Uptake and Release of Choline in Cultures of Human Glioma Cells

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Human glioma cells (138MG) have a low-affinity uptake system for choline (Km = 20 μM; Vmax = 56 pmol/min/10⁶ cells). The uptake is reduced by acetylcholine, hemicholinium-3, HgCl₂, and phosphodiesterase inhibitors. Release of [³H]choline from preloaded cultures showed two pools with half-lives of 1.3 and 160 min. Choline release was stimulated by 8-bromo-cAMP or isobutylmethylxanthine. The results suggest that release of choline occurs by a facilitated diffusion transport system and is increased by elevations of intracellular cAMP.

KEY WORDS: choline transport; cell cultures; glioma cells; cAMP; cGMP; phosphodiesterase inhibitors.

INTRODUCTION

The existence of functional interactions between glia cells and neurons is well documented today (Stewart and Rosenberg, 1979). Astrocytes may play an important nutritive role by supplying the surrounding neurons with substances essential for nerve cell function (Hamberger et al., 1976). In a previous paper it was suggested that choline could be released from astrocytes in response to increased neuronal activity (Walum, 1979). This suggestion was based on choline counter-transport experiments in cultures of human glioma cells (138MG). The present work was undertaken to further elucidate the properties of the membrane transport system for choline in these cells.

METHODS

Cultures

The human glioma cell line 138MG (Pontén and MacIntyre, 1968; Pfeiffer et al., 1977) was grown in 150-cm² tissue culture plastic flasks in Ham's F10 medium (Ham,
1963) supplemented with 9% newborn calf serum, 4% fetal calf serum, 50 units of penicillin, and 50 μg of streptomycin per ml. Ten days before experiments cells were trypsinized (0.25% trypsin in a Ca²⁺-Mg²⁺-free phosphate buffer solution, pH 7.0), pooled, and plated in 21-cm² tissue culture plastic dishes at a density of about 50,000 cells/cm². The cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂ in air. The medium in both flasks (30 ml) and dishes (4 ml) was changed twice a week.

**Determination of Cell Number and Total Protein**

In each experiment the number of cells was determined in duplicate dishes with a Model B Coulter counter. The cells were washed five times in succession with 5 ml of phosphate-buffered saline (Dulbecco and Vogt, 1954) supplemented with 1 mg D-glucose/ml (PBS) and suspended in 2.0 ml of trypsin solution prior to counting. Each dish was counted three times. In parallel, cells were collected by scraping and assayed for total protein according to Lowry et al. (1951).

**Measurement of Total and Nonspecific Uptake**

The rate of total choline uptake was determined as has been described for hexoses (Walum, 1975). In brief, the cultures were washed twice, each time for 30 sec with 5 ml of warm (37°C) PBS, and incubated for various periods of time at 37 or 27°C in 2.0 ml of PBS, containing [³H]choline (1.0 μCi/ml) plus various concentrations of nonradioactive choline. The uptake at 0°C was taken as a measure of nonspecific uptake (simple diffusion and nonspecific adherence to cells and dishes). The incubation was terminated by removal of the incubation solution and subsequent washing of the cells for 15 sec with three portions of an ice-cold 0.9% NaCl solution containing 0.1 mM HgCl₂.

The cells were extracted in 1.0 ml of 0.3 M NaOH, then transferred to scintillation vials, together with a subsequent wash of the culture dish with 1.0 ml of 0.3 M HCl. Radioactivity was determined in PBS or Unisolve I in a Nuclear Chicago Mark III or Packard Tri-Carb 2450 liquid scintillation counter. The counting efficiency was 48–52%.

**Determination of Specific Uptake**

The value for specific uptake was obtained by subtracting the value for nonspecific uptake from those for total uptake. A Prime computer was used to calculate the least-squares fit of the Lineweaver–Burk plot of the concentration curve. Values for \(K_m\) and \(V_{max}\) were calculated from the equation of the line.

**Efflux Experiments**

Cultures were incubated for 2 hr at 37°C in complete F10 medium containing [³H]choline (1.0 μCi/ml; the standard concentration of nonradioactive choline in F10 medium is 5 μM). The uptake was terminated by three rapid washes with 5 ml of ice-cold PBS and the cultures were subsequently continuously perfused for 5 or 10 min