Neuroprotective Effects of Selective Serotonin Reuptake Inhibitors

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Abstract

Serotonin reuptake inhibitors (SSRIs) have been suggested to have a neuroprotective effect. This claim is is largely clinical and not previously supported by evidence based data. This area of neuroscience has not been well studied at the cellular level. This study offers a three part laboratory examination of the effect of SSRIs on glia cells. The investigation found that serotonin reuptake inhibitors had a detectable protective effect on glia when tested under various classic methods. The examination of cell morphology, use of vital dyes, cell death and growth rates were consistent with a neuroprotective class effect. The data suggest that serotonin reuptake inhibitors are, in fact neuroprotective at normal physiologic dosages.

Keywords: Neuroprotective; Serotonin; Glia neuron

Introduction

Serotonin reuptake inhibitors (SSRIs) are used by millions of people each year [1,2]. This class of medications are now frequently and successfully used to reduce depression [1-3]. Although some studies suggest that Serotonin Reuptake Inhibitors may have a protective effect upon neural tissue, the area of cellular longevity remains largely unexplored [1,2]. Some authors have noted that sertraline may have a gross neuroprotective factor *via* a serotonergic mechanism, but these assertions have been based on clinical evidence subject to errors in self report [1]. Little research has been presented on the beneficial effects of these agents at the cellular level beyond the ability to reduce depressive symptoms. The effect of SSRIs at the intercellular level remains largely undiscussed. This study focuses on glial cells exposed to commonly used SSRI agents. In this paper we examine the potential neuroprotective effects of several of these medications on human glia. The glial cells are vital to the normal function of human brain and have been used to estimate effects of medications on neural tissue in the past [4]. The study of neuroglia was, therefore, considered an ideal method for examination of the effects of medications on cellular health and longevity.

Methodology

We used a classic methodology to evaluate the longevity of glial material [5-9]. There are three basic methods of evaluating cell longevity. Normal glia undergo morphological changes with disease or deterioration. As these cells age or become dysfunctional they tend to change shape and become slightly more spheroid as cell death approaches. The percentage of cells undergoing this morphological change in a given in a colony can be calculated and used as an estimate of health or aging within the group. This rate of change can then be used in a case control comparison of a given exposure to pharmaceutical agents. Another common feature of healthy glia is their ability to absorb vital compounds (or in this case vital dyes). The rate of absorption of vital dye is a reliable measure of cell health than can be compared between colonies exposed to pharmaceuticals and those that

are not. Another method of comparing healthy sets of cells to nonhealthy set is to assess the amount of protein consumed in control populations compared to exposed cells. The combination of these three methods give a good prospective to the relative health of various cell communities. These techniques are described in greater detail the following paragraph.

Cells

Human glioma cells were purchased from the American Type Culture Collection. These cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with serum purchased from GIBCO Laboratories [6]. The cells were settled at 37°C in a 5% CO_2-water vapor saturated atmosphere and cultured in polystyrene multi-well trays.

Drugs

The drugs tested were fluoxetine, citalopram, paroxetine, and sertraline. Glial cells were exposed to physiologic concentrations of these chemicals and the effects were compared to identical cells in simple environments, but without exposure to pharmaceuticals of any kind. Several reliable and dependable methods have been developed to estimate cell health and longevity [6]. The relative degree of neuroprotective effect for each study drug was objectively compared and measured by three standards. Drugs tested were provided by the Research Biochemical International Company. The three testing methods were: 1) evaluation of morphological changes, 2) uptake of dye by cells and 3) potential inhibition of cell growth in normal aging. The details of testing methods were as noted below:

Morphological effects

Cultures of glioma cells were treated with the experimental agents in DMEM and 5% calf serum. Rapidly dividing cells (doubling about every forty-five minutes) were treated for 1 h at 37°C and then examined with an inverted microscope at 40X magnification.

Normal aging and some neurotoxic effects are well known to cause cellular rounding due to withdrawal of cytoplasmic processes. Healthy,

dividing cell cultures contain a small percentage of spherical cells as the result of mitosis and spontaneous cell death. Values in excess of this amount indicate an aging or toxic environment. In our control cultures, approximately 17% of the cells were spherical. Individual medications in solution were added to trays, and the cells were incubated for 1 h at 37°C, trypsin fixed, and stained with giemsa stain. We expected that spherical cells would exist at 17% in environment of equal ambient value and that neurotrophic effects would result in less than 17% spherical cells.

Uptake of vital dye

Cultures of cells (5×10^4 cells/well) were treated with our experimental agents for 2.5 h at 37°C. Neutral red (0.1 g/l; 0.1 ml) was added to 0.9 ml DMEM and the cultures were incubated for an additional hour so that the dye could be taken up by the living cells. Cells not staining with neutral red were counted as dead [7]. 100 cells were counted in random microscopic fields for each determination. The computed percent inhibition was compared to the drug concentration for each chemical compared to saline solutions.

Inhibition of cell growth

Normal cell cultures have a predictable growth rate. We assessed the rate of cell growth in normal physiologic condition and compared rates of growth to solutions containing SSRIs at average therapeutic concentrations. Human glioma cells were plated at 5×10^4 cells/well in 0.5 ml DMEM containing 2.5% (v/v) fetal calf serum. The cultures were then incubated for 2 h at 37°C. A second aliquot of DMEM medium containing various concentrations of study drugs was added to each well. These cultures were incubated for 72 h at 37°C to allow for cell growth. The medium in the wells was decanted and the cell cultures washed four times with Hanks balanced salt solution and dried. Cell growth was measured by determining the amount of cell protein used per well using a method slightly modified from that described by Bradford [8]. The percent inhibition caused by each drug concentration was calculated using the values of the cultures without medication as the control.

Statistics

Simple drug concentrations for each drug were pooled for each particular agent in three experiments. Between drug comparison for each test were done using student's t-test statistic. Comparisons of multi-drug outcomes against single drug trials were done by multivariate analysis.

Results

All SSRIs tested appeared to have significant neuroprotective effects on human glia compared to untreated concentrations of saline (p<.001, in each case). Significant differences in the effects of various medications were not found. This suggests a possible class effect for SSRIs.

The data would predict that cell aging and death may be slowed down by exposure to SSRIs (Tables 1-4) [10,11].

	RAW SCORE	SIGNIFICANCE[*]
Control Culture	17%	-
Paroxetine	27%	-
Citalopram	28%	P<.001
Sertraline	25%	P<.001
Fluoxetine	26%	P<.001
[*]T-Test Comparison To Control		

Table 1: Morphological changes.

	%/48 h	SIGNIFICANCE[*]
Control Culture	2.2%/48 h	-
Paroxetine	1.03%/48 h	P<.01
Citalopram	1.02%/48 h	P<.01
Sertraline	1.08%/48 h	P<.01
Fluoxetine	1.07%/48 h	P<.01
[*]T-Test Comparison To Control		

Table 2: Cell death rates.

		SIGNIFICANCE[*]
Control Culture	3%/h	-
Paroxetine	4.9%/h	P<.001
Citalopram	5.9%/h	P<.001
Sertraline	6.7%/h	P<.001
Fluoxetine	4.6%/h	P<.001
[*]By T-Test Comparison		

Table 3: Cell growth rates.

DRUG	VARIATION	F STATISTIC	SIGNIFICANCE
Paroxetine	0.04121	1	-
Citalopram	0.08712	0.907	-
Sertraline	0.24723	0.813	-
Fluoxetine	0.26718	0.982	-

Table 4: Relative toxicity *via* multivariate analysis (Contribution to Variance).

Discussion

The protection of brain tissue is an area of intense interest and increasing research. The reduction of cell death has potential benefit in a variety of neurological conditions. Neurodegenerative models of schizophrenia have existed for a number of years. Premature neuron loss is considered a likely factor in the etiology and course of this disorder. The neurodegenerative models describe schizophrenia and Parkinson's disease with progressively unfavorable neurodegenerative trajectory. The primary evidence supporting this model came from observations of progressive changes in gray matter as well as measurements of morphological, neuronal samples. At this time

treatment focus on reducing the symptoms of degenerative diseases, but little is available to protect cells from damage early in the course of the illness. Neurodegenerative diseases are progressive neurological disorders characterized by death of specific nerve cells. Representative examples include Alzheimer's disease which compromises cognitive and memory functions of patients, and Parkinson's disease, which is a progressive movement disorder exhibiting symptoms such as tremors, memory issues or psychosis. As noted, schizophrenia is another neurodegenerative disease with only symptomatic treatment. It would be premature to assume that SSRIs could slow all forms of neurodegeneration, but the data shown in this study suggests that they could play a role.

Psychiatric neuroscience is challenged by a variety of neurodegenerative diseases ranging from dementia to schizophrenia. A feature common to many of these conditions is premature cell death. If a method of slowing cell loss could be found, it would greatly advance our ability to combat degenerate disease.

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