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 Ẑxed, and stained with giemsa stain. We expected that spherical cells would exist at 17% in environment of equal ambient value and that neurotrophic eẉects 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 Ẑelds for each determination. HẊe 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. HẊe 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. HẊese cultures were incubated for 72 h at 37°C to allow for cell growth. HẊe 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 modiẐed from that described by Bradford [8]. HẊe 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