Chloroquine, a novel inhibitor of amino acid transport by rat renal brush border membrane vesicles

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Summary. Chloroquine is an antimalarial and antirheumatic lysosomotropic drug which inhibits taurine uptake into and increases efflux from cultured human lymphoblastoid cells. It inhibits taurine uptake by rat lung slices and affects the uptake and release of cystine from cystinotic fibroblasts. Speculations on its mode of action include a proton gradient effect, a non-specific alteration in membrane integrity, and membrane stabilization. In this study, the effect of chloroquine on the uptake of several amino acids by rat renal brush border membrane vesicles (BBMV) was examined. Chloroquine significantly inhibited the secondary active, NaCl-dependent component of 10 μM taurine uptake at all concentrations tested, but did not change equilibrium values. Analysis of these data indicated that the inhibition was non-competitive. Taurine uptake was reduced at all osmolarities tested, but inhibition was greatest at the lowest osmolarity. Taurine efflux was not affected by chloroquine, nor was the NaCl-independent diffusional component of taurine transport. Chloroquine (1 mM) inhibited uptake of the imino acids L-proline and glycine, and the dibasic amino acid L-lysine. It inhibited the uptake of D-glucose, but not the neutral α-amino acids L-alanine or L-methionine. Uptake of the dicarboxylic amino acids, L-glutamic acid and L-aspartic acid, was slightly enhanced. With regard to amino acid uptake by BBMV, these findings may support some of the currently proposed mechanisms of the action of chloroquine but further studies are indicated to determine why it affects the initial rate of active amino acid transport.

Keywords: Amino acids – Chloroquine – Renal membrane transport

Introduction

The antimalarial drug chloroquine is known to affect intracellular exocytic pathways (Tsai et al., 1990), cause an elevation in intralysosomal pH (Ohkum and Poole, 1978), and inhibit the activities of cathepsin B (Wibo and Poole,
In addition to its effect on intracellular protein degradation (States et al., 1983), this agent can also stabilize plasma membranes (Go and Lee, 1983), alter transmembrane proton gradients (MacIntyre and Cutler, 1988) and alter the pattern of insertion of transport proteins or receptors into membrane sites of action (McAbee et al., 1990). The alteration in intralysosomal or intravesicular pH induced by chloroquine may be responsible for the redirection of secretory proteins to surface membranes other than those to which they were originally directed (Parczyk and Kondor-Koch, 1989).

Chloroquine can inhibit the accumulation of organic solutes, including amino acids, in transporting epithelial cells, and it inhibits the uptake of cystine by cystinotic fibroblasts (States et al., 1983, Theogene and Lemons, 1980). Chloroquine also reduces uptake of the β-amino acid taurine by 50% in rat lung slices (Lewis et al., 1990) and in cultured human lymphoblastoid cell lines (Tallan et al., 1983, Tallan and Schneidman, 1984). Because changes in intracellular pH, intralysosomal pH, and in transporter protein import may occur following chloroquine exposure to transporting cells, it would be of interest to discern if this antimalarial agent would affect amino acid transport by isolated membranes, devoid of intracellular enzymes, organelles and microtubular structures. This report examines the effect of chloroquine on uptake of several L-amino acids, taurine, and D-glucose by rat renal cortex brush border membrane vesicles (BBMV).

**Materials and methods**

**Animals**

Male Sprague-Dawley rats (Harlan Sprague-Dawley Inc., Indianapolis, Indiana), aged 56–60 days and weighing 200–250 g each, were used in all studies. Experiments were conducted according to the National Institutes of Health Guide for Care and Use of Laboratory Animals. In some experiments, animals were maintained on a low methionine and taurine diet (LTD) (Custom Low Taurine Diet, ICN Biochemicals, Cleveland, Ohio) for 14 days prior to sacrifice.

**Membrane vesicle preparation**

Renal cortex brush border membrane vesicles were prepared by a modification of the method of Booth and Kenny (1974). Rats were decapitated; the kidneys were removed, decapsulated, and placed in a mixture of 0.05 mM mannitol, 1 mM MgSO₄, 2 mM tris (hydroxyethyl) aminomethane (Tris), and 2 mM N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES), (pH 7.1) (THM·MgSO₄). Renal cortex samples weighing 1.5 to 6.0 g each (wet weight) were homogenized in 20 volumes of the THM·MgSO₄ for 1 min with a Polytron (Kinematica, Switzerland) at setting no. 4 for 45 sec and setting no. 7 for 15 sec. MgCl₂ was added to the homogenate to a final concentration of 10 mM and stirred on ice for 15 min to aggregate the intracellular and basolateral membranes. Repeated centrifugations and an additional MgCl₂ precipitation step were performed, as described elsewhere (Chesney et al., 1983, Chesney et al., 1985, Zelikovic et al., 1989). The final membrane preparation was suspended in 396 mM mannitol, 1 mM MgSO₄, 2 mM HEPES-Tris (pH 7.35), and contained approximately 10 mg protein/ml. In all experiments membrane vesicles were used for uptake studies immediately after preparation.