Sigma-2 receptor modulators including CT1812 modulate low-density lipoprotein uptake in primary rat neurons

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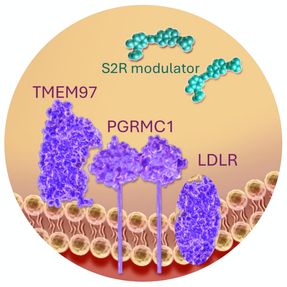
Poster #1507

Introduction

The sigma-2 receptor (S2R, TMEM97) and low-density lipoprotein receptor (LDLR) complex regulates the metabolism of low-density lipoprotein (LDL). Previously, we demonstrated that S2R modulators increased LDL uptake in human retinal pigment epithelium (RPE) cells, and rescue α-synuclein and amyloid-β oligomer-induced trafficking deficits in primary rat neuronal culture[1,2,3,4]. Given that dysregulated lipid metabolism in the brain is a key factor associated with Alzheimer's disease (AD) Parkinson's disease (PD), and dementia with Lewy bodies (DLB) we hypothesized that S2R may modulate this process in neurons as well, and assessed the effects of S2R modulation on LDL transport in rat primary neurons.



Schema 1. The sigma-2 receptor complex is comprised of TMEM97 and PGRMC1 and interacts with the LDL receptor (LDLR)

Methods

Embryonic (E18) rat primary cortical hippocampal neurons were plated in 384 well microplates and cultured for 21 days. Neuronal population and maturity were characterized by microtubule-associated protein 2 (MAP2) staining.

Cells were treated with vehicle, sigma 1 receptor (S1R), or S2R selective modulators at 1, 3, and 10 μM (see schemas below). U18666A, an intra-cellular cholesterol transport inhibitor, was used as a positive control. After 3 hours of treatment with fluorescently tagged LDL-DyLight550 (LDL-DyL), cells were immunostained with MAP2 and high-content imaging analysis was used to assess the uptake of LDL-DyL.

Wildtype and TMEM97 knockout HEK-293T cells were seeded in 96 well plates and cultured for 24 hours prior to compound treatment with S2R modulator and LDL-DyLight 550 assay.

Cell culture → Sr1/ Sr2 compound treatment (16/24 hr) → LDL DyLight Assay (3 hr)

Results

S2R, but not S1R, modulators increase LDL uptake in a concentration dependent manner

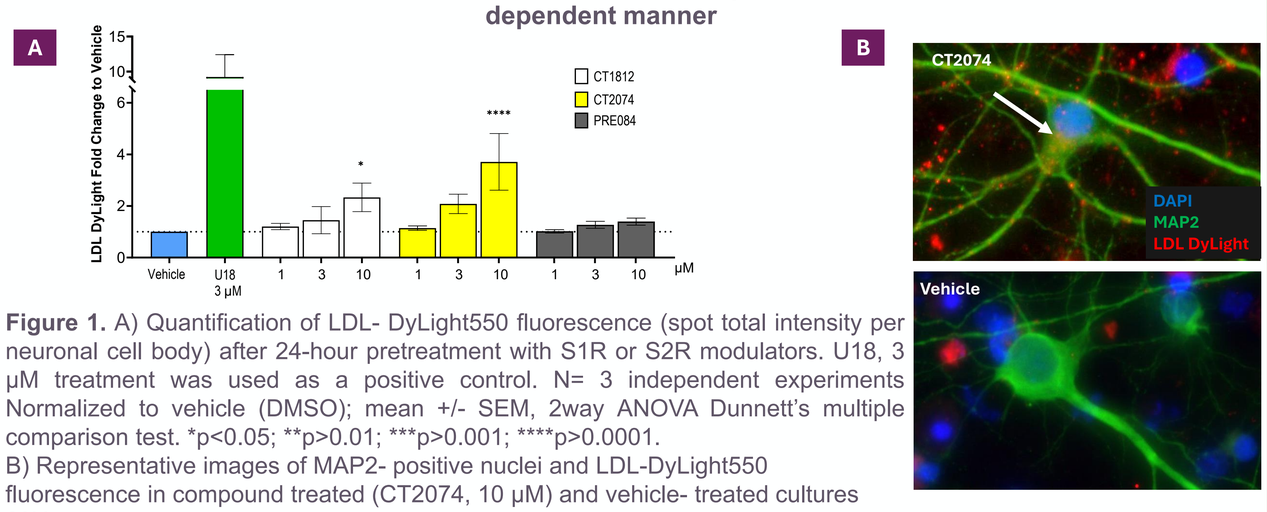


Figure 1. A) Quantification of LDL- DyLight550 fluorescence (spot total intensity per neuronal cell body) after 24-hour pretreatment with S1R or S2R modulators. U18, 3 μM treatment was used as a positive control. N= 3 independent experiments Normalized to vehicle (DMSO); mean +/- SEM, 2way ANOVA Dunnett's multiple comparison test. *p<0.05; **p>0.01; ***p>0.001; ****p>0.0001.
B) Representative images of MAP2- positive nuclei and LDL-DyLight550 fluorescence in compound treated (CT2074, 10 μM) and vehicle- treated cultures 60X

LDL uptake is dependent on S2R/TMEM97

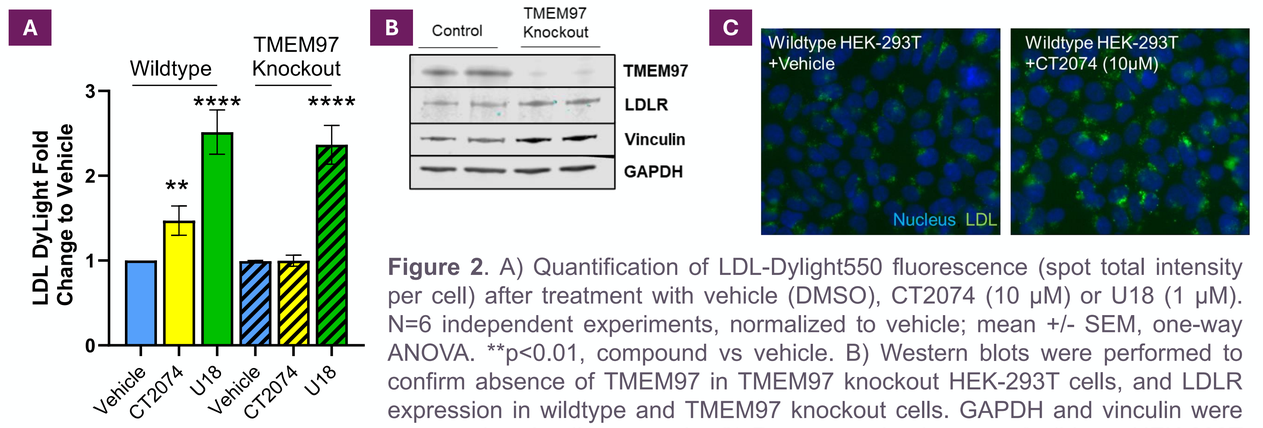


Figure 2. A) Quantification of LDL-Dylight550 fluorescence (spot total intensity per cell) after treatment with vehicle (DMSO), CT2074 (10 μM) or U18 (1 μM). N=6 independent experiments, normalized to vehicle; mean +/- SEM, one-way ANOVA. **p<0.01, compound vs vehicle. B) Western blots were performed to confirm absence of TMEM97 in TMEM97 knockout HEK-293T cells, and LDLR expression in wildtype and TMEM97 knockout cells. GAPDH and vinculin were assessed as loading controls. C) Representative images of wildtype HEK-293T cells treated with vehicle or CT2074, labeled with LDL-DyLight (*green*) and Hoechst (*blue*).

Compound pretreatment is required to increase LDL uptake

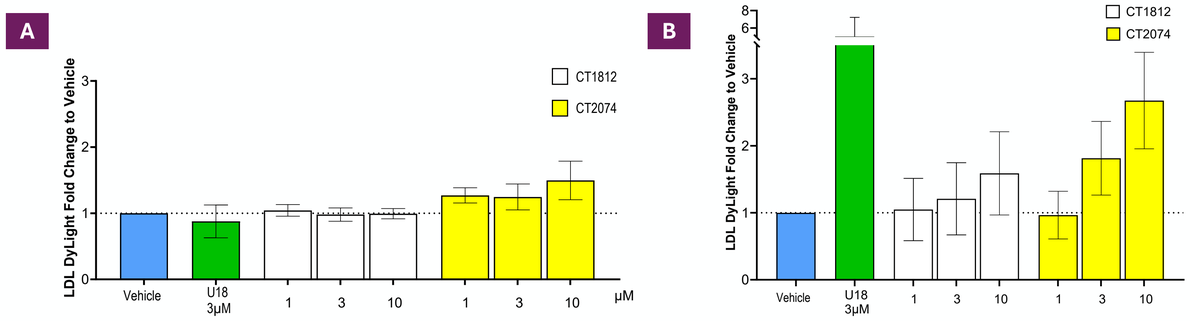


Figure 3. A) Quantification of LDL- DyLight550 fluorescence (spot total intensity per neuronal cell body) after 3-hour concurrent treatment with S2R modulators and LDL-DyLight550. U18, 3 μM treatment was used as a positive control. N= 3 independent experiments Normalized to vehicle (DMSO); mean +/- SEM. B) Quantification of LDL- DyLight550 fluorescence (spot total intensity per neuronal cell body) after 24-hour pretreatment with S2R modulators. U18, 3 μM treatment was used as a positive control. N= 1 experiment normalized to vehicle (DMSO) mean+/- stdev

S2R modulators in combination with PRE084 increase LDL DyLight uptake more than S1R or S2R modulators alone

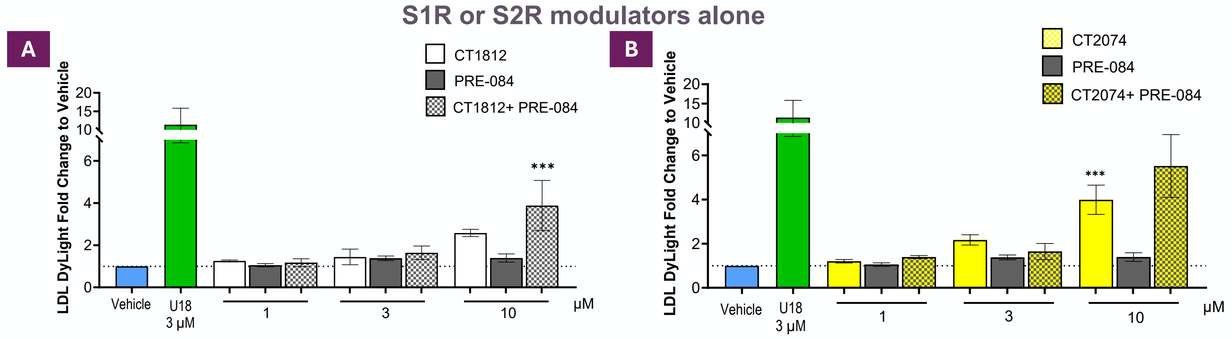


Figure 4. Quantification of LDL- DyLight550 fluorescence (spot total intensity per neuronal cell body) after 24-hour treatment with S1R modulators, alone or in combination with (A) CT1812 or (B) CT2074 and LDL-DyLight550. U18, 3 μM treatment was used as a positive control. N= 3 independent experiments Normalized to vehicle (DMSO); mean +/- SEM, 2way ANOVA Dunnett's multiple comparison test. *p<0.05; **p>0.01; ***p>0.001; ****p>0.0001.

Conclusions

- S2R, but not S1R, modulators increase LDL uptake in a concentration-dependent manner
- We confirm in a neuronal model our previous findings[1] that S2R modulators induce LDL uptake in a TMEM97-dependent manner
- While S1R modulators alone did not induce LDL uptake, a synergistic increase in LDL uptake was observed when both S2R and S1R were added concomitantly
- Overall, our data underline a possible role of S2R modulators in regulating lipid metabolism, a key factor in AD

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References:
1. Lizama BN. et al. Delineating mechanisms of sigma-2 receptor modulators in regulating retinal pigmented epithelial lipid uptake. Poster presented at ARVO 2025; Salt Lake City, UT
2. Limegrover C. et al Sigma-2 receptor antagonists rescue neuronal dysfunction induced by Parkinson's patient brain-derived α-synuclein. *Journal of neuroscience research*, 99(4), 1161–1176.
3. Izzo NJ, et al.. Alzheimer's therapeutics targeting amyloid beta 1-42 oligomers I: Abeta 42 oligomer binding to specific neuronal receptors is displaced by drug candidates that improve cognitive deficits. PLoS One. 2014 Nov 12;9(11):e111898
4. Izzo, N.J. et al. Preclinical and clinical biomarker studies of CT1812: A novel approach to Alzheimer's disease modification. Alzheimers. Dement. 2021; 17(8); 1365-1382.

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