SELECTIVE EARLY ACTIVATION OF A SODIUM DEPENDENT AMINO ACID TRANSPORT SYSTEM IN STIMULATED RAT LYMPHOCYTES

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1. Introduction

Lymphocytes are stimulated to grow into blast-like cells and finally divide when incubated with the plant lectins Concanavalin A (Con A) from the jack bean (*Canavalia enseformis*) or phytohaemagglutinin (PHA) from the red kidney bean (*Phaseolus vulgaris*) [1].

Changes in the properties of the lymphocyte membrane such as the enhancement of the transport of ions [2–5], sugars [4, 6], nucleosides [7], and amino acids [4, 8, 9] are among the earliest observed biochemical events.

We have observed that, in lymphocytes cultured for 1 hr in the presence of lectins, the uptake of 2-aminoisobutyric acid (AIB) increased while that of another amino acid analogue– aminocylopentanecarboxylic acid (ACPC) – was hardly affected [10, 11]. Experiments described in this paper demonstrate that ACPC uptake is mediated by a transport system which is distinct from the AIB transport system. Plant mitogens induce only an early increased activity of a sodium dependent amino acid transport system.

2. Methods and materials

The preparation of a rat lymphocyte cell suspension, the culture conditions, and the stimulation of the cells have been previously described [10]. The transport of AIB and ACPC was determined by a filter method as previously described [11]. Media with different sodium concentrations were prepared by replacing part of the sodium in the Krebs buffer by Tris-HCl; maintaining a pH of 7.2, and an osmolarity of 285 mOsm/ℓ. Concanavalin A was obtained from CalBiochem, Los Angeles. 1-Aminocyclopentane 1-[carboxylic acid-^{14}C] and [1-^{14}C] 2-aminoisobutyric acid were purchased from the Radiochemical Centre, Amersham.

3. Results and discussion

During investigations on the mechanism of the early increased AIB uptake in lymphocytes stimulated with plant lectins [10], we observed that the uptake of another nonmetabolizable amino acid, aminocyclopentanecarboxylic acid (ACPC) was not enhanced. A typical experiment is shown in table 1. There is no increase in the uptake of ACPC after a short incubation (1 hr) of the cells with Con. A.

A different plant lectin, phytohaemagglutinin (PHA), which induces an increased transport of AIB in lymphocytes [7–9] also does not alter the uptake of ACPC [10]. After 24 hr incubation of the lymphocytes with Con A, however, a pronounced increase in the uptake

Table 1
AIB and ACPC uptake in stimulated lymphocytes.

Incubation time (hr)	Amino acid	Uptake of amino acid* Control	+ Con A	Stimulation ratio**
1	AIB	949 ± 66	2023 ± 54	2.1
	ACPC	955 ± 113	866 ± 41	0.9
24	AIB	1144 ± 72	7314 ± 82	6.4
	ACPC	970 ± 83	3198 ± 65	3.3

* Mean dpm/60 min/4 × 10^6 cells ± SD.

** Stimulation ratio = $\frac{\text{mean dpm of the Con A treated samples}}{\text{mean dpm of the control samples}}$

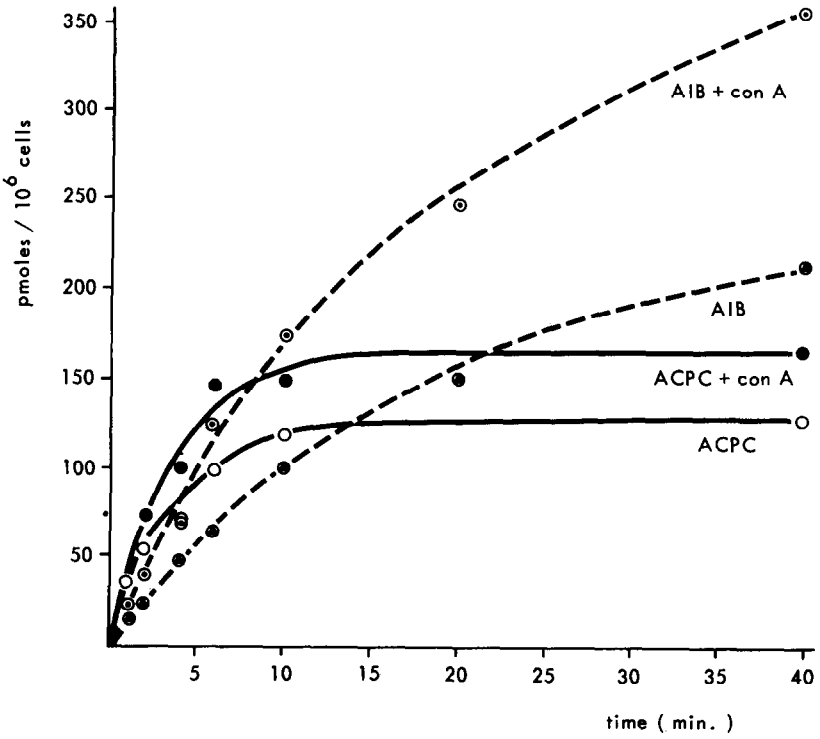

Fig. 1. Time course of ACPC and AIB uptake. Rat lymphocytes were preincubated without and with Con A for 1 hr. After washing and resuspension they were incubated with 0.1 mM ACPC or with 0.1 mM AIB. Uptake of amino acid was determined at the times indicated.

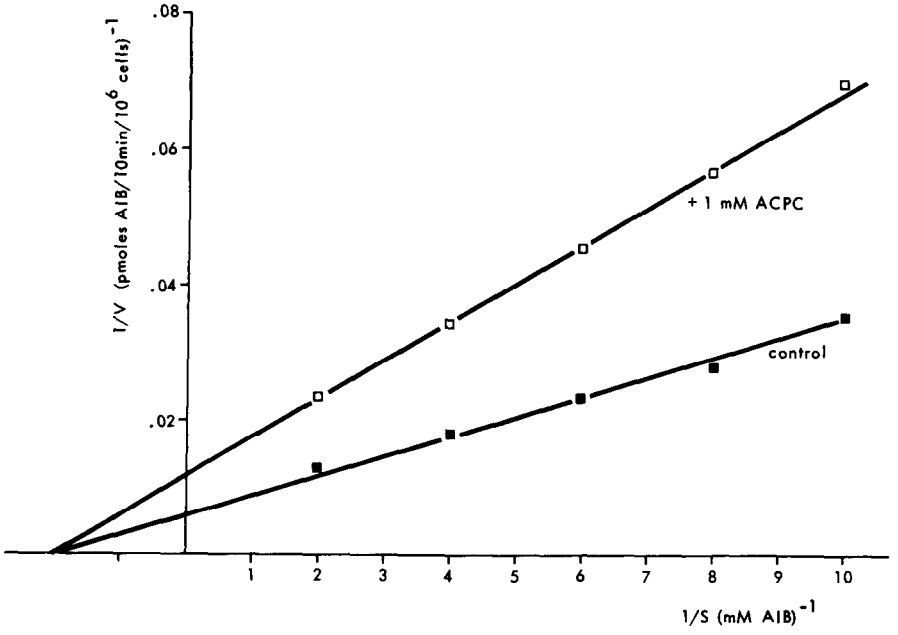

Fig. 2. Effect of ACPC on the rate of AIB uptake. Rat lymphocytes which had been preincubated for 1 hr with Con A were incubated with 1 mM ACPC and different concentrations of AIB. Initial rates of AIB were determined and the results are represented as a double reciprocal plot.

Table 2
ACPC and AIB transport as dependent on extracellular Na^+.

$[Na^+]$ (mM)	Rate of ACPC uptake*				Rate of AIB uptake**			
	Control	(%)	+ Con A	(%)	Control	(%)	+ Con A	(%)
130	4981	100	4710	100	1711	100	4269	100
90	4388	88	4501	96	1361	80	2924	68
40	4621	93	4126	88	1151	68	2093	49

* Mean dpm/2 min/6 × 10^6 cells.
** Mean dpm/10 min/ 6 × 10^6 cells.

of ACPC and AIB can be observed. The question of whether the early increased AIB uptake was due to activation of a distinct amino acid transport system was studied by measuring the kinetics of uptake, the sodium dependence of the uptake, and the inhibition of the rate of AIB uptake by ACPC. The time course of AIB and ACPC transport in resting and stimulated lymphocytes (fig. 1) shows that the kinetics of uptake are different. In unstimulated cells, the initial rate of AIB uptake is rather slow and the steady state is reached in about 60 min [11]; for ACPC, the initial rate of uptake is fast and a steady state is obtained after only 10 min. In cells stimulated by Con A for 1 hr, the initial rate of AIB uptake is increased, but the initial rate of ACPC uptake is not significantly altered. The presence of extracellular Na^+ is required for the transport of many amino acids [12]. The sodium dependence of the rate of AIB transport has been demonstrated in lymphocytes of the rabbit [13] and the rat ([11] and table 2). The initial rate of ACPC uptake in resting rat lymphocytes is almost independent of the concentration of extracellular Na^+ (table 2). Stimulation of the lymphocytes by Con A does not change the sensitivity of the ACPC uptake towards Na^+. In human lymphocytes and granulocytes [14], and in human and rat reticulocytes [15], ACPC transport was also found to be independent of the extracellular sodium concentration.

Further evidence for the existence of independent transport systems for AIB and ACPC stems from experiments in which AIB transport rates were measured in the presence of 1 mM ACPC in lymphocytes stimulated for 1 hr by Con A. The double reciprocal plot (fig. 2) shows that ACPC is a non-competitive inhibitor of the AIB uptake, indicating that AIB and ACPC do not share a common amino acid transport system.

Our results strongly suggest that in rat lymphocytes AIB and ACPC transport are mediated by distinct amino acid transport systems. Shortly after addition of Con A or PHA, only the sodium dependent AIB transport system is activated. Increased activity of the sodium independent ACPC system is only observed after prolonged stimulation at a time that cell enlargement sets in. Experiments with natural amino acids show [16] that these can also be classified into a Na^+ dependent type of uptake, which is increased early after stimulation and a Na^+ independent type of uptake which is not enhanced early after stimulation.

The induction of a sodium dependent amino acid transport system has also been observed in sea urchin eggs early after fertilization [17]. The reverse, a specific decrease in the activity of a Na^+ dependent amino acid transport system, has been found during the maturation of reticulocytes into erythrocytes [15, 18]. The biological significance of this phenomenon is obscure at present. It may be related to the level of metabolic activity in mammalian cells and/or to the state of differentiation.

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