Enhanced Stability of the Na⁺-Dependent Amino Acid-Transport System after Lymphocyte Activation

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The Na⁺-dependent transport system for the amino acid proline in unstimulated lymphocytes decays with a half-life of about 4 h when protein synthesis is inhibited by cycloheximide, but the system in mitogen-activated lymphocytes is more stable. However, the early increase in activity of this transport system after mitogen addition is not due to a direct effect on its stability, but is a secondary effect requiring protein synthesis.

An early consequence of the addition of mitogenic lectins to cultured lymphocytes is an increase in the rate of uptake of many different metabolites (Ling & Kay, 1975). Amongst these changes in membrane transport is a large and progressive increase in the Na⁺-dependent uptake of the amino acid analogue 2-aminoisobutyric acid (Mendelsohn *et al.*, 1971; Van den Berg & Betel, 1973) and of some natural amino acids, including proline (Borghetti *et al.*, 1979a). The $V_{\max}$ of the transport system is increased, but the K_m is unchanged, an observation compatible with an increase in the number of transport sites per cell. There are contradictory reports in the literature about the dependence of the early increase in 2-aminoisobutyric acid transport on protein synthesis (Mendelsohn *et al.*, 1971; Van den Berg & Betel, 1973), but as the half-life of the amino acid-transport systems can be quite short in other cells (Tews *et al.*, 1975; Franchi-Gazzola *et al.*, 1973; Phang *et al.*, 1975), one possible explanation for the increase in amino acid-transport sites would be an increase in the stability of the transport systems after activation.

We have therefore determined the half-life of these systems in lymphocytes, and found that this is indeed altered after mitogen addition. However, the earliest increase in amino acid transport is too large and too rapid to be accounted for by any change in the stability of the systems responsible, and is, in our hands, completely dependent on protein synthesis.

Experimental

Lymphocytes were purified from defibrinated pig blood and incubated at 2×10^6 /ml in Eagle's minimal essential medium containing 15% autologous serum as previously described (Kay *et al.*, 1975). The mitogen phytohaemagglutinin-P (Difco Laboratories, Detroit, MI, U.S.A.) was added where indicated at a final concentration of 10 μ g/ml.

The initial rate of Na⁺-dependent uptake of [³H]-proline was determined as described by Borghetti *et al.* (1979a). All determinations were corrected for the amount of extracellular radioactivity trapped in the cell pellet, determined in each experiment by addition of [³H]proline to cells maintained at 0°C and then processed as normal. The extracellular radioactivity trapped after the washing procedure used was very low (5–50 c.p.m. above background). Four or five replicate determinations were made for each value, and results, expressed as pmol of proline taken up/min per 10^7 cells, are shown with the S.E.M.

Results and Discussion

Addition of 7 μ M-cycloheximide, a concentration that inhibits lymphocyte protein synthesis by over 90% (Kay & Korner, 1966), resulted in a fall in the rate of [³H]proline uptake by unstimulated lymphocytes which began immediately (Fig. 1). The decay was approximately exponential, and the half-life of the proline-transport system under these conditions varied from 3 to 5 h in different experiments.

After incubation with phytohaemagglutinin for 20 h the rate of uptake of [³H]proline increased to more than ten times the initial rate. At this time the rate had reached a maximum value, and it was not further enhanced by additional incubation for a further 8 h (Fig. 1). When cycloheximide was added 20 h after the addition of phytohaemagglutinin the decay of [³H]proline uptake occurred more slowly than in unstimulated lymphocytes (Fig. 1), so that even after 8 h in the presence of the drug the rate of uptake was still half the initial rate. The kinetics of this decay were complex. No significant fall in the rate of [³H]proline uptake was seen in the first 2 h after addition of cycloheximide, but thereafter the decay in activity was approximately exponential, with a half-life comparable with that seen in un-

Fig. 1. Decay of $[^3\text{H}]$ proline uptake after addition of cycloheximide or actinomycin

Cycloheximide or actinomycin was added at 0h, and the initial rate of $[^3\text{H}]$ proline uptake was determined at the times shown thereafter. The rate of uptake of $[^3\text{H}]$ proline at the beginning of the experiment was 3.63 ± 0.22 pmol/min per 10^7 cells for unstimulated lymphocytes (closed symbols) and 50.29 ± 1.46 pmol/min per 10^7 cells for lymphocytes preincubated with phytohaemagglutinin for 20h (open symbols). The inhibitors added were none (□), $7 \mu\text{M}$ -cycloheximide (●, ○) and $0.8 \mu\text{M}$ -actinomycin (△).

stimulated lymphocytes. Addition of $0.8 \mu\text{M}$ -actinomycin had very similar effects to addition of cycloheximide, except that the decay of $[^3\text{H}]$ proline uptake was slightly delayed (Fig. 1). This result was not unexpected, as inhibition of lymphocyte RNA synthesis results in unusually rapid inhibition of protein synthesis (Kay, 1967).

The more rapid decay of $[^3\text{H}]$ proline transport in unstimulated lymphocytes after addition of cycloheximide was not due to any greater sensitivity of unstimulated lymphocytes to the antibiotic, as protein synthesis in unstimulated and phytohaemagglutinin-activated lymphocytes is equally susceptible to inhibition (Kay & Korner, 1966). The simplest explanation for these kinetic changes is that a labile protein component essential for the proline-transport system is rate-limiting in unstimulated

Table 1. Na^+ -dependent $[^3\text{H}]$ proline uptake by lymphocytes incubated with or without phytohaemagglutinin and cycloheximide for 4h

Phytohaemagglutinin	Cycloheximide	$[^3\text{H}]$ Proline uptake (pmol/min per 10^7 cells)
—	—	4.55 ± 0.14
—	+	2.43 ± 0.36
+	—	16.38 ± 0.06
+	+	2.98 ± 0.27

cells but present in excess after incubation with mitogen for 20h.

Decreased lability of the proline-transport system could clearly account for a part of the increase in transport seen after phytohaemagglutinin addition. However, the 3.6-fold increase in the rate of $[^3\text{H}]$ -proline uptake seen 4h after addition of phytohaemagglutinin (Table 1) was too large to be explained by even complete stabilization of the transport system at this early time. If cycloheximide was added immediately before phytohaemagglutinin, then this early increase was abolished (Table 1), showing that it was completely dependent on protein synthesis. This observation is in agreement with the results of Van den Berg & Betel (1973), who studied the effect of concanavalin A on 2-aminoisobutyric acid transport in rat lymph-node lymphocytes, with the effects of addition of serum on Na^+ -dependent amino acid transport in avian fibroblasts (Tramacere *et al.*, 1977; Borghetti *et al.*, 1979b), and with the effects of insulin on amino acid transport in bone (Phang *et al.*, 1975), heart cells (Elsas *et al.*, 1975) and liver (Kletzien *et al.*, 1976), but contrasts with the report by Mendelsohn *et al.* (1971) that the increase in 2-aminoisobutyric acid transport induced by phytohaemagglutinin in human blood lymphocytes is unaffected by inhibition of protein synthesis.

The requirement for protein synthesis to maintain the rate of $[^3\text{H}]$ proline uptake in lymphocytes and for the early increase seen after mitogen addition could indicate that a labile protein is essential for proline uptake, and that the increased uptake after mitogen addition is due to increased synthesis of this protein. An alternative explanation, that the rate of proline uptake is coupled loosely to the rate of protein synthesis, perhaps through consequent changes in the intracellular amino acid pools, cannot at present be eliminated. However, in either case it is clear that the increase in Na^+ -dependent amino acid transport differs from some of the other changes in membrane transport seen early after mitogen addition (Ling & Kay, 1975) in that it is not due to a direct effect of the mitogen acting at

the cell membrane, but is mediated by some intracellular mechanism involving the control of protein synthesis.

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