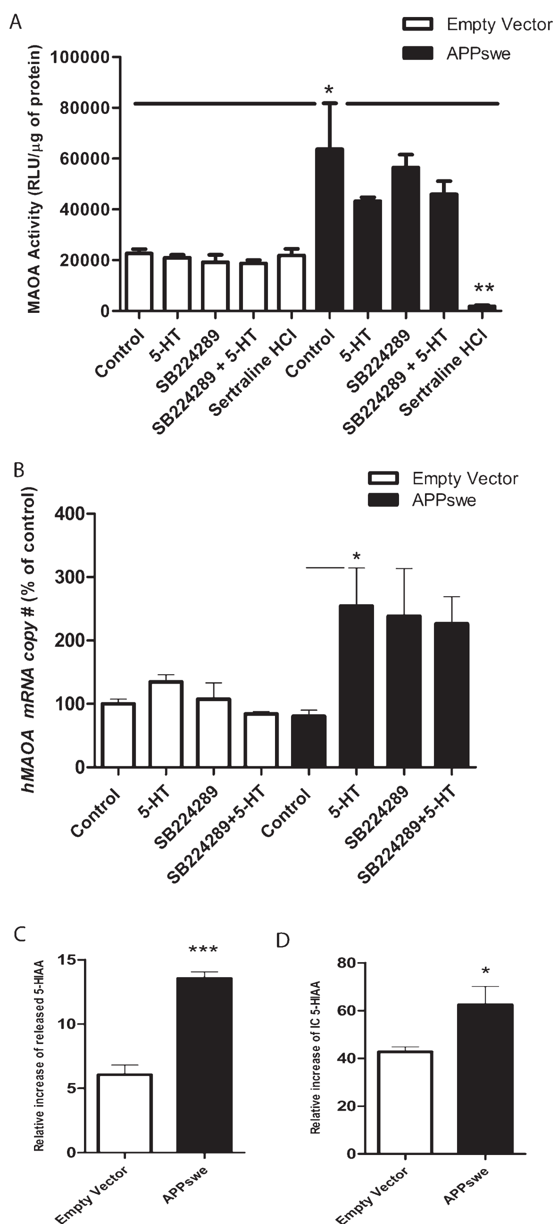

Fig. 3. Modulation outcomes in terms of MAOA alterations and 5-HIAA folds. A) MAOA enzyme activity and B) gene expression were measured after 5-HT, sertraline, and 5-HT1B modulation. Relative increase of 5-HIAA after 5-HT treatment in APPswe cells was measured in (C) cell medium (released) and (D) intracellular. Data is represented as mean ± Standard error of mean (SEM). $^{*}p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$. Difference was assessed Kruskall- Wallis test followed by Dunn's *post hoc* to compare different groups.

tonergic effector molecules including downstream post-synaptic systems in a neuroblastoma cell line overexpressing the APPswe mutation of familial AD. Our findings support the hypothesis that the amyloid changes associated with the APPswe mutation affect the 5-HT1B receptor and related molecules of the serotonergic system, with potential relevance for treatment with serotonergic antidepressants.

A main finding was that the SSRI sertraline induced a significant increase of 5-HT1B mRNA, combined with an increased turnover and reduced intracellular levels of 5-HT in the APPswe cells compared to the control cells.

Several other relevant differences in the 5-HT1B dynamics were found in APPswe cells: First, the 5-HT1B antagonist induced an increased SERT gene expression in control cells, possibly a compensatory up-regulation secondary to a reduction of SERT after 5-HT1B antagonism. This was not observed in APPswe cells (Fig. 1C). Second, p11 gene expression was increased, with a (non-significant) additional increase after treatment with the 5-HT1B antagonist in APPswe compared to control cells (Fig. 1E). Third, MAOA gene expression was increased after adding 5-HT in APPswe (Fig. 3B). Finally, there were indications that the MAPK phosphorylation was altered, and that the kinase-inhibition by the 5-HT1B antagonist was lost, in APPswe cells (Figs. 4 and 5).

These findings have potential clinical and therapeutic relevance in AD. The observed changes may be implicated in the mechanisms underlying the increased occurrence of depression [19], as well as the lack of effect of SSRIs and other antidepressants in AD [27], and thus provides important background information relevant for the development of novel antidepressants for people with AD.

Elevated 5-HT1B activity has been implicated in the etiology and treatment of depression [11], and desensitization of the 5-HT1A and 5-HT1B, autoregulatory effect, which results in increased 5-HT release, seems to be a requirement for the clinical effect of SSRI [10]. Accordingly, the large increase of 5-HT1B gene expression observed in the APPswe model may counteract the normal increased 5-HT release required for the clinical effect of long-term sertraline treatment.

Other observed serotonergic changes may also be relevant for the effect of antidepressants in AD. At baseline, MAOA activity is increased in APPswe cells, and MAO gene expression increased selectively in APPswe cells after treatment with 5-HT. Together with a trend toward reduced MAPK activity, these changes may both increase risk for depression as well as negatively influence response to antidepressants in AD.

On the other hand, p11 gene expression was increased (Fig. 1E), possibly as a compensation

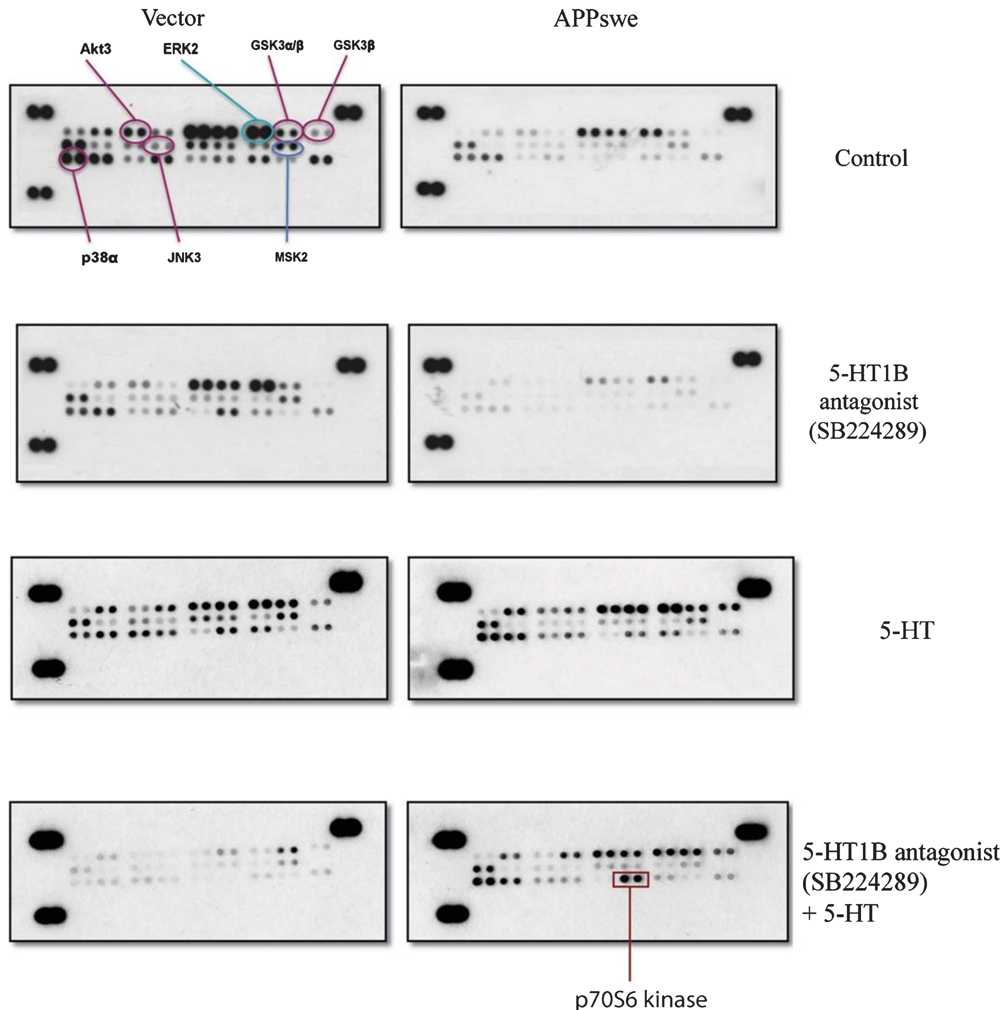

Fig. 4. MAPK phosphorylation patterns after 5-HT and the 5-HT1B receptor blockade assessed by MAPK array. Different patterns of MAPK phosphorylation after 5-HT1B receptor modulation between APPswe cells and empty vector transfected cells. MAPK arrays show the effect of 5-HT, SB224289 and the combination of each in APPswe and empty vector cells. 150 micrograms of protein are added to each group after modulation with SB224289 alone or followed by 5-HT.

for the reduced p11 protein level in AD that we had shown previously [29]. Similarly, the reduced 5-HT1B expressions at baseline might be compensatory effect for lower extracellular 5-HT levels in APPswe, and these changes may reduce the risk of depression and possibly enhance the effect of serotonergic antidepressants. Our findings suggest that 5-HT1B antagonism, in combination with 5-HT1A antagonism, which have been shown to potentiate antidepressant effect in non-AD patients [1, 3], may counteract the SSRI-induced increase of mRNA 5-HT1B, and thus might be particularly relevant for antidepressant therapy in people with AD. The lack of increased SERT expression in AD after 5-HT antagonist supports the potential usefulness of this strategy. On the other hand, 5-HT1B antagonist reduced the downstream effectors in both cell groups, which might argue against an antidepressant effect of this drug class, although the inhibition was lost after addition of 5-HT in the APPswe cells. Further studies of AD animal models are needed to shed more light on whether 5-HT1B antagonism alone, or together with an SSRI, may improve depression.

Biomarkers reflecting the accumulation of Aβ deposition in brain are the earliest detectable sign of AD in healthy elderly [38, 40] and studies both in autosomal dominant AD and late-onset AD suggest that tangle formation occurs after deposition of

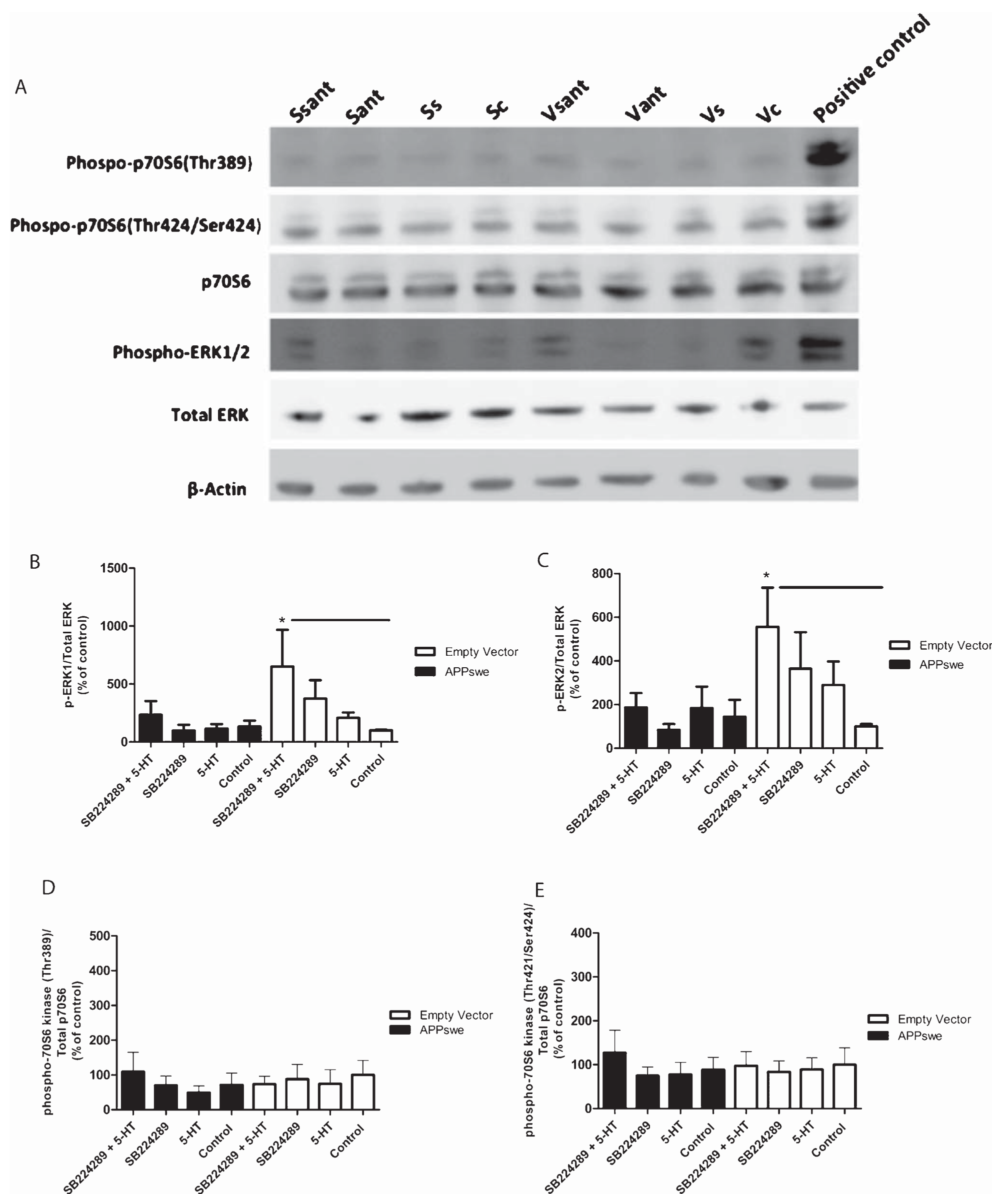

Fig. 5. ERK and p70S6 kinases after 5-HT and the 5-HT1B receptor blockade assessed by western blotting. Western blotting for the phospho-p44/42 MAPK (p-ERK1/2) (Thr202/Tyr204), phospho-p70S6 kinase (Thr389), phospho-p70S6 kinase (Thr421-Ser424), total ERK and p70s6 kinases to show the effect on phosphorylated proteins levels after treatment with either SB224289 1 μM, 5-HT 10 μM, or a combination of both. VC stands for empty vector transfected (control) cells treated with vehicle alone, VS for control cells treated with 5-HT, Vant for control cells treated with the 5-HT1B antagonist SB224289 and Vsant for control cells treated with both 5-HT and SB224289. SC stands for APPswe cells treated with vehicle alone, SS for APPwe treated with 5-HT, Sant for APPswe treated with the 5-HT1B antagonist SB224289 and Ssant for APPswe treated with both 5-HT and SB224289.

Aβ in brain [38]. Based on this, we chose a cellular model where the production of Aβ is increased partly [28] mimicking the disrupted metabolism of Aβ in AD. Previous studies demonstrate a link between the serotonergic system and Aβ production and clearance [41, 42]. Our findings suggest that amyloid species such as $A\beta_{42}$ and $A\beta_{40}$, which are high in APP-swe mutation models, alter the serotoninergic system integrity at gene expression, 5-HT transmission or MAPK signal transduction levels.

There are some limitations that need to be discussed. Firstly, we studied only acute effects of

sertraline and 5-HT1B modulations, and thus the effects of chronic treatment in the APPswe model, arguably more interesting than the acute effect from a clinical perspective, are not known. Secondly, we did not apply different dose and time point scales, including different concentrations and duration of the treatment condition, which are required to clarify the effect of the compounds in AD cells. This limits the conclusions that can be drawn by our studies. Thirdly, this adherent type of cell line doesn't necessarily form histological synapses, and no synaptosome fraction can be derived, making extrapolation of these findings to the 5-HT pathway in the human central nervous, challenging. Fourthly, no uptake studies were performed to understand SERT mediated 5-HT clearance. Only a single time point and concentration was used, however these were chosen based on previous reports. Although we did not observe the expected reduction of 5-HT after 5-HT1B antagonist treatments, in contrast to another study [43], this discrepancy could be attributed to different cell-line types used in the two different studies and remains to be resolved. Moreover, the lack of and additional control cell overexpressing the APP wild type, without the APPswe mutation, is another limitation of this study.

Finally, these preliminary findings need to be supported by similar experiments in other AD cell models as well as AD *in vivo* models. A cell model overexpressing the wild type of APP gene would have provided additional information about the mechanisms driving the effects on the 5-HT system.

In summary, we observed altered transcriptional activity of SERT, p11, MAO and MAPK to changes in 5-HT1B receptor activities in APPswe neurons with potential clinical relevance. In particular, the increased gene expression of 5-HT1B after sertraline may inhibit the cellular changes needed for an antidepressant effect to occur. Further pharmacological studies of the 5-HT1B and related molecules in *in vivo* AD models are required.

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SUPPLEMENTARY MATERIAL

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