Introduction

Neurons are capable of synthesizing serotonin (5HT) from tryptophan. Once synthesized, 5HT is transported into vesicles via vesicular monoamine transporters (VMAT)(3) and stored in vesicles in the presynaptic neuron. Calcium influx into the presynaptic neuron allows for the calcium-dependent fusion of the vesicles with the plasma membrane. 5HT is consequently released into the synapse. The presynaptic neuron also contains sodium-dependent 5HT transmembrane transport proteins (SERT), which are responsible for the reuptake of 5HT in the presynaptic neuron.(4) Once 5HT, upon reuptake, either proceeds through degradation via a mitochondrial monoamine oxidase system (MAO) or is sent back into vesicles via VMAT. This cycle of synthesis, release, inhibition, and reuptake has been well-chronicled.

Astrocytes have been archaically viewed as support cells for neurons in the brain. However, recent studies have demonstrated the important functioning of these cells in the regulation of neurotransmitter release and uptake. The primary focus of this study is the uptake and release of 5HT by astrocytes. The study is being guided by the system of release and uptake in neurons.

Astrocytes have been found to release neurotransmitters, specifically glutamate, in a calcium-dependent manner.(8) Astrocytes have also been found to uptake 5HT in a sodium-dependent and antipressor-sensitive manner. Upon uptake by astrocytes, 5HT may proceed through degradation via a monoamine oxidase system(7) and vesicle repackaging via VMAT as in neurons. This study will first aim to determine if astrocytes up-regulate the uptake of 5HT in the presence of fluoxetine via greater membrane densities of SERT. The study will further seek to determine if serotonin is released in a calcium-dependent manner by astrocytes, which may suggest vesicle packaging of 5HT upon reuptake.

Materials & Methods

- Dissociation and Isolation of Rat Cerebral Astrocytes
  - Papain Dissociation System from Worthington Biochemical Corporation will be used to dissociate rat cortical astrocytes
  - Cells dissociated will be cultured to allow for astrocyte propagation
  - Astrocytes will be identified morphologically and through GFAP staining techniques

- Human astrocytes from ScienCell Research Laboratories may be cultured once procedures and assays have been run successfully on dissociated and isolated rat astrocytes

- Serotonin assay will be obtained for determination of serotonin uptake from or release into culture medium

- Fluoxetine will be used as SSRI

- Western blotting will be used to identify the amount of SERT protein in cells cultured with and without fluoxetine

- Hydroxyindole acetic acid assay will be obtained to test for metabolite of 5HT deamination by MAO

- MAOI will be obtained to verify use of MAO system on 5HT

Discussion

This project will begin by looking at the affects of fluoxetine on serotonin uptake proteins. 5HT uptake by cultured astrocytes will be verified through the use of a standard 5HT assay. Once 5HT uptake has been verified, a Western Blot will be used to determine the amount of SERT protein produced per plate density of astrocyte. The cells will then be cultured in exposure to fluoxetine. A Western Blot for SERT will then be run on the fluoxetine-exposed cells. Up-regulation by the astrocytes for 5HT uptake should produce more SERT proteins when exposed to fluoxetine. An assay for 5-hydroxyindole acetic acid may be performed to determine if 5HT proceeds through degradation by a MAO system following uptake.

The project will next proceed to determination of serotonin synthesis, release, and regulation. Astrocytes will be cultured in tryptophan enriched medium. A 5HT assay will be performed to test for the presence of 5HT. If the assay is positive for 5HT, then the astrocytes will be exposed to an MAOI. The 5HT assay will once again be run to determine if there is an increase in the amount of 5HT released upon exposure to MAOI. If 5HT release is increased, this will suggest that 5HT is regulated by a MAO system. If the initial 5HT assay is negative for 5HT release, then the cells will be shocked to induce calcium influx. Once calcium influx has been triggered, the 5HT assay will be run to test for 5HT release after calcium influx. If 5HT is released, this will suggest that 5HT is released in a calcium-dependent manner, as is seen in neurons. After this calcium-mediated event, the astrocytes will again be exposed to an MAOI to determine the role of a MAO system in the regulation of 5HT release.

References