Amino Acids (1995) 8: 141–158

Amino
Acids

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

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Accepted July 19, 1994

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,

1974) and α-galactosidase (DeGroot et al., 1981). 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 $MgSO_4$, 2 mM tris (hydroxyethyl) aminomethane (Tris), and 2 mM N-2-hydroxyethylpiperazine-N′-2-ethane sulfonic acid (HEPES), (pH 7.1) (THM·$MgSO_4$). Renal cortex samples weighing 1.5 to 6.0 g each (wet weight) were homogenized in 20 volumes of the THM·$MgSO_4$ for 1 min with a Polytron (Kinematica, Switzerland) at setting no. 4 for 45 sec and setting no. 7 for 15 sec. $MgCl_2$ 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 $MgCl_2$ 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 $MgSO_4$, 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.

Enzyme and protein determinations

Membrane purity was routinely assessed from the enrichment of various membrane marker enzymes relative to the homogenate. Enzymes assayed included γ-glutamyl transferase, a brush border membrane marker (Pillion et al., 1976), 5-nucleotidase, a marker for plasma membranes (Dixon and Purdom, 1954), ouabain-inhibitable Na^+K^+ATPase as a basolateral membrane marker (Post and Sen, 1967), malate dehydrogenase to indicate microsomal membranes (Lowry and Passoneau, 1972), succinyl cytochrome c reductase as a mitochondrial marker (Tisdale, 1967), and N-acetyl-β-D-glucosaminidase to indicate lysosomes (Leaback and Walker, 1961). Protein was determined by the method of Bradford (1976) using the BioRad Coomassie Brilliant Blue dye reagent.

Amino acid uptake studies

Uptake of radiolabelled taurine and other amino acids by vesicles in the presence and absence of chloroquine was assayed by a Millipore rapid filtration technique (Chesney et al., 1973). In general, incubation of the membranes was initiated by adding freshly prepared vesicles to media containing various salts and known amounts of radiolabelled and unlabelled amino acid to achieve the desired final concentration. Experimental uptake media contained varying concentrations of ^{3}H-amino acid, 2 mM HEPES-Tris (pH 7.35), 1 mM $MgSO_4$, 100 mM NaCl, and mannitol to balance osmolarity with that of the intravesicular medium (300 mOsm). At specific time intervals, 50 μL aliquots of the incubation mixture were placed onto pre-wetted 0.45 μm Millipore filters (HAWP) and washed with 3 volumes (3.0 mL each) of an ice-cold "stop" solution, which contained 2 mM HEPES-Tris, 1 mM $MgSO_4$, and 150 mM NaCl. All incubations were performed in triplicate and each experiment was performed a minimum of three times. Data are expressed as picomoles amino acid/mg protein retained by the vesicles on the filters. Filters were dried overnight in scintillation vials, then dissolved in a toluene-based cocktail and measured for radioactivity in a liquid scintillation analyzer (Packard Tri-Carb 2000CA).

To measure the effect of chloroquine on taurine efflux, vesicles were incubated with taurine as described above for 1 hour, when equilibrium is known to be achieved (Chesney et al., 1983, Chesney et al., 1985, Zelikovic et al., 1989). The preparation was then centrifuged, the supernatant removed, and the vesicles resuspended as rapidly as possible in taurine-free media. Samples were removed at specific time intervals and placed on filters, then washed, as described for uptake experiments. Nonspecific retention of radioactivity to detergent-lysed (0.1% SDS) membranes in the presence or absence of chloroquine never exceeded 1–1.5% of the total amount found.

Analytical

Data comparisons were made with Statview 512$^+$ software package using repeated measures ANOVA and Student's t test for grouped, independent data (Daniel, 1983). Data are expressed as mean $\pm$ SE.

Materials

[^{3}H]-taurine (25.6 Ci/mmole), [^{3}H]-glycine (49.0 Ci/mmol), [^{3}H]-L-lysine (97.4 Ci/mmol), [^{3}H]-L-methionine (80.0 Ci/mmole), [^{3}H]-L-glutamic acid (25.0 Ci/mmol), [^{14}C]-L-aspartic acid (0.23 Ci/mmol) and [^{3}H]-D-glucose (15.5 Ci/mmol) were purchased from Du Pont, New England Nuclear Corp. (Boston, MA). [^{3}H]-L-alanine (36.0 Ci/mmol) and [^{3}H]-L-proline (60.0 Ci/mmol) were purchased from ICN Radiochemicals (Irving, CA). Radiochemical purity was confirmed by one-dimensional thin layer chromatography (TLC). Chloroquine, diphosphate salt, was obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals used to prepare media were reagent grade.

Results

Analyses of brush border membrane preparations showed an enrichment ratio of γ-glutamyl transferase of 9.6 ± 0.5 relative to the starting homogenate. The enrichment ratio of 5′-nucleotidase was 7.7 ± 0.8. Enrichment of other marker enzymes was not evident.

Uptake of 10 μM taurine was examined in the presence of 0, 100, 250, 500, 750, and 1000 μM chloroquine. Taurine uptake at this concentration is energized by an external NaCl gradient [MacIntyre and Cutler, 1988]. Because taurine accumulation was inhibited in a dose-dependent fashion (Fig. 1), chloroquine appears to significantly inhibit the active, NaCl-dependent component of taurine uptake at all concentrations tested, except for 100 μM chloroquine at 15 sec, where statistical significance was not quite achieved, perhaps because it was obscured by experimental variation. The final intravesicular taurine concentration at equilibrium (45 min) was not affected by the presence of chloroquine at any concentration examined. In contrast, although uptake of 500 μM taurine was decreased slightly, it was not significantly inhibited by chloroquine (Fig. 2), indicating that the NaCl-independent diffusional component of taurine uptake by BBMV was unaffected.

After preloading vesicles with taurine for 60 min, the presence of chloroquine neither augmented nor inhibited taurine efflux. No effect of chloroquine on taurine efflux was found whether this agent was present in the intravesicular or extravesicular media, or both (data not shown). A low level of taurine binding occurs when 10 μM taurine interacts with detergent-treated

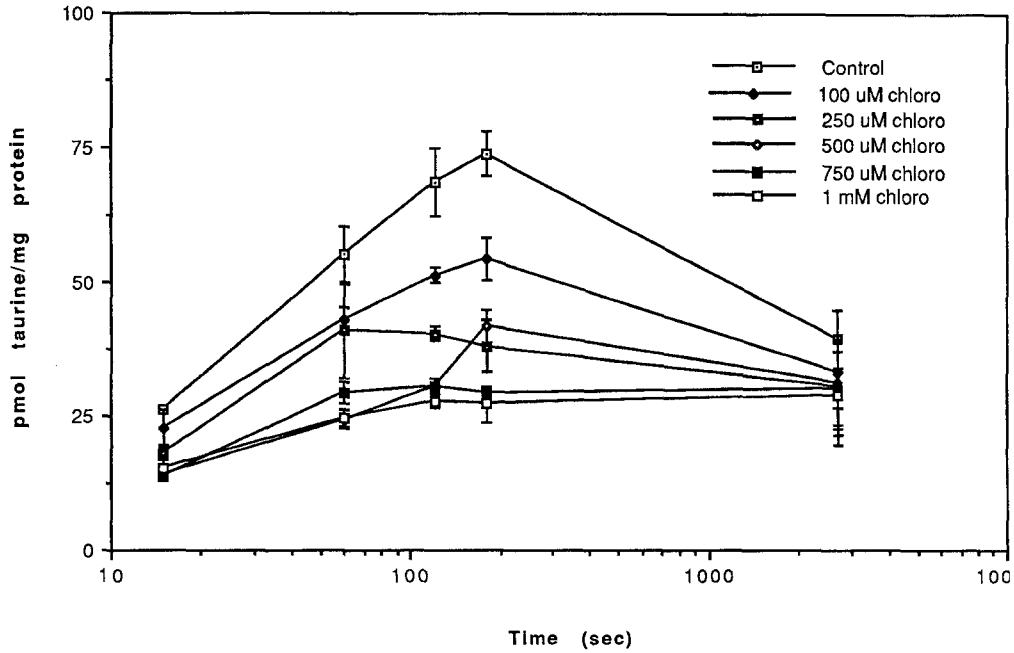


Fig. 1. Time course of 10 μM taurine uptake in the presence of several concentrations of chloroquine (0, 100, 250, 500, 750, 1000 μM). Data are the mean ± SE of three determinations performed in triplicate

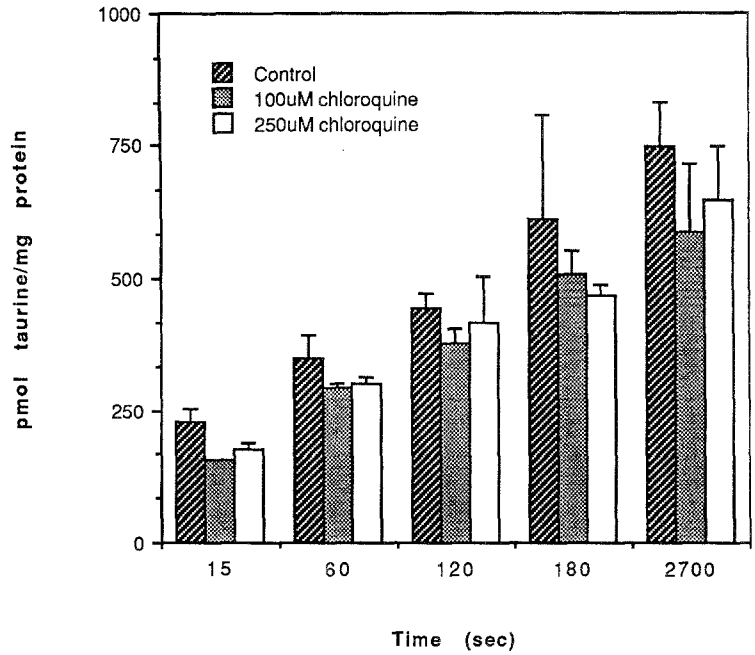
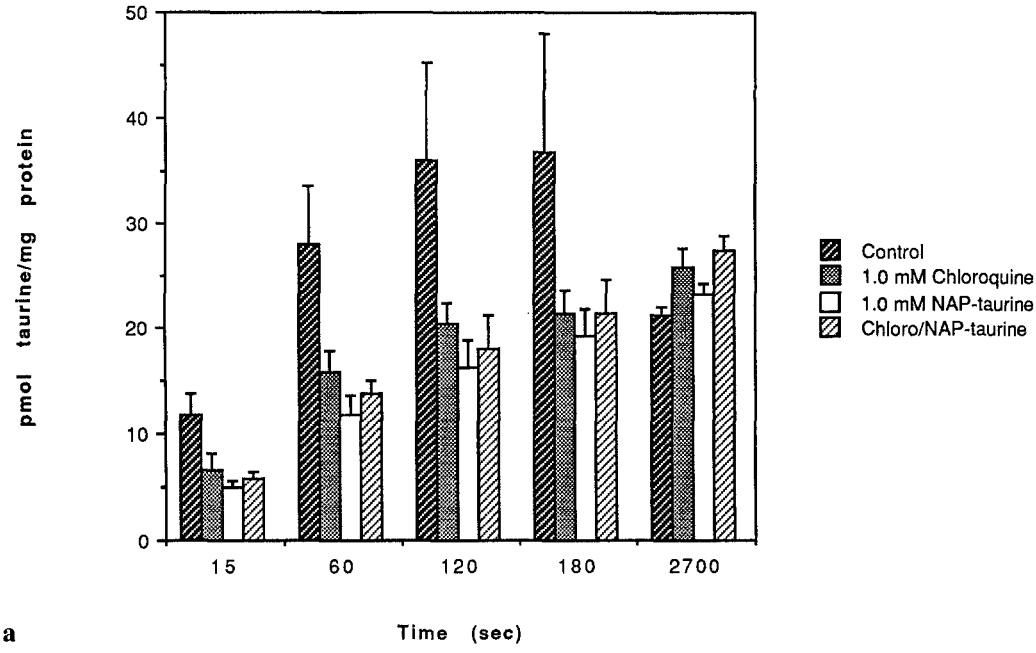


Fig. 2. Uptake of 500 μM taurine in the presence and absence of chloroquine. No statistically significant differences were found. Data are the mean ± SE of three determinations performed in triplicate

lysed membranes, and approximately 2–3 picomoles taurine are bound per mg vesicle protein (<3% of peak taurine uptake by intact BBMV). This degree of binding is unaffected by the presence of 250 μM chloroquine when examined for up to 45 min (data not shown).

The inhibitory effect of chloroquine on the active NaCl-dependent component of taurine uptake was examined in conjunction with that of N-(4-azido-2-nitrophenyl) 2-amino sulfonic acid (NAP-taurine), a photo-affinity probe which has been shown to inhibit the initial rate of secondary active transport of taurine (Zelikovic et al., 1989), to determine if these inhibitory effects were additive. Control values for taurine uptake were significantly higher than were those for uptake in the presence of either inhibitor, but inhibition of uptake in the presence of both agents together was not significantly different from that of either agent alone (Fig. 3a), indicating that both inhibitors may act at a similar site. When DIDS (4,4′-diissothiocyanatostilbene-2,2-disulfonic acid), another inhibitor of both taurine uptake and the anion exchanger (Zelikovic et al., 1989) was examined, it was found that 0.5 mM DIDS inhibited initial rate (15 sec) uptake by 30% ($p < 0.047$), whereas chloroquine alone or chloroquine/DIDS together inhibited taurine uptake by more than 60% ($p < 0.0002$ and $p < 0.0001$, respectively, at 15 sec) (Fig. 3b). Hence, neither DIDS nor NAP-taurine increase the extent of chloroquine inhibition.

The effect of chloroquine on 10 μM taurine uptake was examined in BBMV from rats which had been fed a low methionine and taurine diet (LTD) for 14 days prior to sacrifice. Uptake of taurine is enhanced by dietary



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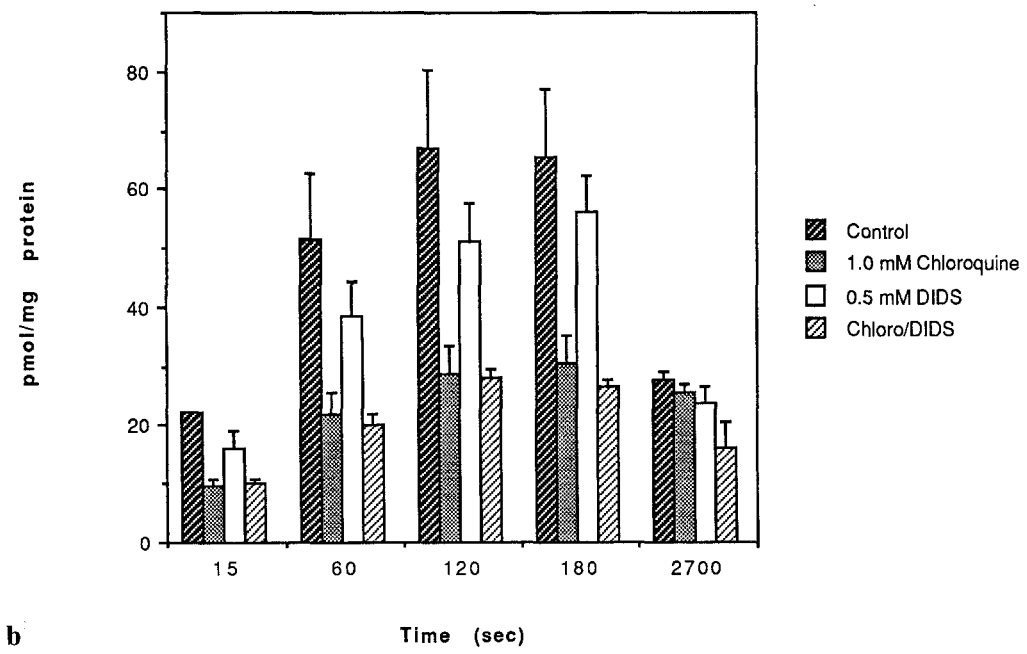

b

Fig. 3. Uptake of 10 μM taurine in the presence of (**a**) 1.0 mM chloroquine and/or 1.0 mM NAP-taurine and (**b**) chloroquine and/or 0.5 mM DIDS. Data are the mean ± SE of three determinations performed in triplicate

restriction of sulfur amino acids, a phenomenon known as the renal adaptive response (Chesney et al., 1983, Chesney et al., 1985). Uptake of taurine was enhanced in the LTD-fed rats as compared to normal taurine diet (NTD)-fed rats, as previously shown (Zelikovic et al., 1989). No difference in the extent of inhibition was found between groups in the presence of 100 μM

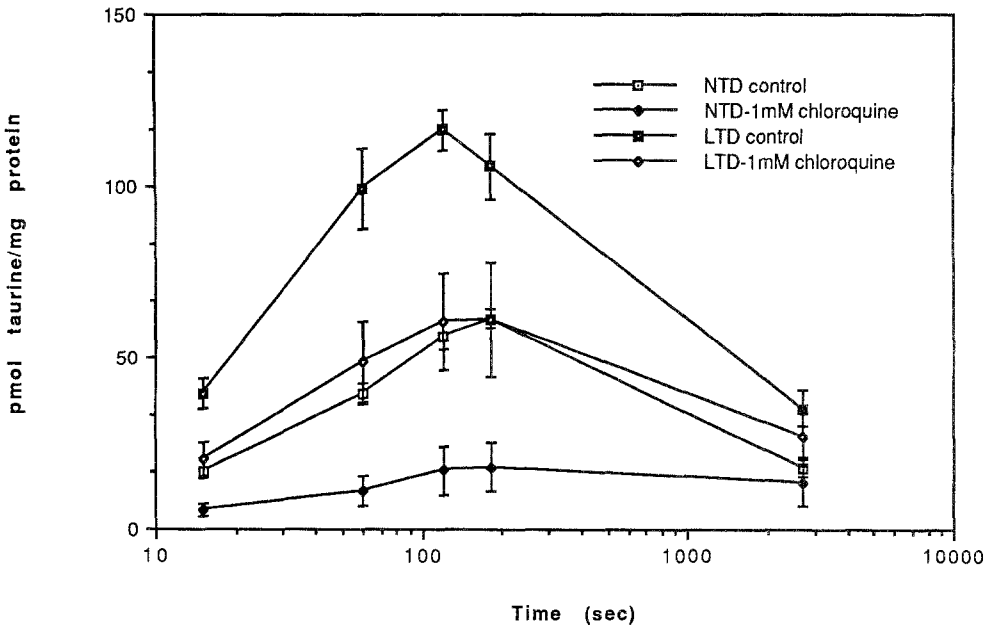

Fig. 4. Taurine uptake in the presence and absence of chloroquine by BBMV prepared from rats fed low or normal taurine diet for 14 days prior to sacrifice. Data are the mean ± SE of three determinations performed in triplicate

Table 1. Kinetic characteristics of taurine accumulation: influence of various concentrations of chloroquine

[Chloroquine]	V_{max} (pmol/mg protein/15 s)	K_m (μM)
0 mM	135.79	64.16
0.25 mM	118.51*	61.13
0.5 mM	105.69*	60.37
1.0 mM	88.01*	73.08

* Different from control value (0 mM) by $p < 0.05$ or less.

chloroquine, but a significant difference in percent inhibition was evident between groups at 15 sec when 1 mM chloroquine was employed: NTD – 69.7 ± 1.1% vs LTD – 47.6 ± 1.9%, $P < 0.0001$ (Fig. 4). The enhanced taurine transporter activity which resulted from feeding rats LTD was inhibited by chloroquine.

Experiments were performed to characterize the nature of the observed inhibition. Uptake of several concentrations of taurine (10, 50, 100, 150, 200, 250 μM) was examined at 15 sec in the presence of several concentrations of chloroquine (0, 50, 250, 500, 1000 μM). Analysis of data by the Lineweaver-Burk method yielded lines which intersected close to, but not on, the y-axis (Fig. 5) and gave similar K_m values but different V_{max} values (Table 1), indicating that though the inhibition may be mixed, it appears to be primarily non-competitive in nature.

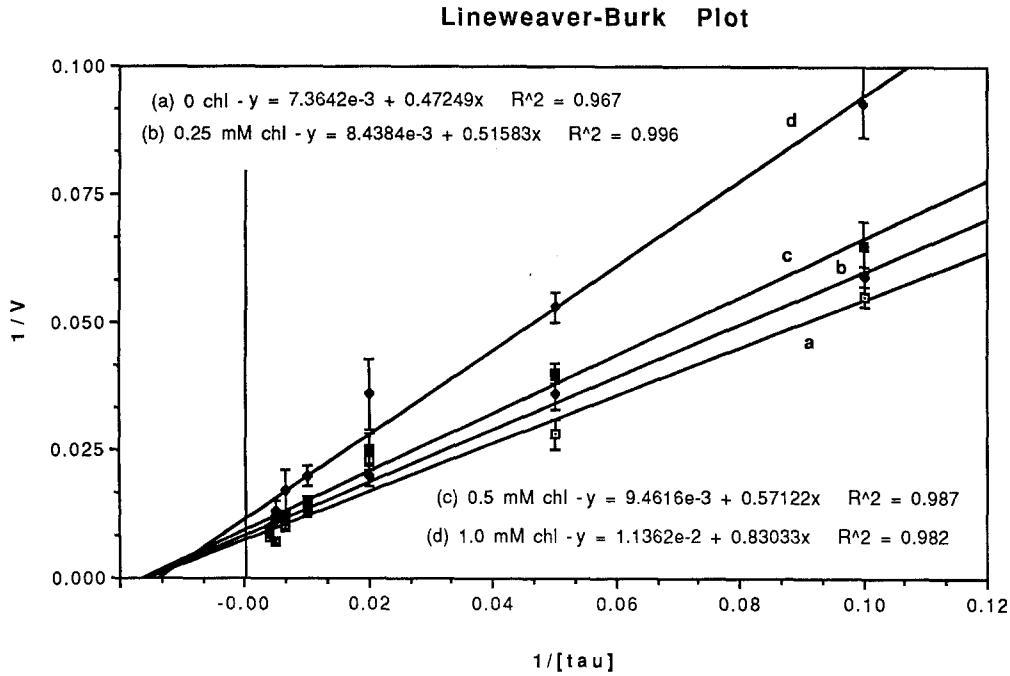

Fig. 5. Lineweaver-Burk plot of the reciprocal rate of taurine uptake in the presence of several concentrations of chloroquine. Data are the mean ± SE of three determinations performed in triplicate

To examine whether chloroquine inhibition of taurine uptake was a chloride-dependent effect, uptake was tested in the presence of 100 mM $NaNO_3$ replacing NaCl in the incubation medium. All other media constituents were as indicated above. Very little inhibition of taurine uptake was observed in the absence of Cl^- (Fig. 6), indicating that the effect of chloroquine on taurine uptake is chloride-dependent. Although it might appear that chloroquine counteracted the effect of nitrate on the size of the vesicles at equilibrium, in fact this difference was not significant, but is the result of experimental variation; in 2 of the 3 experiments the values obtained in the presence of chloroquine were very similar to those obtained in its absence. Uptake of taurine was not examined in Na^+-free, Cl^--containing media because so little taurine transport occurs under these conditions that any inhibitory effects are not measurable by these methods.

Some investigators have suggested that chloroquine may influence biologic processes by stabilization of membranes (Go and Lee, 1990). To examine this possibility, the effect of increasing external osmolarity on the chloroquine inhibition of taurine accumulation was measured. Mannitol was added to the external medium to achieve final osmolarities of 200, 300, 400, 500, 750 and 900 mOsm. Taurine uptake was greatest in the presence of 200 mOsm and least in the presence of 900 mOsm in the medium, presumably because the vesicles behave as osmometers and intravesicular volume is greatest at the lowest osmolarity (Chesney et al., 1973). Vesicle volume at equilibrium can be estimated using the following equation:

$$\frac{\text{pmol taurine retained on filter/mg protein}}{\text{pmol taurine}/\mu\text{l total uptake solution applied}} = \mu\text{l/mg}$$

The reduction in the initial rate of 10 μM taurine uptake in the presence of 1.0 mM chloroquine is indicated in Fig. 7a. Taurine uptake is reduced at all osmolarities, but the extent of inhibition is greatest at the lowest osmolarities. That is, inhibition is more pronounced when the vesicular volume is greatest. At equilibrium, however, there is no influence of chloroquine on taurine uptake (Fig. 7b). Hence, the inhibitory effect of chloroquine on taurine uptake at different external osmolarities pertains only to initial uptake.

The effect of 1.0 mM chloroquine on the uptake of several other amino acids was evaluated in the presence of NaCl in order to optimize conditions for uptake (Fass et al., 1977, Thierry et al., 1981, Lynch and McGiven, 1987, Vorum et al., 1988, Chesney et al., 1986); representative amino acids from each class (Zelikovic and Chesney, 1989) were utilized. Chloroquine inhibited the uptake of the imino acid L-proline, (Fig. 8a) and the shared amino acid transporter and neutral amino acid molecule, glycine (Fig. 8b). Inhibition of the dibasic amino acid, L-lysine, was also found (Fig. 9). Uptake of the neutral α-amino acids, L-alanine and L-methionine, was not affected by chloroquine (Fig. 10a,b). Uptake of the dicarboxylic amino acids, L-glutamic acid and L-aspartic acid, was slightly but significantly enhanced (Fig. 11) (data for L-aspartic acid not shown). That the apparent stimulation of uptake of L-glutamic acid was not due to increased binding of the amino acid to the membranes in the presence of chloroquine was confirmed by measuring

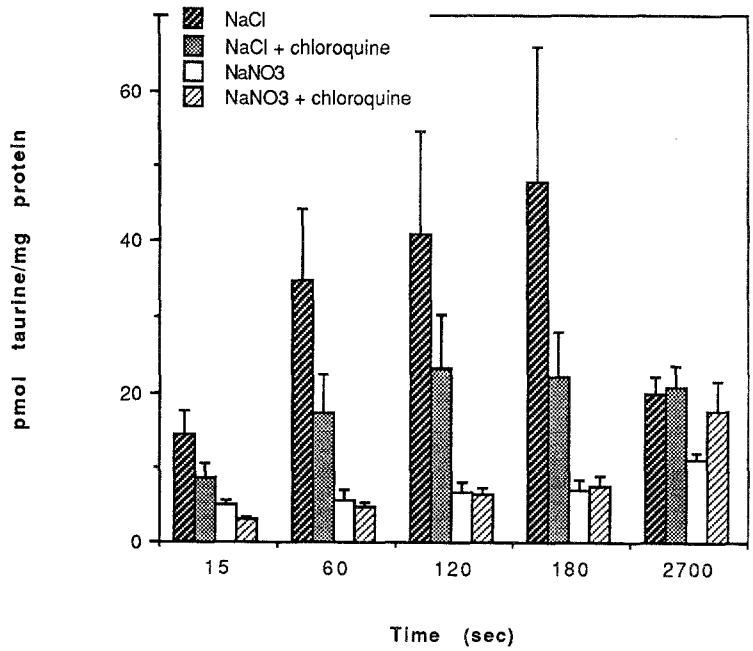

Fig. 6. Taurine uptake with 100 mM $NaNO_3$ replacing NaCl in the external media and in the presence and absence of 1.0 mM chloroquine. Data are the mean ± SE of three determinations performed in triplicate

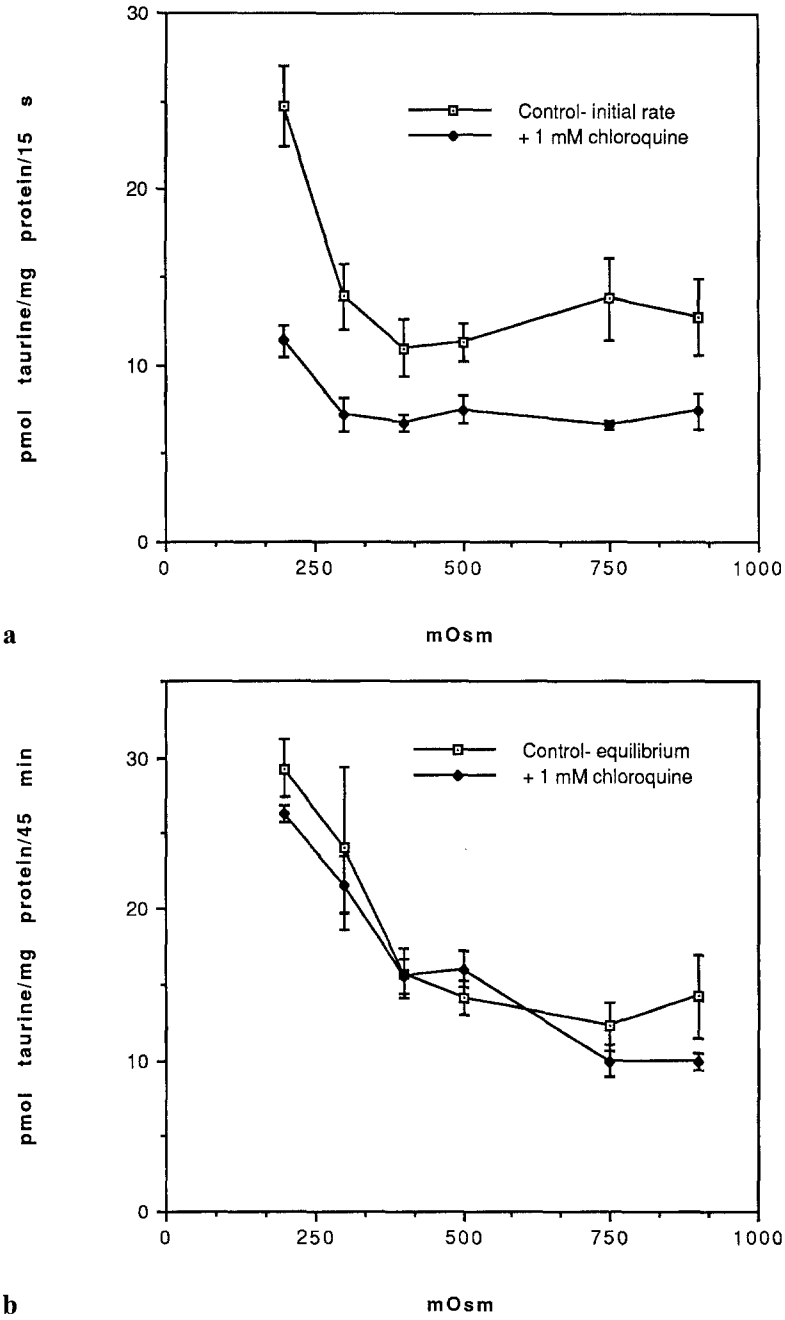

Fig. 7. Influence of extravesicular osmolarity on (**a**) initial rate and (**b**) equilibrium taurine uptake. Data are the mean ± SE of three determinations performed in triplicate

the amount of ^{3}H-L-glutamic acid bound to detergent-lysed membranes in the presence and absence of chloroquine. D-glucose uptake was also inhibited by chloroquine (Fig. 12). As is the case for taurine, transported by the β-amino acid carrier, chloroquine inhibition of glycine and D-glucose pertains only to the initial rate of uptake. The effect of chloroquine on L-proline and L-lysine

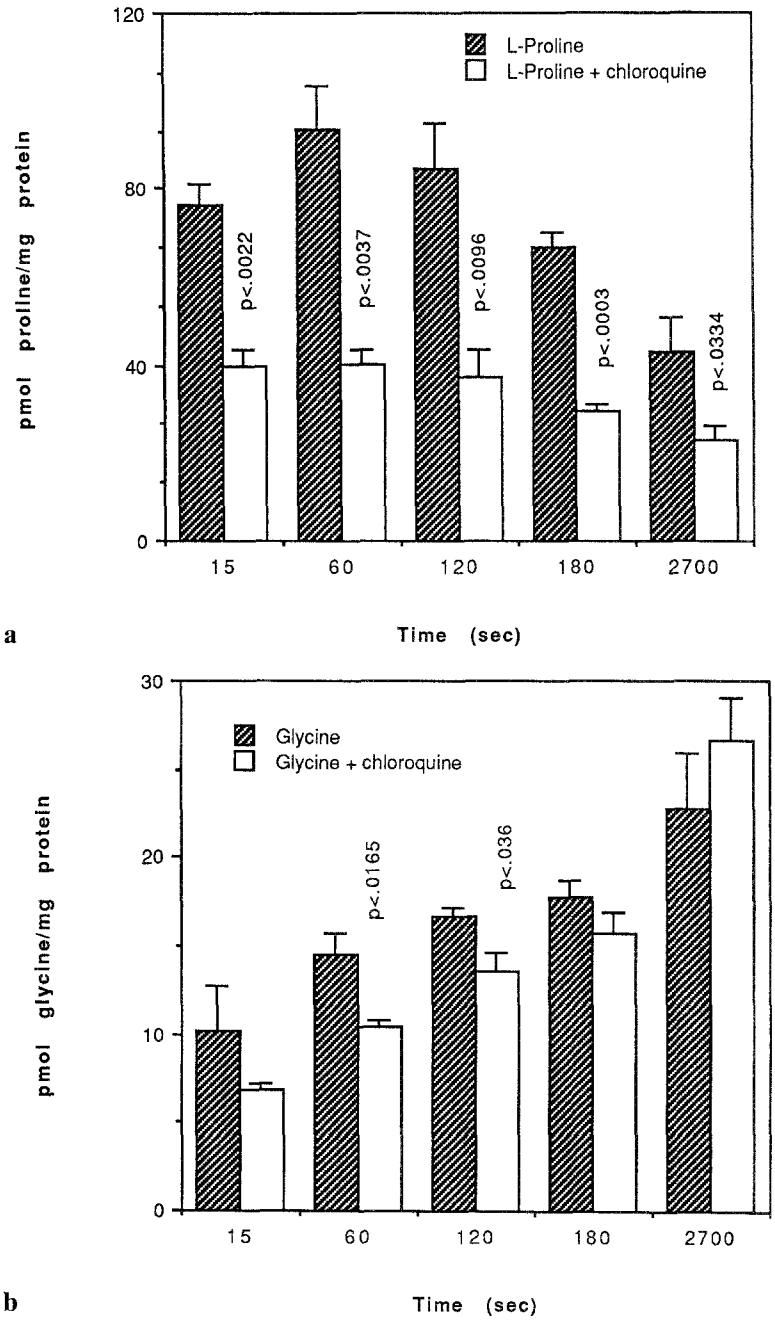

Fig. 8. Effect of 1.0 mM chloroquine on (**a**) 20 μM L-proline and (**b**) 20 μM glycine uptake. Data are the mean ± SE of three determinations performed in triplicate for each amino acid

appears to be especially long-lived, as accumulation of these amino acids is significantly inhibited at the 45 min time point commonly used to represent equilibrium. The stimulation of L-glutamic and L-aspartic acids by chloroquine pertains only to the initial rate of uptake.

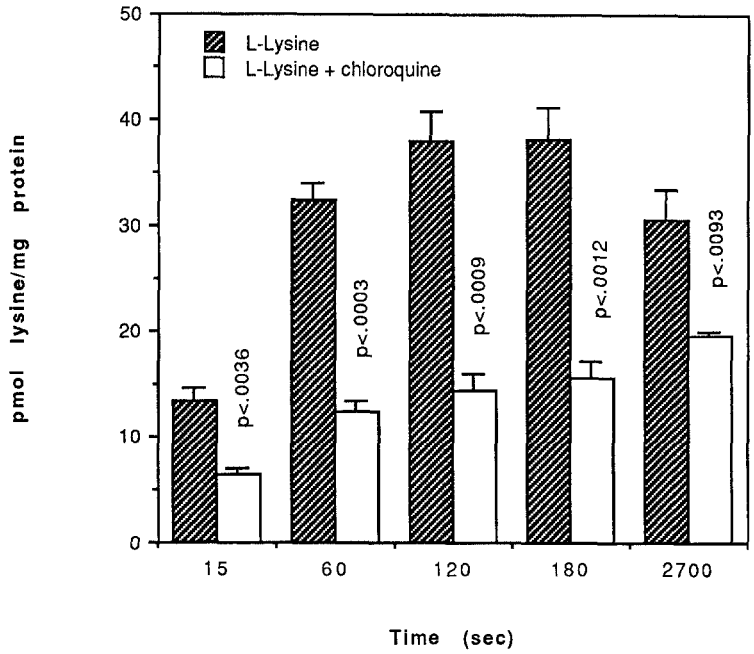

Fig. 9. Effect of 1.0 mM chloroquine on 20 μM L-lysine uptake. Data are the mean ± SE of three determinations performed in triplicate

Discussion

Many of the effects of the agent chloroquine are dependent upon its interaction with intracellular organelles and metabolic processes, including lysosomes, intracellular trafficking, and proton gradient changes (Tsai et al., 1990, Ohkum and Poole, 1978, Wibo and Poole, 1974, DeGroot et al., 1981). In this study, chloroquine appears to influence organic solute transport in an isolated membrane preparation, with inhibition of the initial rate of uptake of amino acids (proline, glycine), dibasic amino acids (L-lysine), β-amino acids (taurine), and a hexose (D-glucose). The dicarboxylic amino acids (L-glutamic and L-aspartic) are enhanced in their initial rate of uptake by chloroquine. No influence was found for the neutral L-amino acids.

Uptake of taurine, a model probe of amino acid transport, is very specifically influenced by chloroquine in a dose-dependent manner. Chloroquine inhibits the initial NaCl-dependent uptake, but not taurine efflux, binding, equilibrium uptake, or NaCl-independent accumulation. Because this study examined transport in isolated membranes which are devoid of cell components, subcellular organelles, and proton gradients, the mechanism of chloroquine inhibition is of particular interest. The proton gradient effect of chloroquine is important, particularly in terms of the uptake of cystine, its metabolism and intracellular translocation in normal and cystinotic fibroblasts (Danpure et al., 1986), or in terms of the vesicular trafficking of secretory proteins to membrane sites (Parczyk and Kondor-Koch, 1989). However, in this study the intravesicular and extravesicular pH were set at the same level to avoid a pH gradient across the apical membrane surface. Hence, this

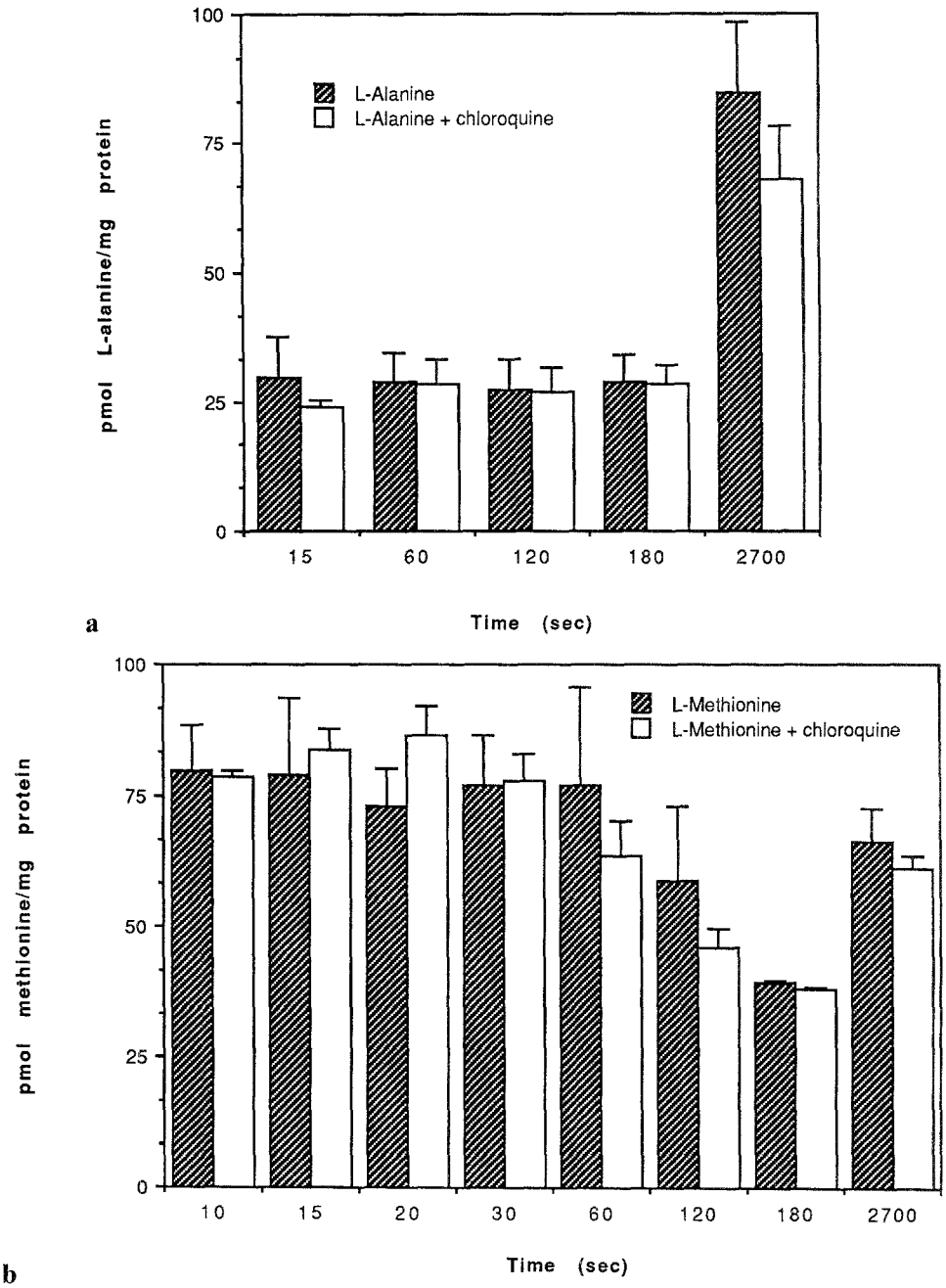

Fig. 10. Effect of 1.0 mM chloroquine on (**a**) 20 μM L-alanine and (**b**) 20 μM L-methionine uptake. Data are the mean of three determinations performed in triplicate

mechanism is unlikely to affect amino acid transport in vesicles. A second proposed mechanism is a non-specific alteration in membrane integrity (McAbee et al., 1990, Thoene and Lemons, 1980). If this mechanism were relevant, it would be expected that all components of taurine transport (uptake, efflux, binding, Na^+ and Cl^--independent accumulation and equilibrium) would be inhibited by chloroquine rather than only the initial

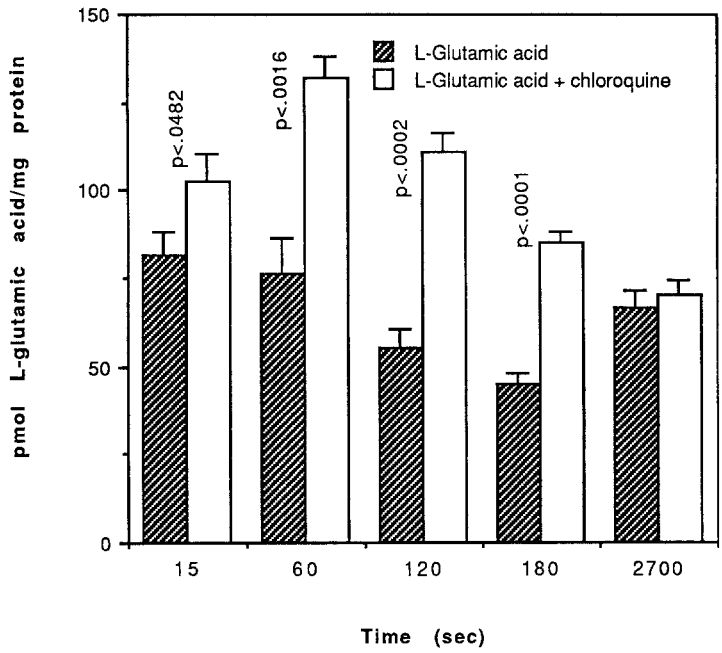

Fig. 11. Effect of 1.0 mM chloroquine on 20 μM L-glutamic acid uptake. Data are the mean of four determinations performed in triplicate

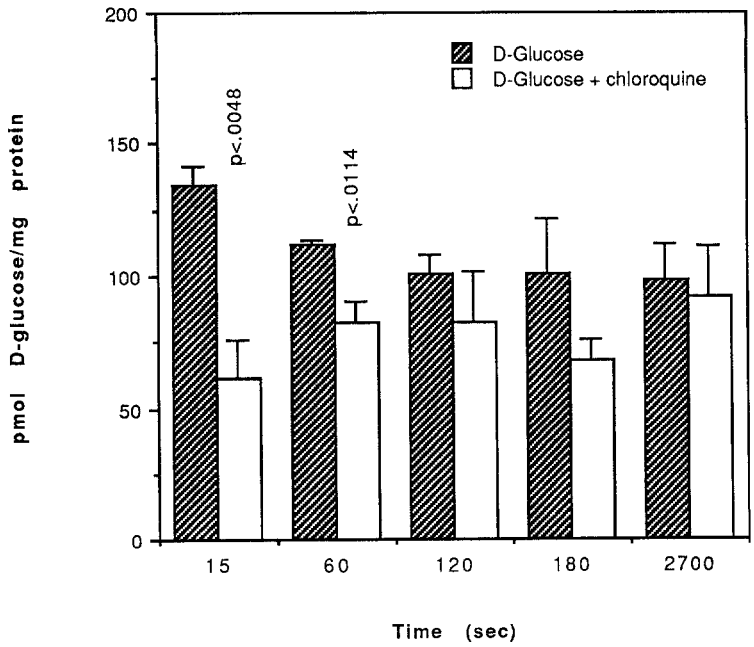

Fig. 12. Effect of 1.0 mM chloroquine on 50 μM D-glucose uptake. Data are the mean of three determinations performed in triplicate

NaCl-dependent component. Likewise, the finding of selective inhibition of several group-specific transporters, the lack of influence on the large group of neutral α-amino acids, and the stimulation of dicarboxylic acid uptake makes this mechanism less likely.

The mode of membrane stabilization is the predominant mechanism of chloroquine action (Tsai et al., 1990, Ohkum and Poole, 1978, Wibo and Poole, 1974, DeGroot et al., 1981, States et al., 1983, Go and Lee, 1990). Chloroquine's activity on lysosomal membranes is well established, which makes this agent particularly effective in terms of its antimalarial and antirheumatic lysosomotropic properties (Colobo and Bertini, 1988). It is unclear whether chloroquine can stabilize the brush border membrane and whether such an action would account for the findings noted above in terms of amino acid and D-glucose transport. If chloroquine influenced renal membranes in terms of their stability, then this agent might influence insertion and anchoring of transporter proteins into the apical membrane of the proximal tubule (Kotal et al., 1988). This effect could potentially influence the transport of organic solutes into vesicles and would allow for increases, reductions, or no change in transporter protein activity. Nevertheless, the precise mechanism of chloroquine on organic solute transport in isolated membrane vesicles is unknown and is the subject of further study.

Chloroquine inhibits accumulation of taurine by lung slices from the rat (Lewis et al., 1990), cultured lymphoblasts (Tallan et al., 1983), and rat cerebral cortex (Schmidt et al., 1975). Taurine serves as a neuromodulator and osmoregulator of the central nervous system, retina and kidney (Tratchman, 1991, Chesney, 1987). Other amino acids also serve as neuromodulators and osmoregulators, including L-glutamic acid, L-proline and glycine (Tratchman, 1991, Strange, 1992). Each of these amino acids appears to be accumulated by a Na^+- and Cl^--dependent mechanism (Chesney et al., 1991, unpublished observations). It is of interest that each of these neuromodulators or osmoregulators (i.e., taurine, glycine, L-proline, and L-glutamic acid) is altered by chloroquine, with inhibition of the former 3 and stimulation of the latter. Because the initial uptake process of each of these 4 amino acids is Na^+- and Cl^--dependent, it is also of interest that chloroquine specifically influences this initial component of transport across the brush border surface. One of the side effects of chloroquine therapy in humans is a retinopathy (Goodman and Gilman, 1990), involving the pigmented retinal epithelium. It is plausible to speculate that chloroquine-induced inhibition of amino acid uptake may be one of the factors which contributes to this retinopathy.

Chloroquine also inhibits uptake of cystine by cystinotic fibroblasts treated with cysteamine (Thoene and Lemons, 1980) in a concentration-dependent fashion (Danpure et al., 1986). Cystine is transported in the kidney by a group-specific, Na^+-independent process together with ornithine, arginine and lysine (Zelikovic and Chesney, 1989). Chloroquine appears to affect this group-specific process in rat kidney brush border membrane vesicles, as evidenced by the inhibition of L-lysine transport noted in the present study.

To summarize, chloroquine appears to be an agent which modulates the uptake of several isolated group-specific transport systems in rat renal brush border membrane vesicles. This effect is an inhibition of the initial rate of Na^+-dependent uptake into vesicles of taurine, proline, glycine, and dibasic amino acids, and a stimulation of dicarboxylic amino acids. While neutral α-amino acid uptake is not affected, the initial rate of D-glucose uptake is

reduced. The mechanism of this modulation is unclear, but may involve membrane stabilization or alterations in anchoring of transporter proteins into membranes. Chloroquine inhibition affects those amino acids which serve as neuromodulators or osmolytes; these amino acids appear to require Na^+ and Cl^- for full uptake. Thus, chloroquine influences those amino acids which subsume the physiologic functions of neuroregulation, osmoregulation and enhancement of specific cations and anions across the renal brush border surface. The membrane effects of chloroquine do not influence taurine efflux, binding, Na^+-independent transport, or equilibrium accumulation. With regard to amino acid uptake by isolated brush border membrane vesicles, these findings support only one of the currently proposed mechanisms of the action of chloroquine, membrane stabilization. Further studies are indicated to determine why this agent influences the initial rate of active amino acid transport.

Acknowledgements

This study was supported in part by NIH Grant DK 37223-07 and The LeBonheur Professorship of Excellence in Pediatrics. We thank Charles Wright and John Sturman for stimulating discussions regarding the interactions of taurine and chloroquine.

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Received February 5, 1994