Vol. 87, No. 1, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 15, 1979 Pages 135-139

FOLATE UPTAKE IN L1210 CELLS: MEDIATION BY AN ADENINE TRANSPORT SYSTEM

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Received January 26,1979

SUMMARY: Uptake of folate by L1210 cells is mediated by a transport system whose primary substrate is adenine. This conclusion is based upon the following evidence: (a) Folate uptake is inhibited competitively by adenine; (b) The K_t for folate transport (430 μM) is comparable to the K_i (450 μM) for folate inhibition of adenine transport; (c) The K_t for adenine transport (21 μM) agrees with the K_i (17 μM) for inhibition of folate transport by adenine; (d) The adenine analogs, 1-methyl-3-isobutylxanthine and 6-mercaptopurine, each inhibit folate and adenine transport to a comparable degree; and (e) Rates of folate and adenine uptake vary in parallel fashion during growth of L1210 cells.

L1210 mouse leukemia cells transport 5-methyltetrahydrofolate, other reduced folates, and Methotrexate (the 4-amino-10-methyl analog of folate) with a high degree of efficiency. Uptake is active and proceeds via a carrier system whose K_t values for various substrates are between 1 and 5 μM (reviewed in (1)). Folate, on the other hand, enters these cells via a separate, non-concentrative transport system which is quite different from that responsible for 5-methyltetrahydrofolate uptake. The relatively high K_t values (100 to 200 μM) reported for folate uptake in L1210 cells (1-3) have suggested, moreover, that folate may be transported by a system for which it is not the primary substrate.

In an effort to clarify this problem, compounds bearing a structural resemblance to portions of the folate molecule have been tested as inhibitors of the uptake of [^{3}H]folate by L1210 cells. While compounds that resemble the amino acid portion of the vitamin (p-aminobenzoylglutamate and glutamate) were ineffective, adenine (an analog of the pteridine portion of folate) was found to inhibit folate uptake. As illustrated in Fig. 1, both the initial

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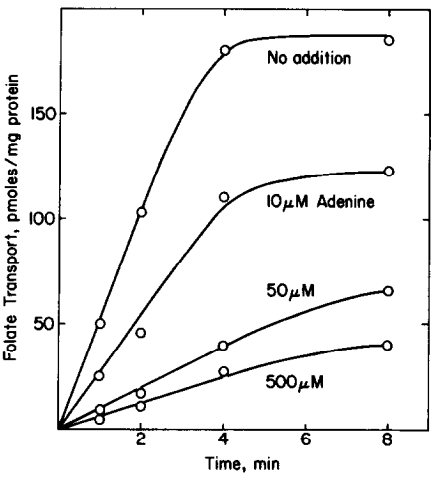

Fig. 1. Adenine inhibition of folate transport. L1210 cells, propagated in culture (4) to a density of 1.0-1.5 x 10^6/ml, were centrifuged at 600 x *g* (5 min, 4°), washed, and suspended in 20 mM Hepes-140 mM KCl, pH 7.4. Assay samples consisted of cells (1.5 x 10^7), 100 nmoles of [3',5'-9(*n*)-^{3}H]folate (30,000 dpm/nmole; Amersham) and the indicated concentrations of adenine in a final volume of 1.0 ml. After incubation at 37° for the indicated time intervals, the cells were diluted with 9 ml of ice-cold 140 mM KCl, and centrifuged at 3,000 x *g* (5 min at 4°). Supernatants were discarded and excess fluid inside the tubes was removed using a cotton swab; radioactivity associated with the cell pellets was determined as described previously (4). Transport (corrected for the amount of labeled folate associated with the cells after 2 min at 4°) was expressed in pmoles/mg protein, the latter being estimated by the biuret reaction (5) using bovine serum albumin as the standard.

rate and steady-state level of folate transport were reduced substantially by increasing levels of adenine. When the folate and adenine concentrations were varied (data not shown), inhibition by adenine was seen to be competitive (K_i = 17 μM). The ability of adenine to reduce the rate of folate transport was also reversible; cells treated for 5 min at 37° with a high level of adenine (1 mM), and then washed twice with 100 volumes of ice-cold buffer, showed no impairment of folate uptake. In separate experiments, adenine was found to be a poor inhibitor (K_i = 1.5 mM) of the 5-methyltetrahydrofolate transport system.

Uptake of [^{3}H]adenine by L1210 cells was linear for 2 min, reached a plateau within a 5-min incubation period and, when measured as a function of

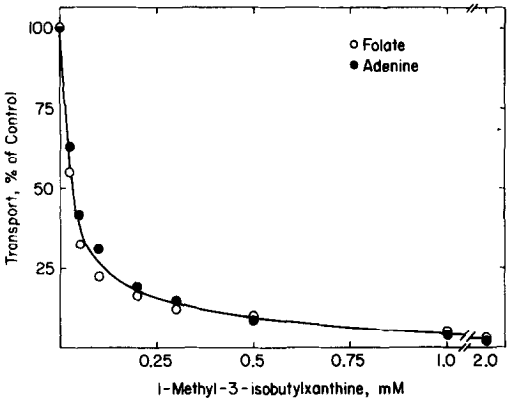

Fig. 2. Inhibition of folate and adenine transport by 1-methyl-3-isobutylxanthine. Experimental procedure as in Fig. 1, except that cells were incubated at 37° with 20 μM [8-^{3}H]adenine (10,500 dpm/nmole; Amersham) for 1 min or with 400 μM [^{3}H]folate for 2 min in the presence of the indicated concentrations of 1-methyl-3-isobutylxanthine (Aldrich). Transport values are expressed as % of control (transport in the absence of inhibitor).

adenine concentration, exhibited saturation kinetics (data not shown). A K_t value of 21 μM for adenine was obtained from the double-reciprocal plot of transport rate vs. adenine concentration. Adenine transport was inhibited by folate with a K_i value (450 μM) equivalent to the K_t for folate transport (430 μM). Pterin and biopterin were comparable to folate as inhibitors of adenine transport, whereas pteridine-6-carboxylate was ineffective.

The relationship between folate and adenine transport could also be demonstrated by the inhibitory effects of certain purine analogs. When adenine and folate uptake were examined in the presence of increasing concentrations of 1-methyl-3-isobutylxanthine, and the results normalized in terms of percent inhibition, both activities were found to decrease in a parallel manner (Fig. 2). The K_i values for 1-methyl-3-isobutylxanthine were 20 and 25 μM for folate and adenine transport, respectively. Similar results (data not shown) were also obtained with 6-mercaptopurine (P-L Biochemicals), except that the K_i values for inhibition of the transport of folate and adenine were 200 and 270 μM, respectively. The xanthine derivative also suppresses the uptake of Methotrexate in L1210 cells (4), but this occurs only at high

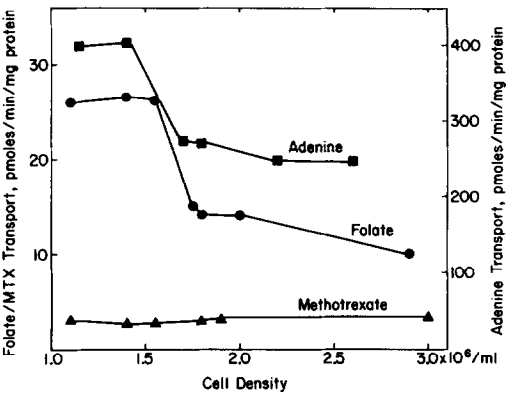

Fig. 3. Transport of folate, adenine and Methotrexate as a function of cell density. L1210 cells were grown to the indicated densities and washed with Hepes-KCl. Transport rates for folate (100 μM) and adenine (100 μM) were determined as described in the legends to Figs. 1 and 2; transport of 2 μM [3',5'-9(*n*)-^{3}H]Methotrexate (240,000 dpm/nmole) was determined as described previously (4). Each point represents the average of 4 determinations.

concentrations (>200 μM) and is due to its ability to increase the level of cyclic AMP. Prostaglandin E_2, which also enhances the cyclic AMP level (4), had no effect upon either adenine or folate transport.

When adenine transport was examined in cells grown to varying densities, the activity decreased significantly as cells progressed from the log to the stationary phase (Fig. 3). This fluctuation, although presently unexplained, was also observed for folate transport. In contrast, uptake of Methotrexate was relatively constant throughout expansion of the cell population.

The above results indicate that uptake of folate is mediated by a transport system whose primary substrate is adenine. The latter assignment is supported by the fact that guanine, uracil, cytosine and thymine showed little capacity to reduce the uptake of adenine. Adenosine and various other purine and pyrimidine ribonucleosides and deoxyribonucleosides were also poor inhibitors of adenine transport, indicating that the adenine/folate system is distinctly different from the nucleoside transport system of L1210 cells described previously by Kessel (6).

The mechanism of adenine transport with regard to metabolism of the internalized substrate was not examined in the present study. It is thus not

yet known whether the passage of adenine into the cell is either accompanied or facilitated by its reaction with 1-pyrophosphoryl-5-phosphorylribose to produce AMP. This reaction is the principal driving force for adenine accumulation in *Escherichia coli* (7).

The presence of a high-affinity transport system for 5-methyltetrahydrofolate in the L1210 lymphoid cell line (and the absence of a specific system for the uptake of folate) is consistent with the fact that 5-methyltetrahydrofolate is the major circulating form of the vitamin in mammals (8,9). Under *in vivo* conditions, therefore, it is likely that the reduced folate compound serves as the primary source of the vitamin. L1210 cells can be propagated *in vitro* using folate instead of 5-methyltetrahydrofolate, but such cells may be non-physiological with respect to the relative activities of: (a) the two transport systems; and (b) the enzymes dihydrofolate reductase and methionine synthetase that are responsible, respectively, for the conversion of folate and 5-methyltetrahydrofolate to the coenzyme, tetrahydrofolate.

ACKNOWLEDGEMENTS

The authors are indebted to Karin Vitols for advice in preparation of the manuscript and to Edward Zevely for technical assistance. This investigation was supported by grants from the National Cancer Institute, National Institutes of Health (CA6522 and CA 23970), American Cancer Society (CH-31), and National Science Foundation (PCM77-23414). G. B. Henderson is the recipient of a Senior Fellowship (D-294) from the California Division of the American Cancer Society.

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