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Amino Acid Transport in Suspension-Cultured Plant Cells[1]

VI. INFLUENCE OF pH BUFFERS, CALCIUM, AND PREINCUBATION MEDIA ON L-LEUCINE UPTAKE

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ABSTRACT

The rate at which L-leucine was transported into suspension-cultured *Nicotiana tabacum* cv Wisconsin 38 cells increased more than 2-fold over a period of hours when the cells were preincubated in a 1% sucrose solution. This increase in uptake rate was eliminated if certain tris buffers were included in the preincubation solution while other buffers had little effect. Calcium could reverse the effect of the inhibitory buffers only if the buffer and calcium were present together from the beginning of the preincubation period. It was the amine group of the inhibitory buffers which was responsible for the inhibition. Preincubation in a complete culture medium (EM Linsmaier, F Skoog 1965 Physiol Plant 18: 100–127) led to minimal changes in L-leucine uptake rate over a 10 hour preincubation period indicating that the uptake rate was stabilized by this medium. The complete medium stabilized the L-leucine uptake rate as a result of its ionic composition and not because of its osmolarity. Most of the increased uptake rate observed after preincubation in a 1% sucrose solution could be inhibited by 2,4-dinitrophenol or carbonyl cyanide *m*-chlorophenyl hydrazone, or high concentrations of L-phenylalanine or L-leucine. Therefore much of the increase could be accounted for by an increase in active transport of L-leucine.

Increases in the rate of solute uptake have been induced in plant cells and tissues by a procedure of washing and preincubation (8, 11, 15, 17, 18). This effect has been observed using a number of species and for a broad range of substrates: phosphate, potassium, chloride, glycine, glucose, and guanosine monophosphate (11); sulfate (10); L-serine (18); malate, glycerate-3-P, uracil, and several amino acids (8); and others. Since Epstein's (5) early report that calcium is required for the integrity of selective ion transport mechanisms, most transport studies have employed media which contain calcium. It has been reported that the high rates of solute uptake which are observed after preincubation are dependent upon calcium in the medium (2, 7, 8, 10, 18). However, other researchers have demonstrated that an enhanced uptake rate can be induced without the addition of calcium to the preincubation solution (6, 11, 15). Transport workers also differ in their use of pH buffers in preincubation and incubation solutions. Some routinely employ pH buffers (2, 7, 8, 10, 15, 18) while others do not (11, 13, 14, 19). Reinhold's laboratory initially employed pH buffers (17), but because buffers were shown to influence the amino acid uptake process, they discontinued the use of buffers (1, 16).

Using suspension-cultured tobacco cells we have investigated the effect of several buffers, calcium, and various other medium components on the enhanced L-leucine uptake rate induced by preincubation in a 1% sucrose solution. Results reported here indicate that certain tris buffers inhibited this increase in uptake rate and that calcium could relieve the tris buffer inhibition of the enhanced uptake rate. Moreover, L-leucine uptake rate was more stable when cells were transferred into a complete culture medium than when transferred into a solution of low ionic strength.

MATERIALS AND METHODS

Cell Culture. *Nicotiana tabacum* cv Wisconsin 38 cells, originally isolated from pith, were maintained in LS medium[3] as previously described (3). Subculturing was performed every 7 d by adding 50 ml of fresh LS medium to a culture flask and then dividing this suspension between two 125-ml Erlenmeyer flasks. Experiments were conducted on the 3rd d after subculturing near the end of the exponential growth phase (4).

Uptake Assay. Under aseptic conditions, three or four flasks of cultured cells were combined, poured through a screen basket (mesh size of 1.5 mm) to remove clumps, and collected by centrifugation (1610*g* for 1 min). Two to 3 ml of cells were transferred to 50-ml tubes. The cells were washed three times by resuspending the cells in about 50 ml of the appropriate solution, shaking gently by hand for 2 min, letting the cells settle for about 4 min, and decanting the wash solution. The same solution, except where noted, was used for washing, preincubating, and measuring uptake except, L-leucine was present in the uptake solution at a concentration of 0.1 mM. After the third wash, cells were resuspended in 50 ml of solution and transferred to 125-ml Erlenmeyer flasks for preincubation under conditions identical to those used for cell culture. After the preincubation period, cells were permitted to settle and the preincubation solution decanted. L-Leucine uptake was measured employing [^{3}H]-L-leucine in a fresh solution identical to the preincubation solution unless otherwise stated. Uptake rate was measured by collecting samples after 6 and 12 min of incubation as previously described

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[3] Abbreviations: LS medium, Linsmaier and Skoog (12) medium (major salts: 20 mM NH_4NO_3, 20 mM KNO_3, 2.5 mM $CaCl_2$, 2.5 mM $MgSO_4$; minor salts: 0.1 mM H_3BO_3, 0.1 mM $MnSO_4$, 30 μM $ZnSO_4$, 5 μM KI, 1 μM Na_2MoO_4, 100 nM $CoCl_2$, 80 nM $CuSO_4$; phosphate: 1 mM KH_2PO_4; iron: 0.1 mM $FeSO_4$, 0.1 mM Na_2 EDTA; organic: 1 μM thiamine, 1μM inositol) supplemented with kinetin (0.1 mg/l), α-naphthaleneactic acid (2.0 mg/l), and sucrose (4%); PEG, polyethylene glycol (200 mol wt); BTP, 1,3-bis[tris(hydroxymethyl)methylamino]propane; BIS-TRIS, bis(2-hydroxyethyl)imino-tris(hydroxymethyl)methane; TAPS, tris(hydroxymethyl)methylaminopropanesulphonic acid; DNP, dinitrophenol; CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone.

(4, 14). All solutions were adjusted to a pH of 5.5 with NaOH or HCl prior to use. Uptake rate was expressed as nmol/(ml HV · min) where HV was the volume of cell debris which pelleted in 1 min at 1610*g* after a sample of cells had been homogenized (3). This standardization parameter has been equated to other standardization parameters (*e.g.* fresh weight, packed cell volume) (3).

Efflux. After a 6 h preincubation period as described above, cells were centrifuged and washed three times with 50 ml of the appropriate solution. Cells were resuspended in 20 ml of the appropriate solution containing [^{3}H]-L-leucine and incubated for 1 h. Cells were then washed three times with 50 ml of the appropriate solution. The loaded, washed cells were resuspended in 20 ml of the appropriate solution and a 0.2 ml sample was taken every 10 min for 1 h. Each 0.2 ml sample was immediately centrifuged to remove cells and 0.1 ml of the supernatant was counted. The efflux rate constant was calculated as previously described (4).

Metabolic Inhibitors. After a 6 h preincubation, cells were incubated in the presence of the metabolic inhibitor for 15 min. After the 15 min incubation, cells were centrifuged, the supernatant was decanted and uptake measured as described above in the appropriate solution containing the metabolic inhibitor.

Chemicals. L-[4,5-^{3}H (N)]Leucine was obtained from ICN; Liquiscint from National Diagnostics; 1,1,1 tris (hydroxymethyl) ethanol from Aldrich; and all other chemicals from Sigma or Fisher Scientific. All chemicals were reagent grade or ACS certified.

RESULTS

Uptake Rate Prior to Preincubation. The L-leucine uptake rate for cells prior to preincubation was estimated by measuring the uptake rate of cells which had been washed three times as in the normal preparation procedure or of cells which had been washed only once (Table I). One wash in low ionic strength solutions reduced the L-leucine uptake rate significantly when compared to the rate in LS medium. However, the rate recovered during the time (approximately 45 min) it took to complete the three wash procedure except when the solution contained 1% sucrose plus BTP.

Uptake Rate after Preincubation. L-Leucine uptake rate markedly increased as a function of time when cells were preincubated in a 1% sucrose solution (Fig. 1). The L-leucine uptake rate exhibited a period of rapid increase which leveled after about 5 h of preincubation. Preincubation in LS medium stabilized the L-leucine uptake rate at close to the zero-time rate with only a gradual increase over the 10 h period (Fig. 1). To better define the stabilization of the L-leucine uptake rate observed in LS medium, uptake rates were measured for cells which had been preincubated in various solutions (Table II). Addition of the organic, hormone, and iron components of LS medium to the 1% sucrose solution did not stabilize the uptake rate (data not shown), while phosphate and major and minor salt components did, to some extent. Potassium, at a concentration similar to that found in LS medium (*i.e.* 20 mM), stabilized the rate at close to the zero time rate observed in LS medium. At 20 mM, sodium also stabilized the uptake rate. However, at higher concentrations these ions depressed the uptake rate. Simulating the osmotic pressure of LS medium with sugars or PEG did not stabilize the L-leucine uptake rate, but, on the contrary, appeared to augment slightly the uptake rate increase normally observed after preincubation in a 1% sucrose solution.

Upon buffering the pH of the 1% sucrose solution with 5.0 mM BTP, the L-leucine uptake rate fell as the preincubation period increased (Fig. 1). The inhibition of the uptake rate by BTP was concentration dependent (Fig. 2). A similar, although much smaller response to BTP was seen when LS medium was employed as the preincubation and incubation solution. LS medium rate was 7.48 ± 0.57. LS medium plus BTP rate was 6.28 ± 0.87. LS medium minus calcium plus BTP rate was 5.20 ± 0.78. When Mes was used as the hydrogen ion buffer, the increased uptake rate associated with preincubation in a 1% sucrose solution was not inhibited (Fig. 2). A host of other buffers were employed to identify any influence they might have on the L-leucine uptake rate (Table III, Fig. 3). Tris and BIS-TRIS behaved like BTP while TAPS, Tes, Hepes, and Tricine behaved like Mes. A compound (1,1,1-Tris[hydroxymethyl] ethanol) structurally related to Tris but lacking the amine group had no

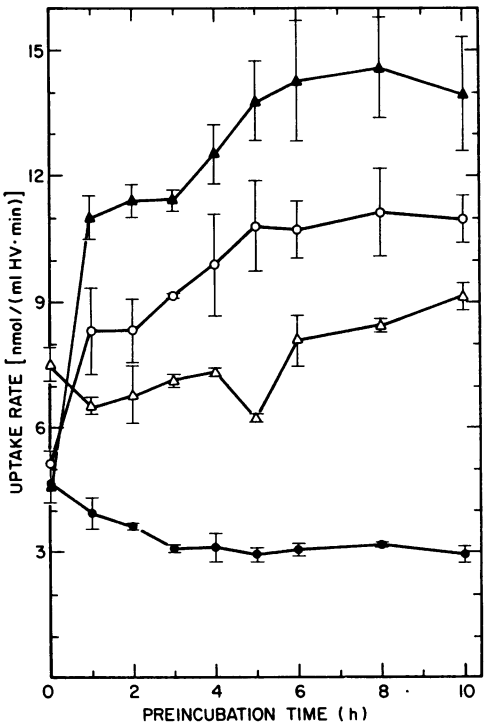

FIG. 1. L-Leucine uptake rate as a function of preincubation time in four different solutions. Cells were settle washed, preincubated for various periods of time and L-leucine uptake rate measured. L-Leucine concentration in the incubation solution was 0.1 mM. Preincubation and incubation solutions: (▲), 1% sucrose; (○), 1% sucrose, 5.0 mM BTP and 0.5 mM $CaCl_2$; (△), LS medium; (●), 1% sucrose and 5.0 mM BTP. The values obtained from the single wash procedure (Table I) were used for the zero time uptake rates.

Table I. *L-Leucine Uptake Rate Prior to Preincubation*

Cells were settle washed once or three times and then the L-leucine uptake rate measured. L-Leucine concentration in the incubation solution was 0.1 mM. In all tables *n* is number of repetitions.

Wash and Incubation Solutions[a]	Treatment	Uptake Rate ± SE (*n*)
		nmol/ml HV·min
No additions	1 wash	4.55 ± 0.39 (12)
5 mM BTP	1 wash	4.64 ± 0.37 (12)
5 mM BTP + 0.5 mM $CaCl_2$	1 wash	5.14 ± 0.35 (12)
LS medium[b]	1 wash	7.53 ± 0.41 (12)
No additions	3 washes	6.94 ± 0.62 (3)
5 mM BTP	3 washes	3.84 ± 0.57 (3)
5 mM BTP + 0.5 mM $CaCl_2$	3 washes	6.65 ± 0.54 (3)
LS medium[b]	3 washes	6.35 ± 0.28 (3)

[a] Solutions contained 1% sucrose plus the indicated compounds. [b] LS medium contained a total of 4% sucrose.

Table II. *Effect of the Ionic Composition and Osmotic Pressure of Preincubation and Incubation Solutions on L-Leucine Uptake Rate*

Cells were settle washed, preincubated for 6 h, and L-leucine uptake rate measured. L-Leucine concentration in the incubation solution was 0.1 mM.

Preincubation and Incubation Solutions[a]	% of Rate in LS Medium	% of Rate in 1% Sucrose	Uptake Rate ± SE (*n*)
			nmol/ml HV·min
LS medium[b,c]	100	53	7.65 ± 0.48 (23)
No additions[c]	189	100	14.49 ± 0.52 (33)
LS major salts	134	71	10.27 ± 0.68 (6)
LS minor salts	146	77	11.16 ± 0.80 (6)
1 mM KH_2PO_4	153	81	11.67 ± 0.51 (6)
20 mM KNO_3	84	44	6.40 ± 0.53 (6)
40 mM KNO_3	64	34	4.80 ± 0.07 (3)
20 mM KCl	97	51	7.43 ± 0.19 (3)
20 mM NaCl	84	44	6.41 ± 1.11 (3)
40 mM NaCl	42	22	3.23 ± 0.60 (3)
Sucrose (3%)[c]	221	117	16.91 ± 0.70 (12)
Sucrose (3.5%) + sorbitol (1.6%)[c]	198	105	15.18 ± 0.53 (6)
PEG (4%)[c]	210	111	16.10 ± 0.93 (3)

[a] Solutions contained 1% sucrose plus the indicated compounds. [b] LS medium contained a total of 4% sucrose. [c] These solutions had the following osmotic pressures: 1% sucrose equaled 0.7 bar, 4% sucrose equaled 2.9 bar, 4% PEG equaled 5.0 bar, 4.5% sucrose plus 1.6% sorbitol equaled 5.3 bar, LS medium equaled 5.4 bar.

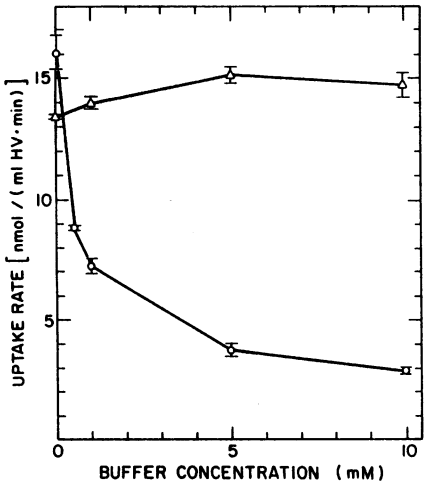

FIG. 2. L-Leucine uptake rate as a function of BTP or Mes concentration. Cells were settle washed, preincubated for 6 h and L-leucine uptake rate measured. L-Leucine concentration in the incubation solution was 0.1 mM. (O), BTP; (Δ), Mes.

effect on the enhancement of the uptake rate, while 1,3 diaminopropane which lacks the tris (hydroxymethyl) methane groups of BTP was somewhat more potent than BTP in inhibiting the L-leucine uptake rate.

Calcium chloride in the preincubation solution counteracted the inhibitory effect of BTP, Tris, and BIS-TRIS thereby allowing for an increase in uptake rate during preincubation (Fig. 1; Table III). This calcium-BTP relationship was also observed using LS medium (see preceding paragraph). The calcium effect was concentration dependent with complete reversal of the effect of 5 mM BTP at 1 mM calcium (data not presented). The calcium-BTP relationship was a calcium effect and not due to anions (Table IV). Magnesium was a moderately effective replacement of calcium. Lanthanum ions alone prevented the increase in L-leucine uptake rate and had only a small influence on the BTP inhibition. The calcium effect was only seen if the BTP and calcium were initially present together indicating that calcium could not readily reverse an established BTP effect (Table V).

Efflux. After a standard 6 h preincubation, cells were loaded with radioactively labeled L-leucine. The loaded cells were washed and the release of label into the medium was measured over a 1 h period. The efflux rate constants were 0.016 ± 0.001 min^{-1} and 0.015 ± 0.003 min^{-1} for cells preincubated in 1% sucrose plus BTP and 1% sucrose, respectively.

Transport Inhibitors. After a standard 6 h preincubation, metabolic inhibitors reduced the L-leucine uptake rate of cells preincubated in 1% sucrose or LS medium to the same extent (Table VI). High concentrations of L-phenylalanine or L-leucine similarly inhibited the uptake rate of 1% sucrose or LS medium preincubated cells. The absolute magnitudes of the inhibited and uninhibited rates were generally twice as great for 1% sucrose as compared to LS medium preincubated cells. The presence of cycloheximide in the preincubation solution also prevented an increase in the L-leucine uptake rate.

DISCUSSION

The data presented established that the L-leucine uptake rate in suspension-cultured tobacco cells was increased more than 2-fold by preincubating the cells in solutions of low ionic strength. These uptake rate increases were not observed when the preincubation solution was a complete culture medium like LS medium. Thus, the complete medium had a stabilizing effect on the L-leucine uptake rate. It was the ionic strength and not the osmotic pressure of the complete medium which was critical since preincubation in nonionic solutions which had an osmotic pressure similar to that of LS medium also permitted the increase in uptake rate. The uptake rate increase in low ionic strength solutions was time dependent and was not dependent upon the presence of calcium in the preincubation solution.

Some researchers who use tris buffers in their preincubation solutions have concluded that calcium is required in the preincubation solution in order to obtain uptake rate increases for amino acids (2, 8, 18). Others have reported that increases in uptake rate do not require the presence of calcium in the preincubation solution (11, 15). We have clearly demonstrated that calcium was only required for increases in the L-leucine uptake rate when certain tris buffers were included in the preincubation

Table III. *Effect of Preincubation and Incubation with Various Buffers on L-Leucine Uptake Rate*

Cells were settle washed, preincubated for 6 h, and the L-leucine uptake rate measured. L-Leucine concentration in the incubation solution was 0.1 mM.

Preincubation and Incubation Solutions[a]	% of Rate in 1% Sucrose	Uptake Rate ± SE (*n*)
		nmol/ml HV·min
No additions	100	13.61 ± 0.41 (28)
5 mM TES	96	13.03 ± 0.89 (6)
5 mM Hepes	94	12.78 ± 0.68 (12)
5 mM TAPS	91	12.41 ± 0.91 (6)
5 mM Tricine	88	11.98 ± 1.11 (5)
5 mM Tris	49	6.63 ± 0.41 (12)
5 mM Tris + 0.5 mM $CaCl_2$	75	10.21 ± 0.54 (12)
5 mM BIS-TRIS	50	6.82 ± 0.33 (6)
5 mM BIS-TRIS + 0.5 mM $CaCl_2$	64	8.69 ± 0.67 (6)
5 mM BTP	24	3.33 ± 0.16 (27)
5 mM BTP + 0.5 mM $CaCl_2$	79	10.75 ± 0.54 (6)
5 mM 1,1,1-Tris(hydroxymethyl)ethanol	112	15.22 ± 1.02 (5)
5 mM 1,3-Diaminopropane	14	1.85 ± 0.28 (6)

[a] Solutions contained 1% sucrose plus indicated compounds.

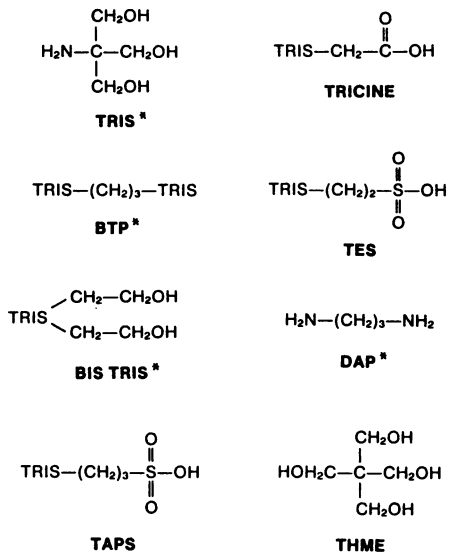

FIG. 3. Chemical structures of tris buffers and related compounds. TRIS in a formula indicates that a molecule of TRIS is linked via the nitrogen atom of TRIS to the carbon atom indicated. DAP, 1,3 diaminopropane. THME, 1,1,1-Tris (hydroxymethyl) ethanol. (*), The compounds which inhibited the increase in L-leucine uptake rate observed when cells were preincubated in a 1% sucrose solution.

solution. Thus, it appears that the reported "calcium effect" is a release of the inhibition of the increase in uptake rate caused by certain tris buffers.

All of the inhibitory buffers we tested have one structural feature in common, an amine group which is active in pH buffering. Without this amine group, the inhibitory effect of Tris was absent (*i.e.* 1,1,1-tris[hydroxymethyl]ethanol). A molecule with just the interior portion of BTP (*i.e.* 1,3-diaminopropane) was slightly more inhibitory than BTP. The inhibitory activity of the tris buffers was lost if a strong acid group is present in close proximity to the amine as is the case with TAPS, Tes, and Tricine (Fig. 3). It is possible that interactions involving nucleophilic attack by the amine nitrogen may be reduced by the influence of the acid group. Alternatively, the acid group may provide an intramolecular charge balance for the predominantly positively charged amine group thereby altering its ionic properties. Incubation of bacteria in solutions containing Tris has been reported to alter membrane structure and function (9). Although there was no significant change in efflux, it is possible that the inhibitory tris buffers were interacting with the plant cell membrane and that calcium ions protected the membrane.

Table IV. *Effect of Calcium Salts, $MgCl_2$ and $LaCl_3$ on BTP Inhibition of the L-leucine Uptake Rate*

Cells were settle washed, preincubated for 6 h, and L-leucine uptake rate measured. L-Leucine concentration in the incubation solution was 0.1 mM.

Preincubation and Incubation Solutions[a]	% of Rate in 1% Sucrose	Uptake Rate ± SE (*n*)
		nmol/ml HV·min
No additions	100	15.40 ± 0.60 (9)
5 mM BTP	22	3.40 ± 0.44 (9)
5 mM BTP + 0.5 mM Ca $(SO_4)_2$	96	14.73 ± 0.96 (3)
5 mM BTP + 0.5 mM Ca $(NO_3)_2$	85	13.13 ± 0.52 (3)
5 mM BTP + 0.5 mM $MgCl_2$	54	8.33 ± 0.70 (6)
5 mM BTP + 0.5 mM $LaCl_3$	37	5.64 ± 0.68 (6)
0.5 mM $LaCl_3$	32	4.98 ± 0.26 (6)

[a] Solutions contained 1% sucrose plus indicated compounds.

It is difficult to determine the mechanism of the tris buffer effect without a clear understanding of the mechanism of the uptake rate enhancement observed upon preincubation of plant cells and tissues in low ionic strength solutions. Such an understanding is not available, but some parameters are known. The development of the enhanced amino acid uptake rate is inhibited by cycloheximide (1, 8, 11) implying that protein synthesis is important to this development. Calcium did not reverse the BTP effect when added after BTP had acted, but BTP reversed the increase in uptake rate when added midway in an 8 h preincubation. Thus BTP acted, directly or indirectly, to interfere with uptake processes, perhaps by disrupting mechanisms employed by cells to respond to their environment and regulate uptake rates.

It is clear from the work reported and cited here that plant cells regulate the rate of solute uptake in response to environmental factors. It would be of interest to know if the increase in L-leucine uptake rate observed after preincubation in solutions with low ionic strength was energy dependent and carrier mediated. If the total uptake rate increase is energy dependent, then metabolic inhibitors like DNP and CCCP should inhibit the

Table V. *Irreversibility of the BTP Effect*

Cells were settle washed and initially preincubated for 4 or 9 h. Those cells which were initially preincubated for 4 h were treated in one of three ways: (a) the L-leucine uptake rate was measured after 4 h, (b) the cells were settle washed after 4 h and preincubated for an additional 4 h in a second solution prior to measurement of L-leucine uptake rate, or (c) after the initial 4 h preincubation period, $CaCl_2$ was added to make the solution 0.5 mM $CaCl_2$ and L-leucine uptake rate was measured 4 h later. L-Leucine uptake rate was measured in an incubation solution identical to the last preincubation solution except for the presence of 0.1 mM L-leucine.

First Preincubation Solution[a]	Treatment	Uptake Rate ± SE (*n*)
		nmol/ml HV·min
No additions	Rate measured after 4 h	12.90 ± 0.85 (6)
5 mM BTP	Rate measured after 4 h	3.14 ± 0.14 (6)
No additions	Rate measured after 9 h	14.33 ± 0.74 (6)
5 mM BTP	Rate measured after 9 h	2.24 ± 0.19 (5)

	Wash	Second Preincubation Solution[a]	
No additions	Yes	No additions	16.27 ± 0.68 (6)
No additions	Yes	5 mM BTP	2.94 ± 0.03 (6)
5 mM BTP	Yes	No additions	4.07 ± 0.27 (6)
5 mM BTP	Yes	0.5 mM $CaCl_2$	2.31 ± 0.21 (6)
No additions	No	Add $CaCl_2$	17.70 ± 0.44 (3)
5 mM BTP	No	Add $CaCl_2$	2.98 ± 0.12 (3)

[a] Solutions contained 1% sucrose plus indicated compounds.

Table VI. *Effect of Transport Inhibitors on L-Leucine Uptake Rate*

Cells were settle washed, preincubated for 6 h, and the L-leucine uptake rate measured. DNP or CCCP was administered 15 min before uptake was measured. Cycloheximide (10 mg/l) was present throughout preincubation and uptake periods. L-phenylalanine and L-leucine were only present during the uptake period. L-Leucine concentration in the incubation solution was 0.1 mM.

Preincubation and Incubation Solutions	% of Rate in 1% Sucrose or LS Medium	Uptake Rate ± SE (*n*)
		nmol/ml HV·min
A. 1% sucrose plus		
No additions	100	16.73 ± 0.78 (11)
0.1 mM DNP	28	4.65 ± 0.40 (6)
0.01 mM CCCP	56	9.33 ± 0.67 (6)
Cycloheximide	47	7.90 ± 0.48 (3)
50 mM L-leu[a]	39	6.54 ± 1.28 (3)
50 mM L-phe	36	6.06 ± 0.65 (3)
B. LS medium plus		
No additions	100	8.29 ± 0.41 (9)
0.1 mM DNP	25	2.06 ± 0.11 (3)
0.01 mM CCCP	44	3.68 ± 0.28 (3)
Cycloheximide	54	4.44 ± 0.18 (3)
50 mM L-Leu[a]	30	2.50 ± 0.10 (3)
50 mM L-phe	34	2.80 ± 0.17 (3)

[a] The concentration of L-leucine in these assays was considered to be 0.1 mM so that the inhibitory influence of the 50 mM L-leucine could be ascertained.

observed increase in uptake rate. If carriers are responsible, then high concentrations of competing amino acids should also inhibit the increased portion of the uptake rate. DNP, CCCP, and high concentrations of L-phenylalanine or L-leucine did inhibit most of the increase in uptake rate observed. Therefore, much of the increased uptake resulted from active transport. However, since all of the increase was not inhibited, it is possible that diffusional uptake also increased during preincubation.

The results reported here indicate that some tris buffers should not be used in amino acid transport studies because of their deleterious effects on L-leucine uptake. Preincubation and measurement of uptake in low ionic strength solutions also significantly influenced the L-leucine uptake rate. Since a complete medium like LS stabilized the uptake rate, a complete medium would be the preferred solution for amino acid transport studies.

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