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Innovation in Schizophrenia Therapeutics: A TAAR1-Centered Approach

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Abstract

Schizophrenia is a debilitating psychiatric disorder characterized by behavioral, cognitive, mood, and psychological impairments. Schizophrenic individuals experience a range of positive symptoms such as hallucinations and delusions, and negative symptoms such as apathy and anhedonia. Current treatment methods largely depend on pharmacologically blocking Dopamine D2 receptors, though about one-third of patients suffer severe side effects or experience a worsening of condition. Dysregulation in glutamate transmission is implicated in the severity of schizophrenia and presents as a target in developing more effective therapeutics. Specifically, glutamate transporters are critical in maintaining the concentration of active glutamate in the cell synapse, which is dysregulated in schizophrenia. Here, we aim to determine whether a novel G-protein coupled receptor, Trace Amine-Associated Receptor 1 (TAAR1), can modulate the activity of the glutamate transporters EAAT1 and EAAT2 in astrocytes, which are specialized glial cells that regulate extracellular glutamate. Both TAAR1 and glutamate transporters were found to be expressed in human astrocyte. We are now assessing whether TAAR1-specific ligands can regulate the localization of the EAAT1 and EAAT2 glutamate transporters on the extracellular extracellular membrane using immunofluorescence techniques, membrane protein biotinylation, and western blotting. The ability of TAAR1-targeted drugs to regulate the membrane localization of glutamate transporters, thereby regulating the ability of astrocytes to clear extracellular glutamate, represents a potential new target in modulating glutamate transmission and the first step in creating a new class of schizophrenia treatments.

Introduction

Schizophrenia

- Impacts 1-3% of the populations
- Characterized by positive symptoms (hallucinations, delusions, racing thoughts) and negative symptoms (apathy, social withdrawal)
- All treatments for schizophrenia block dopamine transmission in the brain
- Most treatments are largely ineffective at providing relief from negative symptoms
- Up to 30% of patients are treatment resistant and do not respond to any available antipsychotics

Trace Amine-Associated Receptor 1 (TAAR1)

- Novel G-Protein coupled receptor that is activated by biogenic amines
- Trace have long been implicated in several psychiatric disorders and are high affinity agonists at TAAR1
- TAAR1 activation modulates the activity the dopamine transporter (DAT)
- TAAR1-mediated stimulation of PKA and PKC cellular signaling pathways modulate DAT function and internalization

Astrocyte glutamate transporters (EAAT1, EAAT2)

- Glutamate transporter EAAT1 and EAAT2 clear extracellular glutamate in the astrocyte cell synapse
- Glutamate transporter inability to clear glutamate leads to “excitotoxicity” and cell death
- Dysfunctional glutamate clearance in astrocytes is implicated in schizophrenia severity
- Stimulation of TAAR1 leads to a decrease in glutamate transporter mRNA and a reduced ability to clear excess glutamate from the cell synapse

Trace Amine-Associated Receptor 1 and astrocyte glutamate transporters

- We hypothesize that TAAR1 activation can regulate glutamate transporter activity in astrocytes
- Glutamate transporter activity is known to be regulated by PKC activation, but the connection to TAAR1 has not been shown

Hypothesis

TAAR1 can be pharmacologically targeted to regulate glutamate transmission as a novel therapeutic approach for the treatment of schizophrenia

Goals

- Validate mRNA expression of TAAR1, EAAT1, and EAAT2 mRNA and protein in human immortalized astrocytes
- Demonstrate that tyramine stimulation of TAAR1 will change the cellular localization of EAAT1 and EAAT2 from the membrane to the cytoplasm
- Demonstrate that TAAR1 activation can modulate EAAT1 and EAAT2 activity

Methods

- PCR for mRNA expression of TAAR1, EAAT1, and EAAT2
- Immunocytochemistry to show protein localization across astrocyte cellular membrane
- Biotinylation of membrane proteins and western blotting to show change in EAAT1 and EAAT2 expression on membrane after TAAR1 stimulation with tyramine
- Glutamate uptake assays to show TAAR1-mediated effects on glutamate transport

Results

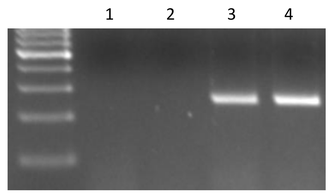

Figure 1: Immortalized human astrocytes express TAAR1 mRNA across multiple treatment types

PCR amplification with EAAT2 specific primers. EAAT2 mRNA expression is amplified after co-treatment with Tyramine and IL-1B.

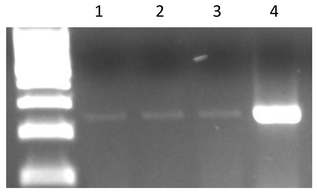

Figure 2: Immortalized human astrocytes express TAAR1 mRNA across multiple treatment types

PCR amplification with TAAR1 specific primers. TAAR1 mRNA expression is consistent across treatment types.

Lane 1: No treatment
Lane 2: Il-1B (cytokine)
Lane 3: Tyramine + IL-1B
Lane 4: Positive control

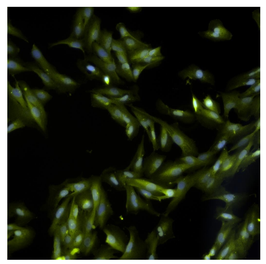

Figure 3: Immortalized human astrocytes stained for EAAT1

Immunocytochemistry shows EAAT1 protein expression in green. Nucleus stained with DAPI in blue.

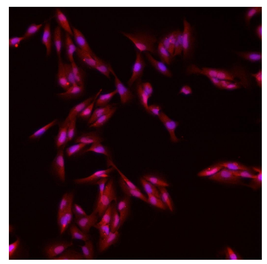

Figure 4: Immortalized human astrocytes stained for EAAT2

Immunocytochemistry shows EAAT2 protein expression in red. Nucleus stained with DAPI in blue.

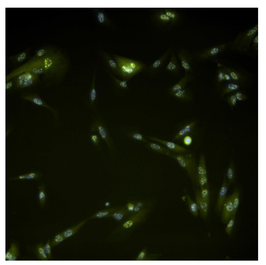

Figure 5: Immortalized Human astrocytes stained for TAAR1

Immunocytochemistry shows TAAR1 protein expression in green. Nucleus stained with DAPI in blue.

Conclusion

- Theses early studies demonstrate TAAR1 and glutamate transporter mRNA and protein expression in an immortalized human astrocytes cell model system.
- Ongoing studies are aimed at determining the cellular itinerary of glutamate transporter localization as well as glutamate transporter kinetic function in response to TAAR1 activation.
- Demonstration of TAAR1 regulation of glutamate transporter function in astrocytes will provide the rationale for further studies which will assess the therapeutic potential of selective TAAR1-targeted compounds to regulate extracellular glutamate levels in brain.

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