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FIRST RUNNER-UP

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Xenia Torroja Soto

in recognition of her research work

Light-Controlled Modulation of Muscarinic Acetylcholine Receptors Using a Novel Subtype-Selective Photoswitchable Ligand

Master's Degree in Neuroscience

Light-Controlled Modulation of Muscarinic Acetylcholine Receptors Using a Novel Subtype-Selective Photoswitchable Ligand

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1. General Context

Traditional pharmacology presents several limitations; particularly, the lack of selectivity at the site of action, leading to unwanted toxic systemic effects. However, in recent years, photopharmacology has emerged as a complementary approach to solve the long-standing issue of off-target toxicity outside the disease location. By designing drugs whose activity can be regulated by light, this strategy enables both selective and reversible modulation of both drug activation (where needed) and inactivation (where potentially toxic). Nonetheless, this research aims at further advancing the development of light-controllable pharmacological tools, improving the safety and efficacy of current treatments.

In particular, this research has represented a significant advancement in the development of a neuronal photoswitchable compound, named neuroswitch, setting this stage for future investigation into its therapeutic potential. To our knowledge, our results provide the first evidence of (1) a subtype-selective muscarinic acetylcholine receptor photoswitchable compound, and (2) a covalently tethered photoswitchable ligand (PTL). PTLs, by attaching to the appropriate anchoring point, enable native ligands to serve as the functional group while minimizing off-target pharmacological effects. Finally, our neuroswitch may have therapeutic value for diseases involving the dysfunction of these receptors, including neurological, gastrointestinal, type II diabetes, and associated metabolic disorders.

2. Objective of the study

The goal of the study was to characterize and analyze the pharmacological activity of our neuroswitch in three different biological systems: human embryonic kidney tsA201 (HEK-tsA201) cells, rat hippocampal neuronal cultures, and rat gastrointestinal smooth muscle tissue. Experiments were designed to evaluate whether: (i) the neuroswitch was able to specifically activate the desired subtype; (ii) the neuroswitch's activity was controlled by light; and (iii) the neuroswitch was able to covalently bind to the receptor.

3. Methodology

HEK-tsA cells were expressed with the main subtypes of muscarinic receptors. On the other hand, rat neuronal cultures and gastrointestinal smooth muscle tissue offered different distributions of endogenous receptors.

For cell experiments, fluorescence and calcium imaging experiments were performed. A fluorescent calcium indicator was used to determine changes in intracellular calcium concentrations upon receptor activation. By irradiation at specific wavelengths at different intervals, the neuroswitch was also able to convert into its non-active or active state.

For rat gastrointestinal tissue, a smooth muscle contractility assay was performed. In this case, the effect of the neuroswitch, both in its active or non-active state, on the amplitude and frequency of the contraction was measured. Finally, changes in contraction induced by cycles of light exposure were also measured.

4. Main findings

The neuroswitch was able to activate one receptor subtype preferentially to others. This receptor also showed the lowest compound concentration required to produce 50 % of receptor activation (EC_{50}). Notably, it revealed that the compound in its active form was five to ten times more potent at the target receptor (EC_{50} = 90 nM) than at the other subtypes (> 400 nM).

On all biological systems, clear reversible photoswitching of the neuroswitch was observed. Moreover, the neuroswitch remained covalently bound to the receptors as a PTL in both cultured neurons and rat smooth muscle gastrointestinal tissue assays.

In cultured HEK-tsA201 cells and neurons, the non-illuminated form induced a greater response than the illuminated-form, meaning that it was most active at the dark-adapted state. Interestingly, the response was reversed during the smooth muscle contractility assay (neuroswitch active in its illuminated state), which may be due to the higher complexity of neuronal circuits *in vivo*.

5. Conclusion and relevance

This research unveiled a novel photoswitchable compound, demonstrating that it can preferentially and reversibly control a muscarinic receptor subtype using light. In addition, the fact that it remains covalently tethered to target allows drug action to be effectively limited to defined cell types within physiological systems. Therefore, by restricting drug activity both to specific tissues and time windows through light stimulation, this approach allows minimization of systemic toxicity, thus offering to improve the safety and efficacy of current treatments.

In summary, the findings from this research lay the foundations for future studies, moving closer to the development of innovative, targeted treatments for diseases involving muscarinic receptor dysfunction.