Identifying and Testing Novel Sigma-1 Receptor Ligands for Neurodegenerative Diseases.

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Neurodegenerative diseases (NDs) are becoming more common due to increased life expectancy. There is currently no drug that can slow or halt the progression of Alzheimer's disease (AD). The sigma-1 receptor (σ 1R) is a chaperone protein located at the junction of the endoplasmic reticulum (ER) and the mitochondria in a region called the mitochondrial-associated membrane (MAM). Activating the σ 1R leads to the chaperoning of ion channels and other proteins involved in cell survival. One of its primary functions in neuron cells is the chaperoning of the IP₃ receptor at the MAM during cellular stress, leading to Ca²⁺ shuttling into the mitochondria, stimulating ATP production, and leading to cell survival, growth, division, or differentiation. The σ 1R is an interesting target for treating NDs such as AD since its activation provides various cell survival benefits, including reducing ER stress and regulating autophagy. There are several σ 1R drugs currently undergoing clinical trials for NDs.

This study aimed to evaluate the σ 1R activity of dipentylammonium (DPA), which has previously been shown to bind to the receptor with nanomolar affinity and have antidepressant-like effects. Furthermore, we hoped to find other novel σ 1R ligands.

We have used *in vitro* (PC-12 and HT-22 cells) and *in vivo* (*C.elegans*, wild type, and mutated strains) techniques to evaluate various σ 1R ligands, particularly DPA. PC-12 cells were cultured in 6-well plates coated with poly-d-lysine and treated with NGF (10 ng/ml) with or without DPA. The resulting neurites were photographed and measured using Image J. The prevention of damage to 7-day NGF (10 ng/ml) differentiated PC-12 neurites caused by 10 μ M A $\beta_{(25-35)}$ by DPA was measured by the MTT assay, and the measurement of neurite length 24 hours after 10 μ M A $\beta_{(25-35)}$ treatment. Immunoprecipitation using an antibody specific to the σ 1R was used to measure colocalization with Binding Immunoglobulin Protein (BiP) visualized using western blotting with a BiP-antibody.

We have shown that the novel σ 1R ligand DPA activates the σ 1R, causing the dissociation of BiP from the σ 1R in HT-22 cells and potentiated NGF-induced neurite outgrowth in PC-12 cells at concentrations between 1 and 100 μ M.

Furthermore, we used the σ 1R-ve mutant strain of *C.elegans* to study the effect of DPA and other potential σ 1R ligands and compared them to other known σ -ligands such as PRE084 and fluoxetine.

Fluoxetine (5 mM) significantly extends the median lifespan of wild-type *C.elegans* (from 14 to 16 days p < 0.05), whereas DPA does not. Fluoxetine (from 9 to 10 days, p < 0.05) and DPA (5mM) (from 9 to 10 days, p < 0.001) could extend the median lifespan, and DPA could prevent paralysis of mutant human A β over-expressing *C.elegans* strains. Neither drug increased the lifespan of the σ 1R KO worm.

Modeling of the C.elegans receptor compared to the human shows a similar structure despite the differences in sequence. Docking analysis of the σ 1R with DPA and other σ 1R ligands suggests that DPA may act as an agonist via both the traditional ligand binding site and as an allosteric activator of the σ 1R.

In conclusion, we have demonstrated that DPA acts via σ 1R activation, which may be a potential starting point for therapeutic development in targeting NDs. Furthermore, we have identified that the σ 1R is required for the lifespan-extending properties of fluoxetine in *C.elegans*.

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