CATECHOLAMINE UPTAKE INTO CULTURED MOUSE ASTROCYTES

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Earlier studies have suggested that glial cells may influence neuronal signalling in the brain. In particular, uptake and metabolism by glial cells may terminate the actions of the catecholaminergic transmitters released from neurons (Henn and Hamberger, 1971; Pelton et al., 1981; Kimelberg and Pelton, 1983). Thus, regulation of glial transport and metabolic activities may provide a mechanism for modulating neurotransmission. Ascorbic acid is stored within catecholaminergic neurons and is secreted with neurotransmitters (O'Neill et al., 1984; Kratzing et al., 1985). One function for ascorbic acid is the retardation of oxidative processes that degrade and inactivate catecholamines; in other words, ascorbic acid may serve as a chemical preservative for catecholamines within neurons and in extracellular fluid. However, because the termination of the neurophysiological actions of catecholamines also is effected by cellular uptake, it is of interest to know if ascorbic acid alters the catecholamine uptake process. Therefore the aims of the present study were to characterize the uptake of norepinephrine in mouse cerebral glial cells and to determine if ascorbic acid could affect uptake rates.

Glial cells were cultured from the cerebral hemispheres of neonatal mice, according to the procedure of Hertz et al. (1982). They were grown in Petri dishes containing serum-supplemented modified Eagle's minimum essential medium. These cells stained positively for

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glial fibrillary acidic protein by the procedure of Wilson et al. (1986). They showed the stellation response to dibutyryl cyclic AMP that is characteristic of astrocytes. The absence of neurons was confirmed by silver staining and microscopical examination of representative cultures.

The cells were used for experiemtns after 2 to 3 weeks in culture. The growth medium was discarded and replaced with serum-free Hepes-buffered incubation medium (pH 7.4, 37 C). The kinetic characteristics of catecholamine uptake were studied during incubation with levo-[7-3H(N)]-norepinephrine (NEN Canada; 0.5 μC/ml incubation medium) in the presence or absence of levo-ascorbic acid (J.T. Baker Chemical Company and Sigma). At the end of the incubation period the cultures were rinsed seven times with ice-cold buffer and the cells were scraped into 1 ml of water. Cell protein was analyzed by the method of Lowry et al. (1951). Aliquots of the harvested cells and of the incubation medium were combined with scintillation cocktail (Biofluor, NEN Canada) and their tritium contents measured by liquid scintillation counting. Cellular uptake rates for tritiated norepinephrine were calculated based on the specific activity of the amine in the medium and were expressed as pmol/mg protein/10 min incubation period. A zero time value, obtained by adding tritiated norepinephrine to the cultures and immediately washing them, was subtracted from all of the uptake data.

Tritiated norepinephrine was taken up from the incubation medium and radioactivity accumulated in the cell cultures (Figure 1). Uptake depended on the extracellular concentration of total (i.e. tritiated and nonradiolabeled) norepinephrine. Kinetic analysis of the uptake mechanism indicated that both saturable and nonsaturable processes were involved. For the saturable component of uptake the half-maximal effective concentration of norepinephrine (Km) was on the order of 0.1 μM and the maximum uptake rate (Vmax) was approximately 1 pmol/mg protein/10 min (Figure 1).

Ascorbic acid competitively inhibited the saturable component of tritiated norepinephrine uptake (Figure 1). The half-maximal inhibitory concentration (IC50) for ascorbic acid was approximately 0.2 μM (Figure 2). Maximal inhibition of saturable norepinephrine uptake was achieved

with about 2 μM ascorbic acid. Ascorbic acid at concentrations of 2 μM or less did not alter the non-saturable component of glial norepinephrine uptake.

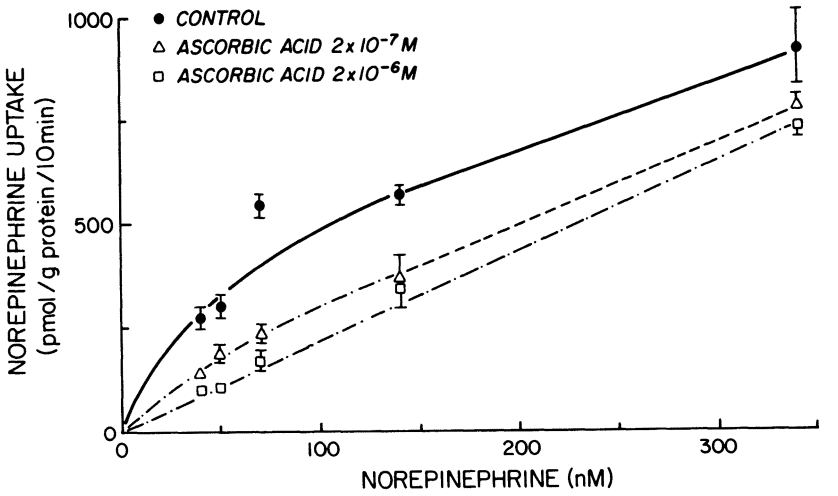

Figure 1. Norepinephrine uptake by mouse cerebral glial cells in the presence and absence of ascorbic acid. Both norepinephrine and ascorbic acid were added to the cells' incubation medium at the beginning of the 10 min incubation period (37 C, pH 7.4). Plotted are the mean ± standard error for triplicate incubations from a representative experiment.

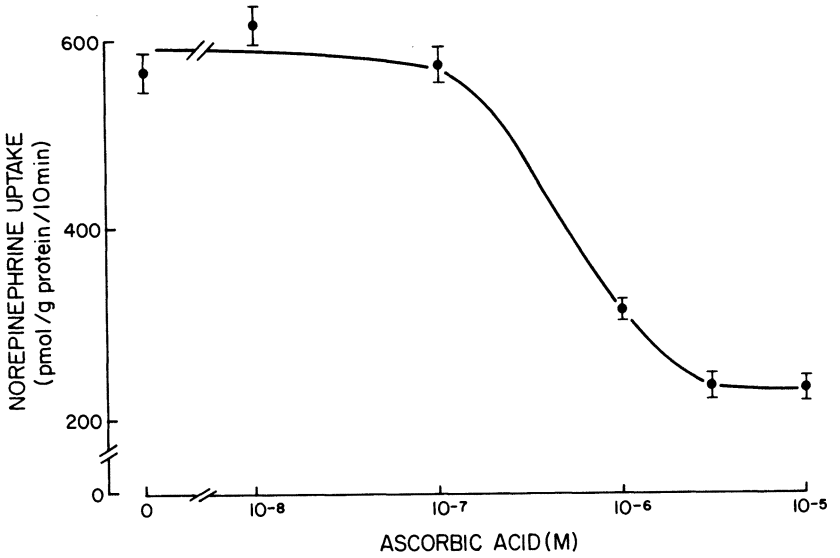

Figure 2. Effect of ascorbic acid on norepinephrine uptake by mouse cerebral glial cells. Both norepinephrine (0.14 uM) and ascorbic acid (0 - 10 μM) were added to the cells' incubation medium at the beginning of the 10 min incubation period (37 C, pH 7.4). Plotted are the mean ± standard error for triplicate incubations from a representative experiment.

The results of the present experiments indicate that mouse cerebral astrocytes can take up norepinephrine from the extracellular fluid. The kinetics of saturable uptake by these murine cells, in the absence of ascorbic acid, resemble those reported for rat cerebral astrocytes (Kimelberg and Pelton, 1983). A novel finding of the

present experiments is that ascorbic acid competitively inhibits the accumulation of tritiated amine in the mouse glial cells. This observation suggests that ascorbic acid may regulate the glial clearance of norepinephrine from the brain's extracellular fluid. Further study is required to learn how this mechanism is involved in regulation of intercellular communication by noradrenergic systems in the central nervous system.

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