Taurine uptake by isolated alveolar macrophages and type II cells

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BANKS, MELANIE A., WILLIAM G. MARTIN, WILLIAM H. PAILES, AND VINCENT CASTRANOVA. *Taurine uptake by isolated alveolar macrophages and type II cells*. J. Appl. Physiol. 66(3): 1079–1086, 1989.—Evidence suggests that taurine may protect cellular membranes against oxidants (Gordon et al., *Am. J. Pathol.* 125: 585–600, 1986). The present study was conducted to determine if alveolar macrophages and type II cells (which are relatively resistant to oxidant injury) possess a specialized transport system for the accumulation of taurine. The results indicate that both cell types contain more taurine than plasma or whole lung. Taurine influx exhibited both carrier-mediated and simple diffusion components. Carrier-mediated uptake displayed saturation kinetics (K_m = 26.3 and 22.5 μM, while V_{max} = 33.2 and 4.9 pmol·10^6 cells^{-1}·min^{-1} for macrophages and type II cells, respectively). Taurine uptake was dependent on extracellular sodium and inhibited by metabolic inhibitors or ouabain. Total taurine uptake by type II cells was lower than that of alveolar macrophages. However, type II cells exhibited a higher intercellular concentration of taurine (14 vs. 4 mM) because of a higher ratio of carrier-mediated uptake to leakage than with alveolar macrophages. It is possible that this specialized transport system for taurine uptake may lend these cells resistant to oxidant injury.

plasma taurine concentration; lung taurine concentration; energy-dependent amino acid uptake by pneumocytes

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TAURINE (2-aminoethanesulfonic acid) is an unusual amino acid found in a wide variety of animal species. Taurine is not incorporated into proteins, but it is abundant in free amino acid pools and is efficiently transported into and concentrated by tissues that are excitable and membrane rich or that generate oxidant compounds. Recently, evidence has accumulated supporting the hypothesis that taurine protects cellular membranes against toxic compounds such as bile acids, xenobiotics, and oxidants. Taurine may exert an antioxidant or protective effect by preventing changes in ion transport and membrane permeability, which result in cellular damage (14, 16, 32).

Exposure to oxidant gases such as hyperoxia, NO_2, or O_3 results in pulmonary injury (5, 7, 22, 24). Oxidant injury to lung tissue results in lipid peroxidation (6, 26) and a depletion of intracellular antioxidants such as glutathione (9, 25) and ascorbic acid (20). Exposure of erythrocytes (30) and fibroblasts (29) to O_3 results in increased membrane ion permeability. Dietary supplementation of the antioxidant vitamins C (21) and E (13) has been shown to protect against lung oxidant injury.

Recently, dietary taurine supplementation has been reported to protect against bronchiolar damage induced by NO_2 (15). Although exposures to O_2, NO_2, and O_3 result in similar morphological and metabolic changes in the lung, the distribution of the lung injury varies. Although hyperoxia induces damage at the levels of the trachea through the distal alveoli, NO_2 and O_3 toxicities primarily involve the airway epithelium and the proximal alveoli, which are located near the terminal bronchioles (8).

Although alveolar type II epithelial cells are relatively resistant to oxidant injury, alveolar type I cells are not (7). In fact, type II cells are thought to repair oxidant-induced damage to the alveolar epithelium by replacing injured type I cells (1, 10, 11). In vivo, alveolar macrophages are relatively insensitive to damage by oxidant gases (19). However, in vitro, alveolar macrophages are more sensitive to oxidant injury than type II cells (28). Whether the relative resistance of these pneumocytes to oxidant injury is related to their taurine content has not been investigated.

In the present study we examined whether alveolar macrophages and type II cells can accumulate taurine. Furthermore, to investigate whether alveolar macrophages and type II cells possess a specialized transport system for taurine, we measured *1*) the taurine content of the cells, *2*) the rates of taurine uptake in the presence of a normal plasma concentration of taurine, *3*) the concentration dependence and kinetic parameters for the transport systems, and *4*) the effects of metabolic inhibitors or ouabain on the transport processes.

METHODS

Isolation of lung cells. Lung cells were isolated as previously described (4, 18). Male Sprague-Dawley rats (200–300 g) were anesthetized with pentobarbital sodium (65 mg/kg body wt). Alveolar macrophages were obtained by pulmonary lavage using 10, 8-ml aliquots of ice-cold calcium- and magnesium-free Hanks' balanced salt solution, concentrated by centrifugation at 550 *g* for 10 min at 4°C (Sorvall RC-3 centrifuge, Sorvall Instruments, Newtown, CT) and resuspended in *N*-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-buffered medium (10 mM HEPES, 5 mM glucose, 1 mM $CaCl_2$, 5 mM KCl, and 145 mM NaCl; pH 7.4). To isolate alveolar type II cells, rats were anesthetized, and the heart and lungs were removed en bloc. The pulmonary vasculature was perfused with phosphate-buffered medium (5 mM KCl, 5 mM glucose, 9.35 mM Na_2HPO_4, 1.9 mM NaH_2PO_4, and 145 mM NaCl; pH = 7.4) to remove blood cells. Free alveolar macrophages were removed by pulmonary lavage with phosphate-buffered medium. The lungs were lavaged once with 8 ml of enzyme solution [phosphate-buffered medium containing 40 U/ml type I elastase, 0.1% collagenase, and 0.018% deoxyribonuclease (DNase), Sigma, St. Louis, MO], filled with 11 ml of this enzyme solution, and incubated for 35 min at 37°C. After enzymatic digestion the lungs were minced with a tissue chopper, and digestion was arrested by incubating the mince with 20 ml of inhibitor solution (25% fetal bovine serum and 0.018% DNase) in phosphate-buffered medium for 10 min at 37°C. The suspension was then strained through nylon mesh, and type II cells were purified by centrifugal elutriation (using a model J-21 centrifuge equipped with a model JR-6 rotor, Beckman Instruments, Fullerton, CA). Debris and small pneumocytes were removed by infusion of phosphate-buffered medium containing 0.5% bovine serum albumin (Sigma) through the elutriator at a flow rate of 10.0 ml/min and a rotor speed of 2,000 rpm. Type II cells were then recovered at a flow rate of 18.9 ml/min and a rotor speed of 2,000 rpm. The alveolar type II pneumocytes were resuspended in HEPES-buffered medium.

The number of cells in each preparation was determined with a Coulter electronic cell counter (model ZB, Coulter Instrument, Hialeah, FL). Cell purity was routinely determined using an electronic cell sizer as reported previously (2, 18). Identity of type II cells was verified by fluorescence microscopy using phosphine 3R and by electron microscopy as shown previously (18). The macrophage fractions contained 91.2 ± 1.1% alveolar macrophages, whereas the type II fractions contained 89.7 ± 1.3% type II cells.

Measurement of lung, plasma, and intracellular taurine. The intracellular concentrations of taurine in alveolar macrophages and type II cells were measured using a high-performance liquid chromatography (HPLC) method (24a). Alveolar macrophages and type II cells were pelleted by centrifugation at 14,000 *g* for 2 min (BHG Hermle microcentrifuge, National Labnet, Woodbridge, NJ), resuspended in a HEPES-buffered medium and were frozen at −4°C until analysis. After thawing, three samples of each cell type, containing $2\text{–}3 \times 10^7$ macrophages or $2\text{–}4 \times 10^7$ type II cells, were disrupted and deproteinized by addition of saturated picric acid (2 g/100 ml water, Fisher Scientific, Pittsburgh, PA). After centrifugation at 15,000 *g* for 10 min, the supernates (500 μl) were removed and placed on dual-bed ion-exchange columns (0.5 cm ID), which were prepared by layering 2.5 cm AG 1-XG (100/200 mesh, chloride form) over 2.5 cm AG 50W-XG (100/400 mesh, hydrogen form) ion-exchange resins. The taurine fraction was collected using 2.0 ml distilled water, evaporated in a drying oven at 70°C, and stored at room temperature until reconstitution with water just before the HPLC analysis.

Each sample was analyzed in triplicate. Samples were reacted for 1 min with an equal volume of derivatizing reagent and were then injected onto a Waters (Milford, MA) C_{18} resolve (5 μm) reversed-phase column, which was attached to an HPLC system consisting of a Perkin-Elmer series 4 liquid chromatograph with a microprocessor-controlled solvent delivery system, a Rheodyne model 7125 injector with a 20-μl injection loop, a Perkin-Elmer PC-75 absorbance detector, and a Varian model 4270 integrator (Perkin-Elmer Instrument, Norwalk, CT). The derivatizing reagent was formulated by adding 0.4 ml β-mercaptoethanol and 50 mg *o*-phthaldehyde (Sigma) to 10 ml of HPLC-grade methanol (Fisher Scientific, Pittsburgh, PA), which was then brought to 100 ml with 0.4 M potassium borate buffer (fluoraldehyde reagent dilutent, pH 10.4, Pierce Chemical, Rockford, IL). The samples were eluted from the HPLC column using a linear gradient of 9–11% methanol in water, which was run at a flow rate of 1.0 ml/min for 10 min to achieve separation of taurine from the internal standard, glutamine (Sigma). The absorbance of the OPA adducts of these amino acids were monitored at 340 nm, at a sensitivity of 0.01 absorbance units full scale. The taurine concentration of the samples was calculated by the peak area-to-weight ratio method from known concentrations of internal (glutamine) and external (taurine) standards in the standard curve. Recovery of taurine from biological samples was previously estimated as 97.2 ± 4.7% (SE).

Taurine contents of whole lung and plasma were also determined using this method. The lungs and heart of four rats were removed en bloc and perfused with 0.9% NaCl to remove blood cells. The lungs were then dissected free of the heart, trachea, main bronchi, and connective tissue and minced by chopping six times with a McElwain tissue chopper (Mickle Engineering, Gomshall, Surrey, UK), which was set at a slice thickness of 0.5 mm. After homogenization (20 passes in a hand-held tissue homogenizer) in 6 vol of phosphate buffer (0.1 M phosphate, pH 7.4), 5 ml of the homogenate were disrupted and deproteinized by boiling for 30 min. The samples were centrifuged at 2,000 *g* for 10 min to remove cellular debris, and the supernates from these samples were centrifuged at 15,000 *g* to remove precipitated proteins. Aliquots of the second supernates (1.0 ml) were applied to the dual-bed ion-exchange columns, and taurine was isolated and quantitated by HPLC, as above. Samples from each rat were analyzed in triplicate.

For determination of plasma taurine, blood samples (6 ml) from four rats were drawn from the inferior vena cava using heparinized syringes. Plasma was obtained by centrifugation (1,400 *g*, 10 min) of whole blood and deproteinized by boiling for 30 min. After centrifugation at 15,000 *g* to remove precipitated protein, 100-μl aliquots of the supernates were diluted to 500 μl with distilled water and applied to the dual-bed ion-exchange columns to isolate taurine. Samples from each rat were analyzed in triplicate by HPLC, as described above.

Measurement of time course of taurine uptake. Taurine uptake was measured using [1,2-^{14}C]taurine (New England Nuclear, Wilmington, DE). Alveolar macrophages ($1–3 \times 10^7$ cells/2.5 ml) or type II cells ($5–7 \times 10^7$ cells/2.5 ml) were preincubated at 37°C for 20 min in HEPES-buffered medium containing 100 μM taurine (the approximate plasma concentration). At the end of the preincubation period, 2×10^{-2} μCi of labeled taurine were added to the incubation mixture. Aliquots of 250 μl were removed, and these samples were allowed to incubate, with shaking, at 37°C for various periods of time (10–120 min). After the appropriate incubation time, a sample was removed from the water bath, and 0.5 ml of ice-cold HEPES-buffered medium was added to stop the reaction. The samples were then centrifuged at 15,000 *g* for 3 min to pellet the cells. The pellets were washed three times by alternate resuspension in 0.5 ml of buffer and centrifugation. After the final wash the pellets were resuspended in 500 μl Soluene (Packard Instrument, Downers Grove, IL) and incubated at 55°C for 1 h to digest the cells. The digested pellet was then added to 10 ml of Scinti-Verse II scintillation fluid (Fisher Scientific, Pittsburgh, PA) and allowed to dissolve overnight. Radioactivity was measured in a Beckman LS-1800 liquid scintillation counter (Fullerton, CA). Taurine uptake was calculated from the counts per min and expressed as nanomoles per 10^6 cells.

Concentration dependence and kinetic parameters of taurine uptake. Isolated alveolar macrophages ($1–2 \times 10^7$ cells) and type II cells ($2–4 \times 10^7$ cells) were pelleted by centrifugation at 15,000 *g* for 3 min and resuspended in 250 μl of HEPES-buffered medium. Samples (250 μl) consisting of 175 μl of buffer containing various taurine concentrations, 25 μl of cells, and 50 μl of radioactive taurine (2×10^{-3} μCi) were incubated, with shaking, for 30 min at 37°C. The final taurine concentration of the samples ranged from 1 to 500 μM. At the end of the incubation period, uptake was stopped by addition of 0.5 ml of ice-cold HEPES-buffered medium to the samples, and the cells were pelleted. The samples were washed twice more by resuspension in HEPES-buffered medium and centrifugation at 15,000 *g* for 3 min. After the final wash, the cells were resuspended in Soluene, digested, and counted as above.

The uptake data were mathematically separated into two components using a curve-stripping technique. The slope of the linear component of influx (i.e., that which occurred at extracellular taurine levels of 100–500 μM) was determined, and a line with the same slope, representing the simple diffusion component of the influx, was drawn through the origin. The carrier-mediated uptake was obtained by subtracting the simple diffusion component from the total flux. The kinetic parameters Michaelis constant (K_m) and maximal velocity (V_{max}) were estimated from double reciprocal plots of the carrier-mediated uptake data.

Effects of extracellular sodium and inhibitors on taurine uptake. Isolated alveolar macrophages ($1–2 \times 10^7$ cells) and type II cells ($2–3 \times 10^7$ cells) were pelleted by centrifugation at 15,000 *g* for 3 min and resuspended in 250 μl of HEPES-buffered medium containing 100 μM taurine (the approximate plasma concentration). Samples consisting of 175 μl of buffer containing various sodium concentrations (23.5 to 15 mM) or inhibitors (1–3 mM), 25 μl of cells and 50 μl of radioactive taurine (2×10^{-3} μCi) were incubated, with shaking, for 30 min at 37°C. At the end of the incubation period, uptake was stopped by addition of 0.5 ml of ice-cold HEPES-buffered medium to the samples, and the cells were pelleted. The samples were washed twice more by resuspension in HEPES-buffered medium and centrifuged at 15,000 *g* for 5 min. After the final wash, the cells were resuspended in Soluene, digested, and counted as above. The percent inhibition was calculated from the counts per minute of each sample compared with this value for a sample with no added inhibitor.

RESULTS

The taurine contents of rat plasma and whole lung are given in Table 1. The intracellular concentrations of taurine in isolated alveolar macrophages and type II cells are given in Table 2. A comparison of these values indicates that, on a molar basis, rat alveolar macrophages contain ~50 times the plasma-taurine content and alveolar type II cells contain ~150 times the plasma-taurine content. The existence of such a gradient suggests that these cells possess a specialized transport system for this nutrient. Both alveolar macrophages and type II cells contain more taurine, on a per gram basis, than whole lung, which consists of over 40 different cell types (27). This suggests that the taurine content of these cells may be related to their specialized functions in the lung.

The time courses for taurine uptake by alveolar macrophages and type II cells in the presence of 100 μM taurine (the approximate plasma concentration) are shown in Fig. 1, *A* and *B*. Taurine uptake displayed two components: the initial component was rapid and of short duration (<30 min), whereas the second component of uptake was less rapid and of longer duration (30–120 min). The rates of taurine influx during both phases of uptake were greater for alveolar macrophages than for type II cells, although alveolar type II cells had a higher taurine content than alveolar macrophages (Table 2). When the data were corrected for differences in the

TABLE 1. *Taurine content of rat plasma and whole lung*

Tissue	Taurine Content
Plasma, μM	100.7±13.1
Lung, μmol/g tissue	0.42±0.09

Values are means ± SE for 12 determinations.

TABLE 2. *Intracellular concentrations of taurine in isolated rat alveolar macrophages and type II cells*

Cell Fraction	Taurine Content		
	nmol/10^6 cells	μmol/g cells*	mM†
Alveolar macrophages	4.19±0.40	0.78±0.14	4.27±0.41
Alveolar type II cells	2.88±0.20	3.91±0.34	14.4±0.95

Values are means ± SE for 9 determinations. * Based on cell weights which were determined by weighing known quantities of pelleted cells in triplicate. † Based on measurements of intracellular water: alveolar macrophages, 0.98 μl/10^6 cells; type II cells, 0.205 μl/10^6 cells (Refs. 3, 18).

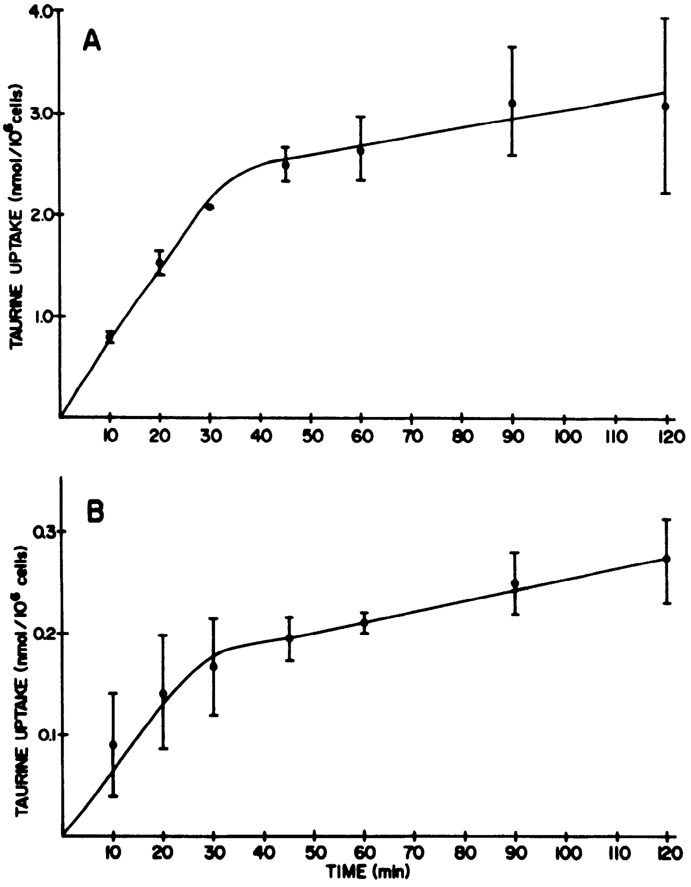

FIG. 1. *A*: time course of taurine uptake by alveolar macrophages. *B*: time course of taurine uptake by alveolar type II cells. Values are means ± SE for 3 experiments.

TABLE 3. *Taurine uptake rates of alveolar macrophages and type II cells adjusted for surface area of the cells*

Cell Fraction	Taurine Uptake,* nmol·10⁶ cells⁻¹· 30 min⁻¹	
	Unadjusted	Adjusted†
Alveolar macrophages	2.08±0.01	2.08±0.01
Type II cells	0.166±0.048	0.406±0.118

Values are means ± SE for 3 determinations. * Rates for the initial component of taurine uptake. † Based on surface areas = 4 πr^2, where r = 7.14 μm for alveolar macrophages and r = 4.27 μm for alveolar type II cells; therefore, ratio of surface area for macrophages/type II cells = 2.441 (Refs. 3, 18).

surface areas of these two cell types, alveolar macrophages still displayed an ~5 times higher initial rate of taurine uptake than type II cells (Table 3).

The concentration dependencies of the taurine transport systems in alveolar macrophages and type II cells are shown in Fig. 2, *A* and *B*, respectively. When the uptake data were mathematically separated into carrier-mediated and simple diffusion components (Fig. 3, *A* and *B*), alveolar macrophages displayed a higher proportion of leakage (22.8% at 100 μM taurine) than type II cells (16.2% at 100 μM taurine). Thus the higher taurine content of alveolar type II cells compared with alveolar macrophages may be due both to differences in the surface area of the cells and to differences in the ratio of carrier-mediated taurine uptake to leakage of taurine from the cells.

The carrier-mediated component of taurine uptake in both cell types displayed saturation kinetics (Fig. 3, *A* and *B*). From plots of $1/v$ (where v is the taurine uptake in pmol·10^6 cells^{-1}·min^{-1}) vs. $1/[s]$ (where $[s]$ is the taurine concentration, μM), K_m and V_{max} for each system were estimated. These values are shown in Table 4.

In other tissues taurine uptake has been shown to depend on the extracellular sodium concentration (32, 33). To determine whether taurine uptake is sodium dependent in alveolar macrophages and type II cells, experiments were performed in media containing different levels of sodium and in the presence of ouabain, an inhibitor of the sodium-potassium pump. The effects of extracellular sodium and of ouabain on taurine uptake by these two types of pneumocytes are shown in Table 5, *A* and *B*. At low (23.5 mM) and intermediate (75.25 mM) levels of extracellular sodium, uptake was decreased by ~72.4 and 22.1%, respectively, from the level obtained in high Na^+ (145 mM) with alveolar macrophages and by 59.9 and 40.1%, respectively, with type II cells. Ouabain, at a concentration of 1 mM, decreased taurine uptake by 92.1% in macrophages and by 85.2% in type II cells. These results indicate that taurine uptake by alveolar macrophages and type II cells is at least partially dependent on the transmembrane sodium gradient.

To determine whether taurine uptake by alveolar macrophages and type II cells is energy dependent, various metabolic inhibitors, including iodoacetic acid, sodium azide, dinitrophenol, and sodium fluoride were added to the medium. Table 6, *A* and *B* summarize the effects of the metabolic inhibitors on taurine uptake by alveolar macrophages and type II cells, respectively. Taurine uptake was inhibited by 76–94% in both cell types by all of the inhibitors tested. These results indicate that taurine uptake by these two types of pneumocytes is at least partially energy dependent.

DISCUSSION

In this investigation we measured the intracellular content of taurine and characterized the transport of this nutrient into alveolar macrophages and alveolar type II cells. Both cell types had a higher taurine content than plasma and whole lung and displayed the ability to accumulate taurine. However, alveolar macrophages displayed a higher rate of taurine uptake than type II cells, whereas the latter had a higher taurine content than the macrophages. This discrepancy may be due to differences in the surface area of the two cell types (and therefore, the number of transporters in the membrane) and in the degree of taurine leakage across the membrane. The carrier-mediated component of uptake in both cell types followed saturation kinetics. In both types of pneumocytes, taurine uptake was dependent on extracellular sodium and was inhibited by ouabain and compounds that interfere with cellular energy metabolism. This suggests that alveolar macrophages and type II cells possess a specialized system for transport of taurine into the cells against a concentration gradient and that the mechanism may involve cotransport of sodium and taurine

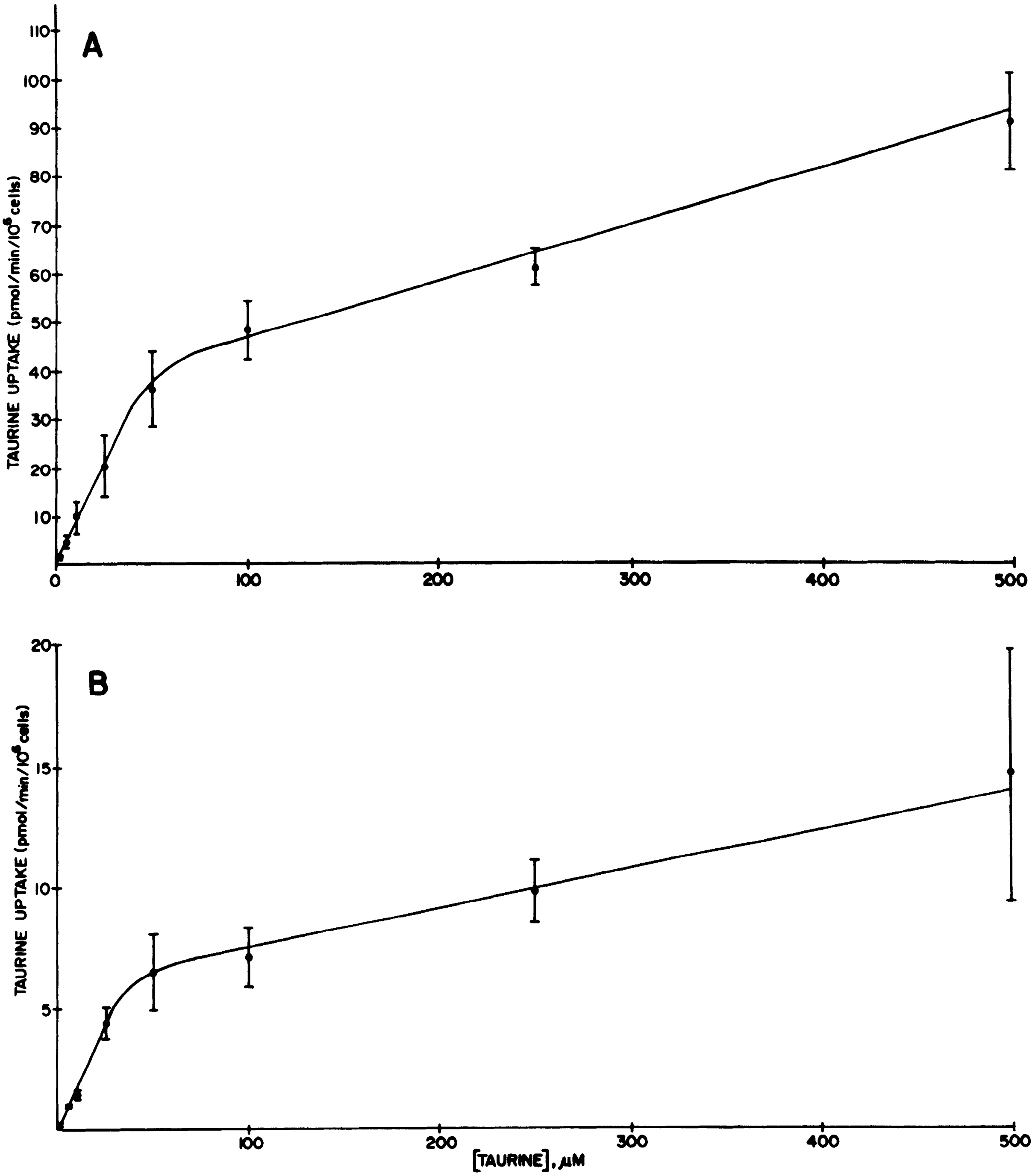

FIG. 2. *A*: taurine uptake as a function of the extracellular taurine concentration in alveolar macrophages. *B*: taurine uptake as a function of the extracellular taurine concentration in alveolar type II cells. Values are means ± SE for 3 experiments.

across the membrane. The direct energy source for the accumulation of taurine seems to be the inwardly directed electrochemical gradient for sodium. This gradient is maintained by the sodium-potassium pump, which is driven by ATP. This transport system is very similar to that described by our laboratory for ascorbate uptake by these pneumocytes (4).

Various mammalian tissues have been reported to accumulate taurine, including platelets, lymphoblastoid cells, and the retina. The taurine content of human platelets is 15 µmol/g; the apparent K_m and V_{max} for taurine uptake are 36 µM and 202 $\text{nmol}\cdot 10^8\ \text{cells}^{-1}\cdot \text{min}^{-1}$, respectively. Taurine uptake is sodium and energy dependent in these cells (32, 33). In human lymphoblastoid cells the K_m is 15–20 µM (32). The approximate normal concentration of taurine in human plasma is 100 µM (32). In the retina, where the taurine concentration is unusually high (50 mM in the rat), taurine uptake has been demonstrated to be sodium and energy dependent (32). Our results with content and uptake of taurine in rat alveolar macrophages and type II cells are consistent with those reported for other cell types in other tissues.

Mechanistic studies of taurine uptake by various tissues with high taurine contents have indicated that tau-

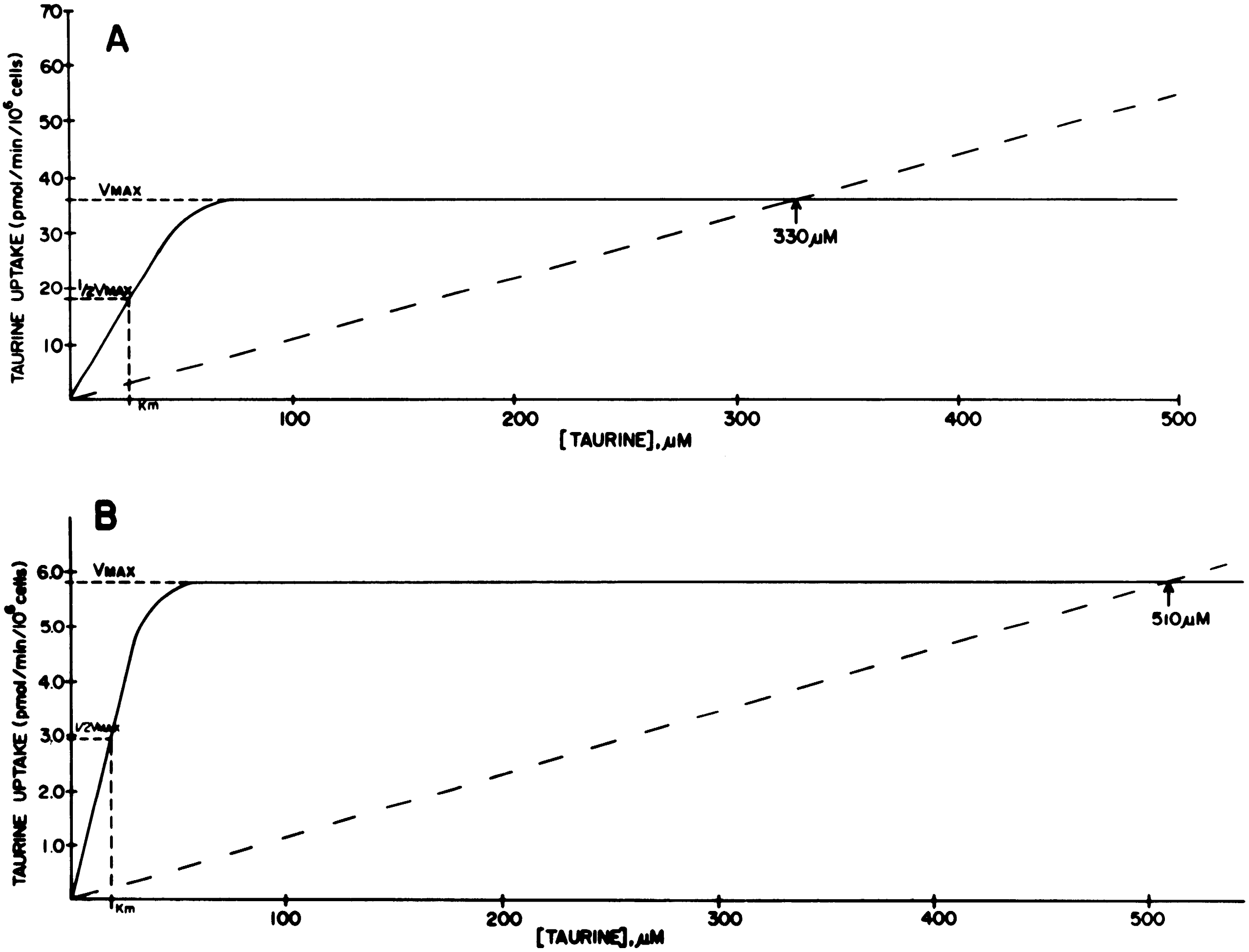

FIG. 3. *A*: taurine uptake as a function of extracellular taurine concentration in alveolar macrophages: carrier-mediated transport (——) and simple diffusion (- - -) components. Data in Fig. 2*A* were mathematically separated into 2 components as in METHODS. *B*: taurine uptake as a function of extracellular taurine concentration in alveolar type II cells: carrier-mediated transport (——) and simple diffusion (- - -) components. Data in Fig. 2*B* were mathematically separated into 2 components as in METHODS. V_{max}, maximal velocity; K_m, Michaelis constant.

TABLE 4. *Concentration dependence of taurine uptake in alveolar macrophages and type II cells*

Cell Fraction	V_{max}, pmol · 10^6 cells^{-1} · min^{-1}	K_m, μM
Alveolar macrophages	33.2	26.3
Alveolar type II cells	4.9	22.5

rine enters the cells by a combination of carrier-mediated transport and simple diffusion (17). Our results with alveolar macrophages and type II cells suggest that similar mechanisms for taurine transport exist in these pneumocytes.

The time course of taurine uptake in these two types of pneumocytes was biphasic, consisting of an initial rapid (0–30 min) component and a second prolonged component (30–120 min) (Fig. 1, *A* and *B*). A similar response has been reported for cation transport in dog erythrocytes (3) and for ascorbate transport in rat alveolar macrophages and type II cells (4).

Although the two types of pneumocytes exhibited similar affinities for taurine, as reflected by the estimated K_m values for the carriers, they exhibited different maximal velocities for taurine uptake. This difference may be accounted for by the difference in surface area between the cells and, thereby the number of taurine transporters. Although correction for surface area does not eliminate the difference in V_{max} (adjusted values: 33.2 in alveolar macrophages vs. 12.0 pmol · 10^6 cells^{-1} · min^{-1} in type II

TABLE 5. *Effects of extracellular sodium and ouabain on taurine uptake by alveolar macrophages on type II cells*

Treatment (Concn)	Taurine Uptake, pmol · 10^6 cells^{-1} · min^{-1}	% Inhibition
A. Alveolar macrophages		
Control (145 mM Na^+)	21.49±1.89	
Intermediate Na^+ (75.25 mM)	16.75±0.42	22.1
Low Na^+ (23.5 mM)	5.94±0.50	72.4
Ouabain (1 mM) + Na^+ (145 mM)	1.69±0.06	92.1
B. Type II cells		
Control (145 mM Na^+)	5.68±1.10	
Intermediate Na^+ (75.25 mM Na^+)	3.40±0.56	40.1
Low Na^+ (23.5 mM Na^+)	2.28±0.64	59.9
Ouabain (1 mM) + Na^+ (145 mM)	0.84±0.44	85.2

Values are means ± SE for 3 experiments. NaCl was replaced by choline chloride in the samples that contained low and intermediate concentrations of sodium.

TABLE 6. *Effects of metabolic inhibitors on taurine uptake by alveolar macrophages and type II cells*

Treatment (Concn)	Taurine Uptake, pmol·10^6 cells^{-1}·mm^{-1}	% Inhibition
A. Alveolar macrophages		
Control (no inhibitor)	21.49±1.89	
Iodoacetic acid (1 mM)	1.88±0.32	91.3
Sodium azide (1 mM)	2.21±0.30	89.7
Dinitrophenol (1 mM)	1.38±0.38	93.6
Sodium fluoride (3 mM)	3.26±0.64	84.8
B. Type II Cells		
Control (no inhibitor)	5.68±1.10	
Iodoacetic acid (1 mM)	0.83±0.39	85.4
Sodium azide (1 mM)	1.09±0.13	80.8
Dinitrophenol (1 mM)	1.14±0.47	79.9
Sodium fluoride (3 mM)	1.36±0.84	76.1

Values are means ± SE for 3 experiments. All samples contained 145 mM NaCl.

cells), the surface area calculated for alveolar macrophages, although larger than that of the type II cells, may still have been underestimated because of the ruffled membrane that these cells possess (4). Additionally, at 100 μM taurine, leakage of taurine across the macrophage membrane, which presumably is a bidirectional process, was estimated to occur at a rate 2.9 times that of the adjusted value for type II cells, i.e., 7.57 vs. 2.61 pmol·10^6 cells^{-1}·min^{-1}, respectively (Fig. 3, *A* and *B*). Therefore more of the taurine that had been transported into the cells may leak back out with alveolar macrophages compared with type II cells.

Overall, type II cells appeared to be less sensitive than alveolar macrophages to the effects of metabolic inhibitors on the transport of taurine. This may reflect a basic metabolic difference between these two cell types. It has been reported that energy generation in type II cells is more dependent on mitochondrial electron transport and less dependent on glycolysis than in alveolar macrophages (12).

Possible factors that could have interfered with our measurements of content and uptake of taurine in these cells include *1*) failure to include exogenous taurine in the media during the cell isolation procedures, *2*) contamination of the cell fractions by other cell types, and *3*) further metabolism of taurine after being transported into the cells. Leakage of taurine out of the cells during the short isolation procedure was unlikely in the case of the alveolar macrophages; it may have been a problem in the case of the longer isolation procedure for type II cells. However, in our study this leakage should have been lessened by maintaining the cells in cold medium (2°C) during the isolation and purification process. Both cell fractions were highly purified. However, a minor contaminant of the alveolar macrophage fraction was erythrocytes and that of the type II fraction was polymorphonuclear leukocytes and to a lesser extent macrophages. Erythrocytes have a low taurine content and therefore are unlikely to take up this nutrient to any large degree, except through simple diffusion (17). Leukocytes, as a class, are taurine rich (17), but whether polymorphonuclear leukocytes specifically contain high levels of taurine has not been investigated. Taurine was previously thought to be an extremely stable metabolic end product that is unlikely to be further metabolized in most cell types (16). However, recent evidence suggests that taurine chloramines are formed in phagocytic cells, such as polymorphonuclear leukocytes, that contain the enzyme myloperoxidase (31, 32). Finally, the plasma-cell gradient may have been underestimated due to leakage of taurine from damaged leukocytes and platelets into the plasma (17).

The exact nutritional role of taurine in the lung is unknown. However, the results of a study by Gordon et al. (15) suggest that taurine may protect pulmonary tissue against oxidant injury. The results of this study suggest that the high taurine content of alveolar macrophages and type II cells may be related to their relative resistance to oxidant injury. We are continuing to investigate the function of taurine in the alveolar region of the lung.

A preliminary report of this work was published in abstract form and presented at the 72nd Annual Meeting of the Federation of American Societies for Experimental Biology, Las Vegas, NV, 1988.

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Received 16 May 1988; accepted in final form 20 September 1988.

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