Biochemical Pharmacology, Vol. 30, No. 21, pp. 2937–2943.
Printed in Great Britain.

0006-2952/81/212937-07 $02.00/0

ACTIVE TRANSPORT OF FOREIGN AMINO ACIDS BY RAT LUNG SLICES*

YI-JONG LIN† and LEWIS S. SCHANKER‡
Department of Pharmacology, University of Missouri, Kansas City, MO 64110, U.S.A.

(*Received* 17 *November* 1980; *accepted* 25 *March* 1981)

Abstract—Uptake of amino acids by rat lung slices was investigated using the ^{14}C-labeled nonmetabolized foreign compounds 1-aminocyclopentanecarboxylic acid (cycloleucine) and α-aminoisobutyric acid (AIB). When incubated with slices suspended in oxygenated Krebs–Ringer phosphate–glucose solution (pH 7.4) at 37°, cycloleucine was accumulated in the tissue against a concentration gradient by a saturable process that was inhibited by anaerobic conditions, low temperature, ouabain, iodoacetic acid, 2,4-dinitrophenol and *N*-ethylmaleimide. Uptake was depressed by AIB, glycine and L-methionine but not by D-ornithine, 3-*O*-methyl-D-glucose, phenol red or disodium cromoglycate. Phenol red enhanced cycloleucine accumulation. AIB was taken up by lung slices by a process similar to that seen with cycloleucine, and uptake was depressed by cycloleucine, glycine and L-methionine but not by D-ornithine. From the initial rates of saturable transport of cycloleucine and AIB, V_{max} and K_m values were calculated. L-Methionine was a competitive inhibitor of the transport of both cycloleucine and AIB.

The nonmetabolized amino acid 1-aminocyclopentanecarboxylic acid (cycloleucine) has been reported to be absorbed from the lungs of the anesthetized rat in part by a carrier-type transport process and in part by diffusion [1]. The carrier-type process showed a transport maximum and was inhibited in a concentration-dependent manner by a number of L- and D-amino acids. Specificity for the carrier process was evident in that the L-form of a given amino acid always produced a stronger inhibition than the D-form, and also in that L-ornithine was a far weaker inhibitor than all other L-compounds studied. Moreover, D-ornithine as well as betaine showed no significant inhibitory activity. The carrier process for cycloleucine appeared to be different from the process responsible for the mediated absorption of phenol red and disodium cromoglycate [2, 3], since a high concentration of these organic anions failed to inhibit absorption of the amino acid. In contrast to results with cycloleucine, α-aminoisobutyric acid (AIB), another model amino acid, appeared to be absorbed from the lungs of anesthetized rats solely by a process of diffusion [1]. This result was of particular interest, since AIB inhibited the mediated absorption of cycloleucine [1] and has also been reported to be accumulated in rat lung slices by a carrier type transport process [4].

The present study shows that cycloleucine is taken up by rat lung slices by an energy-dependent, concentrative, carrier-mediated process and also confirms a similar type uptake for AIB.

MATERIALS AND METHODS

Incubation procedure. Male Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, MA), weighing 150–300 g, were killed by a blow on the head. The lungs were removed, rinsed in cold 0.9% sodium chloride solution, separated into their five lobes, and placed in an ice-cold petri dish. Slices (1 mm thick) were cut with a McIlwain tissue chopper (Brinkmann Instruments, Westbury, NY) from areas of the lungs devoid of major bronchi. Two to three slices (30–85 mg combined weight) were placed in 4.9 to 9.9 ml of Krebs–Ringer phosphate solution (pH 7.4), in which the concentration of calcium ion had been lowered to one-fifth the usual concentration to avoid turbidity [5], and which contained 1 g of glucose/liter. The resulting mixtures, contained in 50-ml beakers, were incubated at 37° in a Dubnoff metabolic shaking incubator (90 oscillations/min) in an atmosphere of 100% oxygen. After an equilibration period of 15 min, Krebs–Ringer phosphate–glucose solution containing a ^{14}C-labeled amino acid, either alone or together with an unlabeled compound, was added to the incubation mixtures in an amount sufficient to give a final volume of 10 ml. Incubation was then continued for various times up to 4 hr. After an incubation period, tissue slices were removed from the beaker, quickly blotted on slightly moistened filter paper, and weighed.

Analytical methods. For the estimation of ^{14}C-labeled amino acids, lung slices or a sample of incubation medium were placed in glass liquid scintillation counting vials, and to each vial was added 0.2 ml of 60% perchloric acid solution followed by 0.4 ml of 30% hydrogen peroxide solution. The vials were then capped tightly, heated at 70° for 1.5 hr,

* This investigation was supported by Public Health Service Research Grant HL-18028 from the National Heart, Lung, and Blood Institute.

† Present address: Department of Pharmacology and Toxicology, Colgate-Palmolive Co., 909 River Road, Piscataway, NJ 08854, U.S.A.

‡ Address all correspondence to: Dr. Lewis S. Schanker, Department of Pharmacology, University of Missouri-Kansas City, 5100 Rockhill Road, Kansas City, MO 64110, U.S.A.

and then cooled to room temperature. To the resulting digest was added 20 ml of a liquid scintillation medium, and radioactivity was measured with a Packard model C2425 Tri-Carb liquid scintillation spectrometer. Net counts were at least five times background and were corrected for quench by comparison with a standard quench correlation curve determined for the liquid scintillation medium used. When known amounts of labeled amino acids were added to tissue or medium and the assays were carried out as described above, recoveries were complete (98–100 per cent).

Results of tissue uptake experiments were expressed as a slice water/medium (S_{H_2O}/M) concentration ratio of compound. To measure the water content of lung slices, tissues were removed from the incubation medium after various times, blotted as described above, weighed, and dried at 100° to constant weight. The water content (percentage of wet tissue weight ±S.E.M.), calculated from the wet and dry tissue weights, was 83 ± 0.2 per cent prior to incubation and 84 ± 0.3 per cent after incubation for 1–6 hr. Since the maximum increase in water content after any period of incubation was less than 2 per cent, all S_{H_2O}/M ratios were calculated using an average slice water content of 84 per cent. Because the volume of the incubation medium was much larger than that of the tissue, the concentration of amino acids in the medium remained virtually constant during an experimental period. In determinations of initial rates of slice uptake of amino acids, accuracy of the data was improved by correction for the amount of amino acid that adhered to the surface of blotted tissue slices in the form of a thin film of incubation fluid. Corrections were based on uptake data obtained at 0.1, 5, 10 and 20 min at 0° (carrier transport was blocked at this temperature) which were extrapolated linearly to zero time. The S_{H_2O}/M ratio thus obtained at zero time (0.39 ± 0.01) was considered the result of amino acid adhering to the slice surface. Heat-denatured lung slices were prepared by placing beakers containing the slices suspended in Krebs–Ringer phosphate solution in a boiling water bath for 30 min.

Statistical evaluations were made using Student's *t*-test [6], and variability was expressed as the mean ±S.E.M.

Binding to lung homogenates. The binding of [^{14}C]cycloleucine to homogenates of lung tissue was estimated by ultrafiltration through Visking cellulose tubing. Details of the procedure have been described previously [7]. Briefly, lung homogenates (20 and 40%, w/v) containing 0.001 mM cycloleucine were prepared in cold Krebs–Ringer phosphate solution using a Tenbroeck glass homogenizer. Cellulose sacs containing the homogenates were centrifuged at 0° for 2 hr at 27,000 *g* in a Sorvall model RC2-B centrifuge. The concentration of cycloleucine in the ultrafiltrate was then compared with that in the tissue homogenate.

Compounds studied. 1-Aminocyclopentane-1-[^{14}C]carboxylic acid ([^{14}C]cycloleucine), sp. act. 60 mCi/mmole, and α-[1-^{14}C]aminoisobutyric acid ([^{14}C]AIB), sp. act. 59 mCi/mmole, were obtained from the Amersham Corp., Arlington Heights, IL. All unlabeled amino acids, ouabain, *N*-ethylmaleimide, and 3-*O*-methyl-D-glucose were obtained from the Sigma Chemical Co., St. Louis, MO; iodoacetic acid, 2,4-dinitrophenol, and tetraethylammonium bromide from Eastman Organic Chemicals, Rochester, NY; and phenol red sodium salt from the J. T. Baker Chemical Co., Phillipsburgh, NJ. Disodium cromoglycate was provided by Fisons Ltd., Loughborough, U.K.

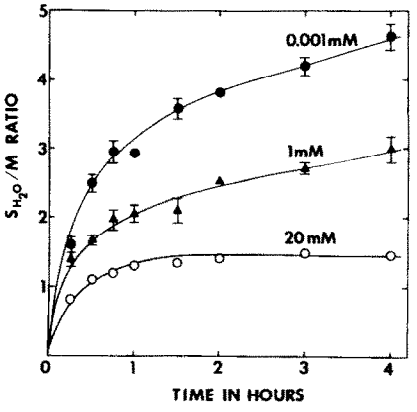

Fig. 1. Uptake of [^{14}C]cycloleucine by rat lung slices as a function of time and concentration. Each point is the mean of three to eight experiments except that the 2-hr values are the means of nine to thirty-four experiments. Vertical brackets indicate S.E.M., and absence of brackets indicates that the S.E.M. was too small to be shown.

RESULTS

Effect of concentration on uptake of cycloleucine by lung slices. When lung slices were incubated with 0.001 mM [^{14}]cycloleucine for various times, the compound was taken up by the tissue against an apparent concentration gradient (Fig. 1). For example, the slice water/medium (S_{H_2O}/M) concentration ratio of compound increased steadily from a value of 1.6 at 0.25 hr to a value of 4.6 after 4 hr of incubation. A steady-state distribution of the compound was not achieved within the 4-hr experimental period.

Uptake of cycloleucine was not proportional to the concentration of compound in the incubation medium (Fig. 1). For example, on raising the concentration from 0.001 mM to 1 mM, the 4-hr S_{H_2O}/M ratio declined from 4.6 to a value of 3.0, and on raising the concentration further, to 20 mM, the ratio declined to a steady-state value of 1.4. These results suggested that the uptake process was saturable.

To assess the quantitative significance of the saturable uptake of cycloleucine, the 2-hr uptake of the amino acid was measured at a variety of concentrations. When the amount of cycloleucine taken up per g of lung tissue was plotted against the concentration of compound in the incubation medium (Fig. 2), a curve was obtained that could be resolved into two components: (1) a linear portion, seen at concentrations above 7.5 mM, and (2) a nonlinear portion, seen at concentrations below 7.5 mM. Thus, at the lower concentrations, uptake appeared to occur primarily by a saturable process, but at higher

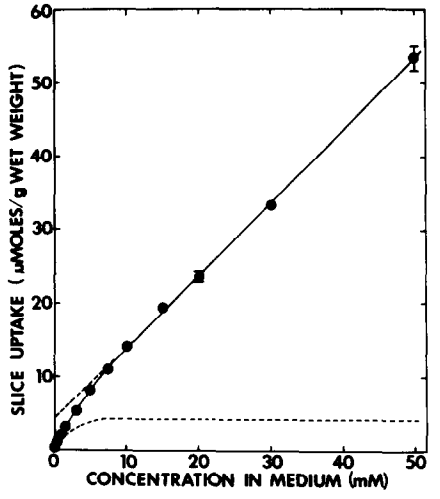

Fig. 2. Effect of concentration on the 2-hr uptake of [^{14}C]cycloleucine by rat lung slices. The curved broken line represents the curve calculated for the saturable uptake component only; the straight broken line is an extrapolation of the straight portion of the solid curve to its intercept on the vertical axis. Each point is the mean of three to seven experiments except that the values for 0.001, 1 and 20 mM cycloleucine are the means of nine to thirty-four experiments. Vertical brackets indicate S.E.M., and absence of brackets indicates that the S.E.M. was too small to be shown.

concentrations, at which the saturable process was approaching saturation, most of the cycloleucine appeared to be taken up by a nonsaturable process such as diffusion. When the linear portion of the curve was extrapolated to its intercept on the vertical axis, or when a curve for only the saturable component of uptake was calculated (Fig. 2), it appeared that the transport maximum for the saturable component was 4.2 μmoles · (g wet weight tissue)$^{-1}$ · 2 hr^{-1} (2.1 μmoles · g^{-1} · hr^{-1}).

Effects of metabolic inhibitors, low temperature, and absence of oxygen on cycloleucine uptake. The uptake of [^{14}C]cycloleucine by lung slices was markedly depressed by a number of metabolic inhibitors and also by anaerobic conditions and low temperature (Table 1). For example, when slices were incubated in a nitrogen atmosphere, the 2-hr S_{H_2O}/M ratio of 0.001 mM cycloleucine was depressed by 57 per cent. Incubation at 0° resulted in a 78 per cent depression of the ratio. In the presence of 10^{-4} M iodoacetic acid, 2,4-dinitrophenol, *N*-ethylmaleimide or ouabain, the S_{H_2O}/M ratio of cycloleucine was depressed by 40–52 per cent; and in the presence of 10^{-3} M concentrations of the same compounds, the ratio was depressed by 57–70 per cent.

Since heat-denatured lung slices showed a S_{H_2O}/M ratio close to unity (0.97 ± 0.008) after both 2 and 4 hr of incubation with 0.001 mM cycloleucine, it was assumed that a ratio of 1.0 represented the passive (non-concentrative) component of uptake that could not be depressed by metabolic inhibitors. Accordingly, the above results were corrected for this component and expressed in terms of a percentage depression of concentrative tissue uptake (Table 1, last column).

Effect of amino acids and other compounds on cycloleucine uptake. To investigate the chemical specificity of the saturable, concentrative uptake process for cycloleucine, the 2-hr uptake of the ^{14}C-labeled compound (0.001 mM) by lung slices was measured in the presence of various other amino acids as well as some chemically unrelated compounds. The results, summarized in Table 2, are expressed both as a percentage depression of the S_{H_2O}/M ratio and as a percentage depression of concentrative tissue uptake. L-Methionine, in concentrations ranging from 0.001 to 100 mM, depressed

Table 1. Effects of metabolic inhibitors, absence of oxygen, and low temperature on 2-hr uptake of 0.001 mM [^{14}C]cycloleucine by rat lung slices

Metabolic inhibitor or experimental condition	Concn (M)	S_{H_2O}/M ratio*	Depression of S_{H_2O}/M ratio (%)	Depression of concentrative tissue uptake† (%)
Control		3.81 ± 0.05 (34)		
Absence of O_2 (100% N_2)		1.65 ± 0.07 (6)	57	77
Low temperature (0°)		0.84 ± 0.01 (8)	78	100
Iodoacetic acid	10^{-4}	1.92 ± 0.14 (5)	50	67
	10^{-3}	1.28 ± 0.08 (3)	66	90
2,4-Dinitrophenol	10^{-4}	2.23 ± 0.21 (6)	41	56
	10^{-3}	1.64 ± 0.05 (3)	57	77
N-Ethylmaleimide	10^{-4}	1.82 ± 0.06 (5)	52	71
	10^{-3}	1.13 ± 0.01 (5)	70	95
Ouabain	10^{-4}	2.29 ± 0.18 (5)	40	54
	10^{-3}	1.46 ± 0.12 (4)	62	84

* Mean ± S.E.M. followed by the number of experiments in parentheses. All values were significantly different from the control value of 3.81 ($P < 0.05$).

† Calculated on the assumption that the passive (non-concentrative) component of tissue uptake resulted in a S_{H_2O}/M ratio of 1.0, since heat-denatured slices showed a ratio of 0.97 ± 0.008 after both 2 and 4 hr of incubation.

Table 2. Effect of amino acids and other compounds on 2-hr uptake of 0.001 mM [^{14}C]cycloleucine by rat lung slices

Compound	Concn (mM)	S_{H_2O}/M ratio*	Depression of S_{H_2O}/M ratio (%)	Depression of concentrative tissue uptake† (%)
Control		3.81 ± 0.05 (34)		
AIB	0.001	3.00 ± 0.10‡ (6)	21	29
	1	1.98 ± 0.16‡ (6)	48	65
	20	1.36 ± 0.02‡ (6)	64	87
Glycine	0.001	3.10 ± 0.16‡ (3)	19	25
	1	2.14 ± 0.09‡ (3)	44	59
	20	1.37 ± 0.04‡ (5)	64	87
L-Isoleucine	50	1.44 ± 0.04‡ (3)	62	84
	100	1.44 ± 0.04‡ (4)	62	84
L-Methionine	0.001	3.27 ± 0.17‡ (3)	14	19
	1	1.36 ± 0.10‡ (3)	64	87
	20	1.13 ± 0.02‡ (8)	70	95
	50	1.03 ± 0.01‡ (8)	73	99
	100	0.98 ± 0.01‡ (6)	74	100
D-Ornithine	1	3.66 ± 0.09 (5)	4	5
	20	3.72 ± 0.14 (5)	2	3
3-*O*-Methyl-D-glucose	20	3.72 ± 0.10 (5)	2	3
Tetraethylammonium	20	3.80 ± 0.11 (4)	0	0
Phenol red	20	6.05 ± 0.36‡ (4)	0	0
Disodium cromoglycate§	20	1.99 ± 0.04§ (5)	1	2

* Mean ± S.E.M. followed by the number of experiments in parentheses.

† See footnote to Table 1.

‡ Significantly different from control value ($P < 0.05$).

§ Since this compound forms insoluble complexes with calcium and magnesium, both ions were omitted from the incubation medium, and this resulted in a control S_{H_2O}/M ratio of 2.01 (S.E.M. ± 0.05 in five experiments).

the S_{H_2O}/M ratio of cycloleucine by 14–74 per cent and depressed concentrative uptake of cycloleucine by 19–100 per cent. Similarly, AIB and glycine, in concentrations ranging from 0.001 to 20 mM, depressed the S_{H_2O}/M ratio by 19–64 per cent and depressed concentrative uptake by 25–87 per cent. L-Isoleucine, studied only at 50 and 100 mM concentrations, depressed concentrative uptake by 84 per cent in both cases. In contrast to these results, no significant inhibition of cycloleucine uptake was seen with 1–20 mM D-ornithine. Similarly, 3-*O*-methyl-D-glucose, the quaternary ammonium compound tetraethylammonium, and the anionic compound disodium cromoglycate failed to inhibit cycloleucine uptake when present in a concentration of 20 mM. The anionic compound phenol red (20 mM) had an unusual effect in that it increased the S_{H_2O}/M ratio of cycloleucine.

Binding of cycloleucine to lung homogenates. To assess the possible binding of [^{14}C]cycloleucine (0.001 mM) to components of lung tissue, diluted homogenates of rat lung [20 and 40% (w/v) in Krebs–Ringer phosphate solution] were subjected to ultrafiltration through a cellulose membrane. Since there was no significant difference between the concentration of cycloleucine in the ultrafiltrate and that in the whole homogenate water (ratio of concentrations, ultrafiltrate/homogenate, was 0.99 ± 0.006), it appeared that binding to macromolecules was negligible.

Uptake of α-aminoisobutyric acid (AIB) by lung slices. When lung slices were incubated with 0.001 mM [^{14}C]AIB for 2 or 4 hr, the resulting S_{H_2O}/M ratios were 8.29 and 10.63, respectively (Table 3), suggesting uptake against a concentration gradient. Uptake of AIB was not proportional to the concentration of compound in the incubation medium. For example, on raising the concentration to 1 and 20 mM, the S_{H_2O}/M ratios declined progressively (Table 3), suggesting that the uptake process was saturable.

To investigate the specificity of the uptake process for AIB, the 2-hr uptake of the compound (0.001 mM) was measured in the presence of various other amino acids. The results, summarized in Table 4, show that cycloleucine, glycine and L-methionine depressed AIB uptake in a concentration-dependent manner. When present in a concentration of 20 mM, these amino acids produced an essentially complete

Table 3. Effect of concentration on uptake of [^{14}C]AIB by rat lung slices

Concn (mM)	Incubation time (hr)	S_{H_2O}/M ratio*
0.001	2	8.29 ± 0.44 (9)
	4	10.63 ± 0.33 (6)
1	2	5.27 ± 0.24 (6)
	4	6.67 ± 0.23 (6)
20	2	1.44 ± 0.03 (6)
	4	1.62 ± 0.02 (6)

* Mean ± S.E.M. followed by the number of experiments in parentheses.

Table 4. Effect of amino acids on 2-hr uptake of 0.001 mM [^{14}C]AIB by rat lung slices

Compound	Concn (mM)	S_{H_2O}/M ratio*	Depression of S_{H_2O}/M ratio (%)	Depression of concentrative tissue uptake† (%)
Control		8.29 ± 0.44 (9)		
Cycloleucine	0.001	8.36 ± 0.29 (3)	0	0
	1	5.16 ± 0.31‡ (3)	38	43
	20	1.14 ± 0.03‡ (3)	86	98
Glycine	0.001	9.23 ± 0.66 (3)	0	0
	1	4.27 ± 0.05‡ (3)	48	55
	20	1.25 ± 0.02‡ (3)	85	97
L-Methionine	0.001	7.60 ± 0.50 (4)	8	9
	1	3.33 ± 0.24‡ (3)	60	68
	20	0.97 ± 0‡ (3)	88	100
D-Ornithine	0.001	8.97 ± 0.09 (3)	0	0
	1	8.96 ± 0.32 (3)	0	0
	20	7.82 ± 0.19 (3)	6	6

* Mean ± S.E.M. followed by the number of experiments in parentheses.

† Calculated on the assumption that the passive (non-concentrative) component of tissue uptake resulted in a S_{H_2O}/M ratio of 1.0.

‡ Significantly different from control value ($P < 0.05$).

inhibition of the concentrative uptake of AIB. In contrast to these results, no significant inhibition of AIB uptake was seen with 20 mM D-ornithine.

Initial rates of uptake of amino acids by lung slices. In Fig. 3 are shown time curves describing the early uptake (5–20 min) of various concentrations of [^{14}C]cycloleucine by rat lung slices. Since the curves were linear, initial rates of uptake could be calculated from the slopes of the regression lines. Initial rates of [^{14}C]AIB uptake were obtained in the same manner (not shown).

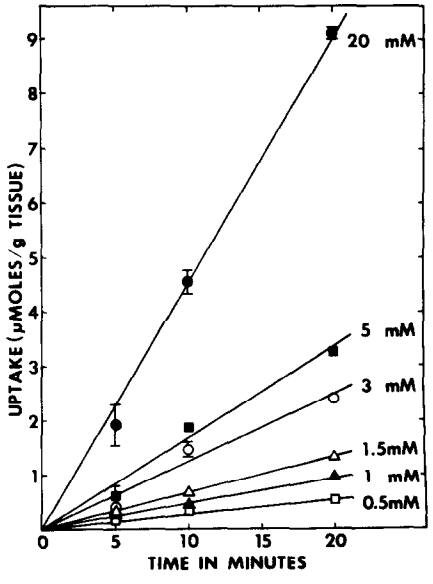

Fig. 3. Time course of early uptake of various concentrations of [^{14}C]cycloleucine by rat lung slices. Curves were calculated by least squares linear regression. Each point is the mean of four to six experiments. Vertical brackets indicate S.E.M., and absence of brackets indicates that the S.E.M. was too small to be shown.

When the initial rate of uptake of cycloleucine was plotted against concentration of compound in the incubation medium (Fig. 4), a curve was obtained that showed a linear portion at concentrations above 5 mM and a nonlinear portion at lower concentrations. Thus, at concentrations below 5 mM, uptake appeared to occur mainly by a saturable process; but at higher concentrations, most of the cycloleucine was taken up by a nonsaturable process such as diffusion. A curve similar to that shown in Fig. 4 was obtained when corresponding data were plotted for AIB (not shown). From the slope of the linear portion of the curves for cycloleucine and AIB, the following apparent diffusion rate constants were calculated: 0.0186 and 0.0152 μmole · (g tissue)$^{-1}$ · min^{-1} · mM^{-1} for cycloleucine and AIB respectively. Uptake resulting from diffusion alone could then be determined for each concentration studied by multiplying the diffusion rate constant by the

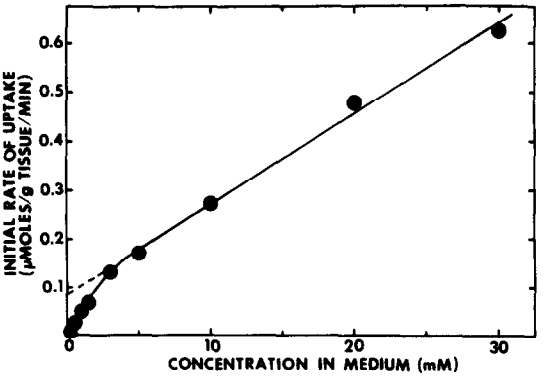

Fig. 4. Initial rates of slice uptake of [^{14}C]cycloleucine at various concentrations. Plotted rates are the slopes of the linear regression lines shown in Fig. 3. Also included are rates for three additional concentrations not shown in Fig. 3.

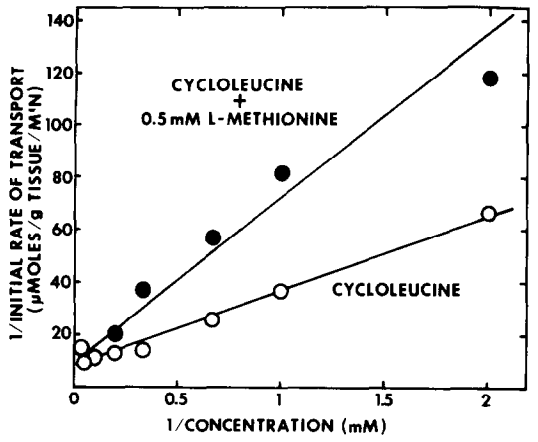

Fig. 5. Double-reciprocal plot of initial rate of saturable uptake (transport) and concentration of [^{14}C]cycloleucine in the presence and absence of 0.5 mM L-methionine. Slice uptake due to the saturable transport process was calculated by subtracting the diffusion component from the overall initial rate of uptake at each concentration (see text).

concentration of amino acid in the medium. This value could then be subtracted from the overall initial rate of uptake at each concentration to give the initial rates of uptake for the saturable process only. In Fig. 5 is shown a double-reciprocal plot describing the relation between the initial rate of saturable uptake and the concentration of cycloleucine. From this plot, the following kinetic parameters were calculated [8]: V_{max}, 0.119 μmole · (g tissue)$^{-1}$ · min^{-1}; and K_m, 3.38 mM. From a double-reciprocal plot of corresponding data for AIB (not shown), the kinetic parameters for this amino acid were: V_{max}, 0.158 μmole · (g tissue)$^{-1}$ · min^{-1}; and K_m, 4.71 mM.

In addition to the studies described above, initial rates of slice uptake for five to six different concentrations (0.5 to 20 mM) of cycloleucine and AIB were determined in the presence of 0.5 mM L-methionine. When the linear uptake data were plotted as in Fig. 3 (not shown), results were corrected for the diffusion component of uptake, and the initial rates of saturable transport were plotted against concentration in double-reciprocal fashion as described above, the resulting kinetic parameters for cycloleucine in the presence of 0.5 mM L-methionine were: V_{max}, 0.119 μmole · (g tissue)$^{-1}$ · min^{-1}; and K_m, 7.63 mM (Fig. 5). The parameters for AIB in the presence of 0.5 mM L-methionine (plot not shown) were: V_{max}, 0.158 μmole · (g tissue)$^{-1}$ · min^{-1}; and K_m, 6.00 mM. It thus appears that, in these lung slices, L-methionine was a competitive inhibitor of the saturable transport of both cycloleucine and AIB.

DISCUSSION

The nonmetabolized amino acid cycloleucine appears to have been accumulated in the rat lung slices by an energy-dependent, carrier-type process that exhibited the general characteristics of active transport. For example, the compound, which showed no binding to components of lung tissue, was taken up against a concentration gradient with tissue levels rising to a value 4.6 times that in the medium after 4 hr of incubation. When the concentration of cycloleucine in the incubation medium was raised, the uptake process tended to become saturated, and S_{H_2O}/M ratios declined toward unity as diffusion became the predominant mechanism of uptake. The saturable transport process showed energy-dependency, since cycloleucine uptake was markedly depressed by anaerobic conditions, low temperature, and low concentrations of iodoacetic acid, dinitrophenol, *N*-ethylmaleimide and ouabain. Specificity for the uptake process was evident in that, while AIB, glycine and L-methionine inhibited cycloleucine transport in a concentration-dependent manner, another amino acid, D-ornithine, showed no inhibitory activity. Moreover, no depression of cycloleucine uptake was seen with high concentrations of the chemically unrelated compounds 3-*O*-methyl-D-glucose, tetraethylammonium, phenol red or disodium cromoglycate. Considering that the latter two compounds are known to be taken up by rat lung slices by an active transport process [5, 9], it appears that cycloleucine is transported by a separate carrier system. Interestingly, phenol red has the unique effect of elevating the S_{H_2O}/M ratio of cycloleucine to a value 1.6 times the control value. It is not known whether this resulted from an increased capacity of the carrier system for cycloleucine, an increased affinity of the carrier system for the amino acid, a change in the rate of diffusion of cycloleucine, or the creation of tissue binding sites for the amino acid.

AIB, another model amino acid that is metabolically stable in the lung [4], appears to have been accumulated in the lung slices by a process similar to that described for cycloleucine. Uptake against an apparent concentration gradient occurred by a saturable process that was inhibited in a concentration-dependent manner by cycloleucine, glycine and L-methionine. Moreover, as with cycloleucine, uptake was not inhibited by high concentrations of D-ornithine. These results with AIB confirm and extend those of Gregorio and Massaro [4], who reported that the compound is accumulated in rat lung slices by a saturable process that is inhibited by anaerobic conditions, cyanide, glycine, proline and valine. Although the values for V_{max}, K_m and diffusion constant reported by the above investigators were different from those obtained in the present study, a direct comparison of results is not possible because of significant differences in experimental conditions including incubation temperature, incubation medium, and gas phase. Moreover, kinetic parameters were based on initial rates of uptake in the present study and on 30–60 min data in the previous investigation.

From the initial rates of slice uptake of cycloleucine and AIB, it has been possible to estimate the kinetic parameters of the carrier-type transport processes. Cycloleucine showed V_{max} [μmole · (g tissue)$^{-1}$ · min^{-1}] and K_m (mM) values of 0.119 and 3.38, respectively, whereas AIB showed values of 0.158 and 4.71, respectively. Lineweaver–Burk plots of the transport of these compounds in the presence and absence of L-methionine showed that the latter amino acid is a competitive inhibitor of the transport

of both cycloleucine and AIB, since the values of V_{max} remained unchanged while those of K_m rose in the presence of the inhibitor. Whether cycloleucine, AIB and L-methionine are all transported by a single carrier system or by several carrier systems of overlapping specificity, as has been suggested for a number of other tissues [10–12], cannot be determined from the results of the present study.

Finally, it is of interest to compare the uptake of model amino acids by lung slices with absorption of the compounds from the lung *in vivo* [1]. In the case of cycloleucine, both processes show certain similarities, whereas, with AIB, the processes show some marked differences. For example, cycloleucine was taken up *in vitro* and is absorbed *in vivo* by a saturable, carrier-type process as well as by a nonsaturable process; moreover, both *in vitro* and *in vivo*, the saturable process is inhibited by AIB, glycine, L-isoleucine and L-methionine but not by D-ornithine, 3-*O*-methyl-D-glucose, tetraethylammonium, phenol red or disodium cromoglycate. In contrast, with AIB, while uptake *in vitro* occurred by both saturable and nonsaturable processes, absorption *in vivo* appears to take place by a nonsaturable process only and at a rate consistent with diffusion through aqueous membrane channels; moreover, the saturable process *in vitro* was inhibited by L-methionine as well as by certain other amino acids, whereas absorption *in vivo* is not inhibited by a high concentration of L-methionine. It is difficult to provide a simple explanation for these results, since absorption *in vivo* appears to involve transport from lumen to epithelial cell followed by transport from epithelial cell to circulation, whereas uptake *in vitro* would represent the difference between uptake and release processes not only by epithelial cells but also by other cell types present in lung slices. Moreover, amino acid accumulated in a 1-mm thick lung slice might be present not only within cells but also in extracellular spaces as a result of uphill transport across certain cells. In addition, the tissue slice technique has the inherent shortcoming that the direction of epithelial transport is uncertain. A more suitable method for investigation of directional transport of amino acids *in vitro* might be provided by an isolated perfused lung preparation [13].

REFERENCES

1. Y.-J. Lin and L. S. Schanker, *Am. J. Physiol.*, **240**, C215 (1981).
2. S. J. Enna and L. S. Schanker, *Life Sci.* **12** (Part I), 231 (1973).
3. T. H. Gardiner and L. S. Schanker, *Xenobiotica* **4**, 725 (1974).
4. C. A. Gregorio and D. Massaro, *J. appl. Physiol.* **42**, 216 (1977).
5. T. H. Gardiner and L. S. Schanker, *J. Pharmac. exp. Ther.* **196**, 455 (1976).
6. W. J. Dixon and F. H. Massey, Jr., *Introduction to Statistical Analysis*, 3rd Edn., p. 116. McGraw-Hill, New York (1969).
7. S. W. Hwang and L. S. Schanker, *Am. J. Physiol.* **225**, 1437 (1973).
8. H. Lineweaver and D. Burk, *J. Am. chem. Soc.* **56**, 658 (1934).
9. T. H. Gardiner and F. R. Goodman, *J. Pharmac. exp. Ther.* **198**, 395 (1976).
10. H. N. Christensen, *Fedn Proc.* **32**, 19 (1973).
11. H. Sershen and A. Lajtha, *J. Neurochem.* **32**, 719 (1979).
12. G. Wiseman, in *Handbook of Physiology. Alimentary Canal* (Ed. C. F. Code), Vol. III, Section 6, p. 1277. American Physiological Society, Washington, DC (1968).
13. J. M. Charles, M. B. Abou-Donia and D. B. Menzel, *Toxicology* **9**, 59 (1978).