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ADAPTIVE REGULATION OF AMINO ACID TRANSPORT ACROSS THE CELL MEMBRANE IN AVIAN AND MAMMALIAN TISSUES

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SUMMARY

The regulation of amino acid transport across the cell membrane by adaptive mechanisms has been studied in a variety of mesenchymal and epithelial cells and tissues of avian and mammalian origin.

Changes in transport activity as a function of time under various in vitro conditions (amino acid dependence, active and inhibited protein synthesis) have been evaluated by measurements of initial entry rates with representative amino acids.

Results and conclusions based on the adopted experimental approach include the following.

(1) An adaptive control mechanism for the transport of neutral amino acids corresponding to the typical substrates of the *A* mediation is operative in (a) mesenchymal cells (fibroblasts, chondroblasts, osteoblasts and myoblasts) from embryonic tissues of avian (chick embryo) origin and (b) mesenchymal cells from immature rat uterus (fibroblasts and smooth muscle cells) and other mammalian tissues (cardiac cells from newborn mouse and rat heart).

(2) Adaptive regulation is restricted to a discrete subgroup of amino acids (L-proline, glycine and the analogue α-aminoisobutyric acid) in rat peritoneal macrophages and thymic lymphocytes.

(3) Adaptive regulation is absent in erythroid cells (human erythrocytes, rabbit erythrocytes and reticulocytes, avian erythrocytes) which lack the *A* mediation and are incapable of active gene transcription.

(4) Adaptive regulation is absent in the epithelial kidney cortex tissue and possibly absent in the epithelial component of liver tissue from adult rats; it is fully operative in the chick embryo crystalline lens, i.e. an epithelial preparation of embryonic origin.

(5) These observations indicate that adaptive control mechanisms of amino acid transport across the cell membrane are quite common among tissues and species and emphasize their broad biological significance in eukaryotes.

INTRODUCTION

The transport of amino acids across the cell membrane in animal tissues is accomplished by a number of systems of mediation whose specific properties include different reactivity to substrates, ion and energy dependence, sensitivity to pH and metabolic inhibitors, capacity for exchange diffusion and transinhibition, etc. [1–5].

Recent studies indicate that the molecular process involved is more complex than a mere sequence of binding-translocation-dissociation between substrate and effector component and includes regulatory mechanisms which continuously adapt the efficiency of transport (and, therefore, the flow of amino acids through the plasma membrane) to the actual needs of the intracellular machinery [6–8]. Among them, a time-dependent adaptive control mechanism for transport system *A* has been found operative in chick embryo heart cells and other muscle tissues [9]. It involves repression-derepression of transport activity by amino acid substrates of this mediation acting at gene transcription coupled to control of transport-protein breakdown (or inactivation) at the cell membrane [10].

Experiments in our laboratory with lymphoid cells [11] and reports by other Authors for immature rat uterus [12], human placenta [13] and newborn rat kidney cortex [14] indicate that this regulation is not restricted to muscle tissue and suggest that it has a large incidence among tissues and species.

The purpose of the present work was to verify the occurrence of adaptive transport systems for amino acids (and to define their reactivity to substrates) in cells and tissues from avian and mammalian sources. The selected biological preparations included isolated cells from chick embryo tissues (fibroblasts from cornea, chondroblasts from cartilaginous pelvic bone, osteoblasts from calvaria, myoblasts from heart, erythrocytes from peripheral blood), immature rat uterus mesenchymal cells, rat peritoneal macrophages, rat thymocytes, human erythrocytes, rabbit erythrocytes and reticulocytes, chick embryo crystalline lenses, rat liver and kidney cortex slices.

The results to be presented indicate that an adaptive control mechanism for the transport of neutral amino acids corresponding to those ascribed to the A mediation [6, 9] is operative in mesenchymal tissue cells (fibroblasts, chondroblasts, osteoblasts, myoblasts). Adaptive regulation is restricted to a discrete subgroup of amino acids (including proline, glycine and α-aminoisobutyric acid) in macrophages and lymphoid cells; it is absent in cells lacking transcription (mammalian and avian erythrocytes, reticulocytes). In experiments with epithelial tissues, adaptive regulation has been unambiguously detected only in an embryonic preparation (chick embryo lens).

MATERIALS AND METHODS

Chemicals

α-Amino-[1-^{14}C]isobutyric acid, L-[U-^{14}C]alanine, L-[U-^{14}C]leucine, L-[U-^{14}C]lysine, L-[U-^{14}C]phenylalanine, L-[U-^{14}C]proline and L-[U-^{14}C]serine were obtained from Radiochemical Centre, Amersham, Bucks, England; [1-^{14}C]glycine came from New England Nuclear Chemicals, Dreieichenhain, West Germany. Each compound was diluted, as appropriate, with unlabelled material. The sources for most materials and reagents used are as listed in the preceding papers on this subject [6, 9, 10].

Preparation of cells and tissues

All the biological models to be described were examined histologically with appropriate staining procedures of smears or sections. Cell suspensions were routinely examined by phase-contrast microscopy.

Corneas have been excised from eye globes of 9-day-old chick embryos by cutting the tissue along the corneal-scleral limbus. At this stage of development the cornea consists of a thick stratum of loosely packed fibroblasts (substantia propria) placed between a two-cell layer outer epithelium and the endothelial monolayer [15]. As estimated by histological examination, fibroblasts represent more than 90 % of the cell population. Cartilaginous pelvic bones were removed from 11-day-old chick embryos, when these rudiments (two per embryo) were thin non-mineralized plates of cartilage [15, 16]. Both surfaces of each plate were carefully cleaned to remove adherent perichondrium before further use. Membrane bone calvaria were dissected from 12-day-old chick embryos. At this stage the tissue appears as a network of well mineralized trabeculae rich in bone-forming osteoblasts without significant hematopoietic tissue [17]. Both surfaces of hemicalvaria were carefully cleaned to remove adherent periosteum and dura before further use. Hearts were obtained from 7-day-old chick embryos, newborn mice and rats as described previously in detail [9, 18, 19]. Uteri were removed from immature female rats (Wistar strain) weighing 50–70 g [20]; uterine horns were cleaned of attached connective tissue and cut in small segments. At this stage the tissue consists of a lamina propria (rich in fibroblasts and poor in uterine glands) covered by a one-cell-layered columnar epithelium (endometrium), and of tunic smooth muscle cells interspersed by numerous fibroblasts (myometrium).

The procedures of cell isolation by collagenase treatment from corneal tissue, pelvic cartilage, calvaria bones, mammalian hearts and uteri (cf. ref. 21) were essentially similar to those previously described for the isolation of cells from chick embryo hearts [19, 22]. Macrophages were obtained by collecting peritoneal exudates from adult rats previously injected with sterile paraffin oil [23]. After proper washing procedures the cell suspension contained 85–90 % macrophages (as estimated by adhesion to glass) (cf. ref. 24); the contaminating cells were polymorphonuclear leukocytes, lymphocytes and mast cells. The isolation of thymocytes from the thymus of 13- to 19-day-old Wistar rats was as described by Goldfine et al. [25]. Avian erythrocytes were obtained from peripheral blood of 18-day-old chick embroys; human erythrocytes were from normal blood donors; reticulocytes were collected by heart puncture from rabbits made anemic with phenylhydrazine as previously indicated [26]; erythrocytes were similarly obtained from normal rabbits. Crystalline lenses were obtained from 8-day-old chick embryos; careful dissection under stereoscopic microscope minimized contamination by non-epithelial tissues. Liver and kidney cortex slices were obtained by hand razor from Sprague-Dawley albino rats weighing approx. 150 g (cf. refs 27 and 28).

Incubation

Incubations were carried out in silicone-treated glass vessels at 37.5 °C under continuous mild stirring [19]. The basic incubation medium was Krebs-Ringer bicarbonate containing 8 mM glucose, in an atmosphere of O_2/CO_2 (95 : 5, v/v). In additional experiments with erythroid cells (avian and mammalian erythrocytes,

reticulocytes) the incubation medium was as described by Wheeler and Christensen [29] with Tris · HCl (pH 7.5) as buffer. Under the conditions selected for incubation the biological preparations mentioned in the preceding paragraph were viable for several hours (and at least 3 h for tissue slices), as assessed by measurements of oxygen consumption and incorporation of labelled amino acids into protein (cf. ref. 19). Erythroid cell viability was indicated by lack of haemolysis. Additions to the medium (amino acids, inhibitors) and washing procedures of the cells before changes in medium composition are specified in Results.

Initial rates of amino acid uptake were measured by transferring samples of cell suspension (or tissues) into flasks containing the desired medium with the appropriate additions (labelled amino acids, inhibitors) and incubating the flask at 37.5 °C for 5 min (cell suspensions), 10 min (lenses), 20 min (kidney cortex slices) or 30 min (liver slices). The means for determining intracellular accumulation of the tracer amino acid and for evaluating the proper corrections to be introduced were as described by Guidotti et al. [18, 19, 22]. Modifications were adopted (a) in experiments with lymphocytes where, at the end of incubation, these small-sized cells were collected on glass fibre filters Whatman GF/A (which replaced AP20 Millipore filters) to avoid cell loss and (b) in experiments with erythroid cells where, at the end of incubation, centrifugation through discontinuous Ficoll gradients replaced filtration to collect these fragile cells, avoiding haemolysis. Chromatographic analysis of tissue extracts and media on silica-gel-coated polyethylene terephthalate sheets [19] was used to identify the tracer amino acid when the incubation exceeded 5 min in experiments with naturally occurring amino acids.

TABLE I

AMINO ACID UPTAKE BY ISOLATED FIBROBLASTS FROM CHICK EMBRYO CORNEAL TISSUE: CHANGES IN TRANSPORT ACTIVITY WITH TIME

Cell suspensions obtained from 9-day-old chick embryo corneas (see text) were incubated for 2 h in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 8 mM glucose. Incubation was at 37.5 °C in an atmosphere of O_2/CO_2 (95 : 5, v/v). Amino acid uptake (initial velocity) was measured by transferring samples of cell suspensions ($5 \cdot 10^6$ cells) into flasks containing Krebs-Ringer bicarbonate supplemented with 8mM glucose and the ^{14}C-labelled amino acid under study (0.1 mM, final concentration) and incubating for 5 min at 37.5 °C. The values are means of 3 separate determinations.

Amino acid	Uptake (μmol/ml cell water in 5 min)		
	Incubation time (h)		Difference (%)
	0	2	
α-Aminoisobutyric acid	0.29	1.35	+366
L-Proline	0.45	1.69	+276
Glycine	0.31	0.71	+129
L-Alanine	1.00	2.19	+119
L-Serine	1.00	2.68	+168
L-Leucine	0.74	0.45	− 39
L-Phenylalanine	0.42	0.28	− 33
L-Lysine	0.64	0.30	− 53

RESULTS

Mesenchymal cells

Changes in amino acid transport activity with time for a variety of mesenchymal cells from avian embryonic tissues upon incubation in Krebs-Ringer bicarbonate buffer are shown in Tables I–IV. In these biological preparations including fibroblasts (from corneas), chondroblasts (from cartilaginous pelvic bones), osteoblasts (from calvaria) and myoblasts (from hearts), increased transport activities have

TABLE II

AMINO ACID UPTAKE BY ISOLATED CHONDROBLASTS FROM CARTILAGINOUS PELVIC BONES OF CHICK EMBRYO: CHANGES IN TRANSPORT ACTIVITY WITH TIME

Cell suspensions were obtained from cartilaginous pelvic bones of 11-day-old chick embryos (see text). Incubations and amino acid uptake measurements (on samples of $3 \cdot 10^6$ cells) were as described in Table I. The values are means of 3 separate determinations.

Amino acid	Uptake (μmol/ml cell water in 5 min)		
	Incubation time (h)		Difference (%)
	0	2	
α-Aminoisobutyric acid	0.61	1.29	+111
L-Proline	0.95	2.14	+125
Glycine	0.46	0.69	+ 50
L-Alanine	1.84	2.99	+ 63
L-Serine	1.86	2.41	+ 30
L-Leucine	0.43	0.26	− 40
L-Phenylalanine	0.21	0.13	− 38
L-Lysine	0.41	0.29	− 29

TABLE III

AMINO ACID UPTAKE BY ISOLATED OSTEOBLASTS FROM MEMBRANE BONE CALVARIA OF CHICK EMBRYO: CHANGES IN TRANSPORT ACTIVITY WITH TIME

Cell suspensions were obtained from calvaria bones of 12-day-old chick embryos (see text). Incubations and amino acid uptake measurements (on samples of $1.5 \cdot 10^6$ cells) were as described in Table I. The values are means of 3 separate determinations.

Amino acid	Uptake (μmol/ml cell water in 5 min)		
	Incubation time (h)		Difference (%)
	0	2	
α-Aminoisobutyric acid	0.49	1.10	+124
L-Proline	0.80	2.26	+183
Glycine	0.60	0.93	+ 55
L-Alanine	1.75	2.99	+ 71
L-Serine	1.84	2.48	+ 35
L-Leucine	0.56	0.36	− 36
L-Phenylalanine	0.22	0.15	− 32
L-Lysine	0.40	0.27	− 32

TABLE IV

AMINO ACID UPTAKE BY ISOLATED MYOBLASTS FROM CHICK EMBRYO HEART TISSUE: CHANGES IN TRANSPORT ACTIVITY WITH TIME

Cell suspensions were obtained from 7-day-old chick embryo hearts (see text). Incubations and amino acid uptake measurements (on samples of 10^7 cells) were as described in Table I. The values are means of 7–11 separate determinations.

Amino acid	Uptake (μmol/ml cell water in 5 min)		
	Incubation time (h)		Difference (%)
	0	2	
α-Aminoisobutyric acid	0.31	0.74	+139
L-Proline	0.44	0.87	+98
Glycine	0.27	0.40	+48
L-Alanine	1.03	1.66	+61
L-Serine	0.68	1.11	+63
L-Leucine	0.58	0.29	−50
L-Phenylalanine	0.25	0.20	−20
L-Lysine	0.71	0.30	−58

been detected with the model amino acid α-aminoisobutyric acid and with the naturally occurring amino acids L-proline, glycine, L-alanine and L-serine. All these amino acids belong to the group taken up, primarily or appreciably, by the *A* transport system [1, 5]. Only minor changes or definite decrements took place with L-leucine, L-phenylalanine and L-lysine, which are preferential substrates of transport systems *L* and Ly^+ [1, 5].

The addition of cycloheximide to the incubation medium at a concentration sufficient to abolish protein synthesis almost completely (cf. ref. 10) prevented the

TABLE V

UPTAKE OF α-AMINOISOBUTYRIC ACID BY ISOLATED MESENCHYMAL CELLS FROM CHICK EMBRYO: CHANGES IN TRANSPORT ACTIVITY UPON INCUBATION IN THE PRESENCE OF CYCLOHEXIMIDE

Cell suspensions (fibroblasts, chondroblasts, osteoblasts and myoblasts) obtained from chick embryo tissues (cf. Tables I–IV) were incubated for 2 h in the absence and in the presence of cycloheximide (2 μg/ml). Conditions of incubation and assay of aminoisobutyrate uptake were as described in Tables I–IV. The values are means of 3 separate determinations.

Cells	α-Aminoisobutyric acid uptake (μmol/ml cell water in 5 min)		
	Incubation time (h)		
	0	2 (Control)	2 (Cycloheximide)
Fibroblasts	0.29	1.35	0.21
Chondroblasts	0.61	1.29	0.33
Osteoblasts	0.49	1.10	0.25
Myoblasts	0.30	0.68	0.16

increase with time of the activity of transport system *A* (as measured by α-aminoisobutyric acid uptake) in all the above-mentioned mesenchymal cell preparations (Table V).

TABLE VI

UPTAKE OF L-PROLINE AND L-ALANINE BY ISOLATED MESENCHYMAL CELLS FROM CHICK EMBRYO: CHANGES IN TRANSPORT ACTIVITY UPON INCUBATION IN THE PRESENCE OF AMINO ACIDS

Cell suspensions (fibroblasts, chondroblasts, osteoblasts and myoblasts) obtained from chick embryo tissues (cf. Tables I–IV)were incubated for 2 h in Krebs-Ringer bicarbonate buffer containing 8 mM glucose, in the presence and absence of individual amino acids at 5 mM concentration. Incubation was at 37.5 °C in an atmosphere of O_2/CO_2 (95 : 5, v/v). L-Proline or L-alanine uptake (initial velocity) was measured at the beginning and at the end of incubation, on samples of cell suspensions which were washed with Krebs-Ringer buffer (to remove the amino acid when present in the incubation medium), transferred into flasks containing the same medium supplemented with 0.1 mM labelled amino acid and incubated for 5 min at 37.5 °C. The values are means of 3 separate determinations.

Amino acid added (5 mM)	L-Proline uptake (μmol/ml cell water in 5 min)		L-Alanine uptake (μmol/ml cell water in 5 min)	
	Incubation time (h)		Incubation time (h)	
	0	2	0	2
Fibroblasts				
None	0.42	1.65	0.92	2.03
α-Aminoisobutyric acid	0.35	0.19	0.77	0.53
L-Proline	–	–	0.88	0.51
L-Alanine	0.38	0.25	–	–
L-Phenylalanine	0.40	1.26	1.05	2.02
L-Lysine	0.44	1.40	1.09	2.14
Chondroblasts				
None	1.17	2.50	1.93	3.05
α-Aminoisobutyric acid	0.98	0.50	1.76	0.91
L-Proline	–	–	1.62	1.06
L-Alanine	1.12	0.69	–	–
L-Phenylalanine	1.09	2.04	1.90	2.96
L-Lysine	1.18	2.22	2.01	3.04
Osteoblasts				
None	0.80	2.26	1.75	2.99
α-Aminoisobutyric acid	0.79	0.46	1.55	0.80
L-Proline	–	–	1.69	1.35
L-Alanine	0.80	0.47	–	–
L-Phenylalanine	0.81	2.11	1.69	2.85
L-Lysine	0.85	2.21	1.93	3.11
Myoblasts				
None	0.36	0.78		
α-Aminoisobutyric acid	0.32	0.18		
L-Proline	–	–		
L-Alanine	0.32	0.22		
L-Phenylalanine	0.37	0.86		
L-Lysine	0.39	1.00		

The addition of individual amino acids to the medium during the incubation period (2 h) had a profound and selective effect on the rate of change in activity of transport system *A* (measured by the uptake of L-proline and L-alanine as representative amino acids) in the same preparations (Table VI). Using proline as tracer, the increase in activity of the system was completely blocked when α-aminoisobutyric acid or alanine (substrates of system *A*) were present during the incubation (and removed before uptake measurement) and was affected little if at all by the addition of phenylalanine or lysine (substrates of systems *L* and Ly^+, respectively). Similarly, with alanine as tracer, the increase in activity of system *A* was abolished upon addition of α-aminoisobutyric acid or proline (substrates of the system) to the medium during the incubation and was not substantially altered by the addition of phenylalanine or lysine.

All these results indicate that the *A* mediation, as identified by Christensen and co-workers in Ehrlich ascites tumour cells [1, 30] and by us in chick embryo heart cells [6, 9], behave as an adaptive transport system (for neutral amino acids) subject to regulation by its substrate molecules (cf. ref. 10) in such mesenchymal cells as fibroblasts, chondroblasts, osteoblasts and myoblasts from embryonic tissues of avian origin.

Comparable results were obtained with mesenchymal cells from immature rat uterus (Table VII), i.e. with a cell population represented mainly by fibroblasts and smooth muscle cells of mammalian origin. Increased transport activities with time have also been observed when isolated cells from newborn mouse and rat hearts were incubated in Krebs-Ringer bicarbonate buffer using α-aminoisobutyric acid (cf. ref. 9), L-proline, glycine and L-alanine as tracer amino acids; minor decrements took place with L-leucine, L-phenylalanine and L-lysine (results not shown).

Changes in amino acid transport activity with time for rat peritoneal macrophages and thymic lymphocytes upon incubation in Krebs-Ringer bicarbonate buffer

TABLE VII

AMINO ACID UPTAKE BY CELLS ISOLATED FROM IMMATURE RAT UTERUS: CHANGES IN TRANSPORT ACTIVITY WITH TIME

Cell suspensions were obtained from uteri of immature female rats (see text). Incubations and amino acid uptake measurements (on samples of $2.5 \cdot 10^6$ cells) were as described in Table I. The values are means of 3 separate determinations.

Amino acid	Uptake (μmol/ml cell water in 5 min)		
	Incubation time (h)		Difference (%)
	0	2	
α-Aminoisobutyric acid	0.30	0.87	+190
L-Proline	0.18	0.86	+378
Glycine	0.24	0.40	+ 67
L-Alanine	1.04	1.74	+ 67
L-Serine	1.00	1.75	+ 75
L-Leucine	0.17	0.15	− 12
L-Phenylalanine	0.20	0.19	− 5
L-Lysine	0.38	0.24	− 37

TABLE VIII

AMINO ACID UPTAKE BY RAT PERITONEAL MACROPHAGES: CHANGES IN TRANSPORT ACTIVITY WITH TIME

Macrophage suspensions were obtained from peritoneal exudates of paraffin oil-injected adult rats (see text). Incubations and amino acid uptake measurements (on samples of $3.5 \cdot 10^6$ cells) were as described in Table I. The values are means of 4–6 separate determinations.

Amino Acid	Uptake (μmol/ml cell water in 5 min)		
	Incubation time (h)		Difference (%)
	0	2	
α-Aminoisobutyric acid	0.11	0.29	+164
L-Proline	0.11	0.28	+155
Glycine	0.09	0.12	+ 33
L-Alanine	0.89	0.74	− 17
L-Serine	0.89	0.51	− 43
L-Leucine	0.15	0.12	− 20
L-Phenylalanine	0.25	0.12	− 52
L-Lysine	0.60	0.30	− 50

are presented in Tables VIII and IX. In both these biological preparations enhancements of transport activity have been detected with the analogue α-aminoisobutyric acid and with the naturally occurring amino acids L-proline and glycine. Surprisingly, transport activity with time did not change (or decreased) using L-alanine or L-serine as tracers (cf. Tables I–IV and VII). Substrates of transport systems *L* and Ly^+ (L-leucine, L-phenylalanine and L-lysine) exhibited usual decrements with time in their initial entry rates. Cycloheximide addition and repressive conditions (presence of amino acid substrates of transport system *A*, including L-alanine and L-serine) during

TABLE IX

AMINO ACID UPTAKE BY ISOLATED RAT THYMOCYTES: CHANGES IN TRANSPORT ACTIVITY WITH TIME

Thymocyte suspensions were obtained from thymus glands of 13- to 19-day-old rats (see text). Incubations and amino acid uptake measurements (on samples of $1.5 \cdot 10^7$ cells) were as described in Table I. The values are means of 6–10 separate determinations.

Amino acid	Uptake (μmol/ml cell water in 5 min)		
	Incubation time (h)		Difference (%)
	0	2	
α-Aminoisobutyric acid	0.107	0.193	+79
L-Proline	0.058	0.114	+97
Glycine	0.038	0.049	+32
L-Alanine	0.290	0.290	0
L-Serine	0.290	0.280	− 4
L-Leucine	0.046	0.038	−19
L-Phenylalanine	0.038	0.035	− 8
L-Lysine	0.251	0.217	−14

TABLE X

AMINO ACID UPTAKE BY HUMAN ERYTHROCYTES: CHANGES IN TRANSPORT ACTIVITY WITH TIME

Erythrocyte suspensions obtained from normal blood donors were incubated for 3 h in a salt mixture containing Tris · HCl buffer as described by Wheeler and Christensen [29]. Incubation was at 37.5 °C in an atmosphere of air. Amino acid uptake measurements (on samples of $2 \cdot 10^8$ cells) were as described in Table I. The values are means of 8–11 separate determinations.

Amino acid	Uptake (μmol/ml cell water in 5 min)		
	Incubation time (h)		Difference (%)
	0	3	
α-Aminoisobutyric acid	0.005	0.005	0
L-Proline	0.015	0.015	0
Glycine	0.009	0.008	−11
L-Alanine	0.022	0.020	− 9
L-Serine	0.023	0.021	− 9
L-Leucine	0.092	0.091	− 1
L-Phenylalanine	0.113	0.113	0
L-Lysine	0.038	0.037	− 3

the incubation (cf. Tables V and VI) abolished the increase in transport activity for α-aminoisobutyric acid, L-proline and glycine in thymocytes and for L-proline (selected as representative tracer amino acid) in macrophages (results not shown).

Tables X–XIII provide data on the transport of a number of amino acids in human, rabbit and chick embryo erythrocytes and rabbit reticulocytes. Some differences in uptake values for individual amino acids have been observed. (a) Substrates of system *L* (L-leucine and L-phenylalanine) exhibited entry rates remarkably faster in

TABLE XI

AMINO ACID UPTAKE BY RABBIT ERYTHROCYTES: CHANGES IN TRANSPORT ACTIVITY WITH TIME

Erythrocyte suspensions were obtained from normal adult rabbits (see text). Incubations and amino acid uptake measurements (on samples of $2 \cdot 10^8$ cells) were as described in Table X. The values are means of five separate determinations.

Amino acid	Uptake (μmol/ml cell water in 5 min)		
	Incubation time (h)		Difference (%)
	0	3	
α-Aminoisobutyric acid	0.025	0.025	0
L-Proline	0.052	0.052	0
Glycine	0.044	0.040	−9
L-Alanine	0.070	0.064	−9
L-Serine	0.048	0.045	−6
L-Leucine	0.012	0.012	0
L-Phenylalanine	0.014	0.014	0
L-Lysine	0.054	0.050	−7

TABLE XII

AMINO ACID UPTAKE BY CHICK EMBRYO ERYTHROCYTES: CHANGES IN TRANSPORT ACTIVITY WITH TIME

Erythrocyte suspensions were obtained from peripheral blood of 18-day-old chick embryos (see text). Incubations and amino acid uptake measurements (on samples of 10^8 cells) were as described in Table X. The values are means of 5–11 separate determinations.

Amino acid	Uptake (μmol/ml cell water in 5 min)		
	Incubation time (h)		Difference (%)
	0	3	
α-Aminoisobutyric acid	0.032	0.031	− 3
L-Proline	0.027	0.026	− 4
Glycine	0.196	0.168	−14
L-Alanine	0.045	0.045	0
L-Serine	0.017	0.015	−12
L–Leucine	0.037	0.033	−11
L–Phenylalanine	0.013	0.012	− 8
L–Lysine	0.065	0.061	− 6

human than in rabbit or avian erythrocytes. (b) The same amino acids were the only ones whose transport rate did not increase in reticulocytes, as compared with corresponding rabbit erythrocytes. (c) Glycine and L-alanine were the amino acids which presented the highest values of transport activity in rabbit reticulocytes (and the more marked decrement in this activity upon cell incubation; this finding is in agreement with the schedule of regression of transport systems during reticulocyte maturation) (cf. ref. 31). (d) Glycine was the amino acid transported at by far the highest rate in chick embryo erythrocytes; this result is likely to reflect the occurrence of a transport system nearly specific to glycine in avian nucleated red blood cells [32–34]. (e) En-

TABLE XIII

AMINO ACID UPTAKE BY RABBIT RETICULOCYTES: CHANGES IN TRANSPORT ACTIVITY WITH TIME

Reticulocytes were obtained from rabbits made anaemic with phenylhydrazine (see text). Incubations and amino acid uptake measurements (on samples of $5 \cdot 10^8$ cells) were as described in Table X. The values are means of four separate determinations.

Amino acid	Uptake (μmol/ml cell water in 5 min)		
	Incubation time (h)		Difference (%)
	0	3	
α-Aminoisobutyric acid	0.082	0.068	−17
L–Proline	0.109	0.080	−27
Glycine	0.639	0.296	−54
L–Alanine	0.503	0.243	−52
L–Leucine	0.018	0.018	0
L–Phenylalanine	0.015	0.015	0
L–Lysine	0.167	0.166	− 1

hancements of transport activity with time were never detected for the amino acids tested (which included substrates of systems *A*, *ASC*, *L* and Ly^+) upon incubation of human, rabbit and avian erythrocytes and rabbit reticulocytes either in the Tris · HCl medium described by Wheeler and Christensen [29] or in Krebs-Ringer bicarbonate buffer (results not shown). Interestingly, the *A* mediation which behaves as an adaptive transport system in mesenchymal cells (cf. Tables I–VII) is conspicuously absent in avian and mammalian erythrocytes and reticulocytes [1, 34, 35]. Moreover, all these cells are incapable of gene transcription, a function required for the adaptive regulation of transport system *A* in chick embryo heart cells [10].

Epithelial tissues

The difficulties in obtaining pure populations of isolated epithelial cells with unimpaired transport activity forced us to investigate the regulation of amino acid transport in tissue preparations in which the epithelial components were prevailing.

First, we started with kidney cortex and liver slices obtained from adult rats. The results are presented in Tables XIV and XV. Only minor decrements of transport activity with time for all the amino acids tested were observed with kidney cortex slices upon their incubation in Krebs-Ringer bicarbonate buffer. This result is in agreement with recent findings of Segal et al. [14]. Definite increases of transport activity with time have been detected with liver slices using α-aminoisobutyric acid (cf. ref. 36), L-proline and glycine as tracers, whereas decrements occurred with L-alanine, L-serine, L-phenylalanine and L-lysine. This pattern is reminiscent of the time-dependent changes of transport activity observed in peritoneal macrophages (see Table VIII), and liver macrophages (Kupffer cells) represent 35 % of the total cells in the mammalian liver [37]. It must be realized that measurements of transport activity

TABLE XIV

A MINO ACID UPTAKE BY RAT KIDNEY CORTEX SLICES: CHANGES IN TRANSPORT ACTIVITY WITH TIME

Kidney cortex slices (from adult rats) were incubated for 2 h in a large volume of Krebs-Ringer bicarbonate buffer containing 8 mM glucose (tissue/medium ratio 1 : 100, w/v), which was renewed at 30-min intervals. Conditions of incubation were as described in Table I. Amino acid uptake was measured by transferring three slices (70–80 mg, wet weight) into flasks containing Krebs-Ringer bicarbonate supplemented with 8 mM glucose and the ^{14}C-labelled amino acid (0.1 mM, final concentration) under study and incubating for 20 min at 37.5 °C. The values are means of 4–8 separate determinations.

Amino acid	Uptake (μmol/ml cell water in 20 min)		
	Incubation time (h)		Difference (%)
	0	2	
α-Aminoisobutyric acid	0.15	0.12	−20
L-Proline	0.21	0.18	−14
Glycine	0.25	0.22	−12
L-Alanine	0.19	0.18	− 5
L-Serine	0.25	0.24	− 4
L-Phenylalanine	0.13	0.11	−15
L-Lysine	0.15	0.15	0

TABLE XV

AMINO ACID UPTAKE BY RAT LIVER SLICES: CHANGES IN TRANSPORT ACTIVITY WITH TIME

Liver slices were obtained from adult rats (see text). Conditions of incubations were as described in Table XIV, except for duration of uptake assay, which was 30 min. The values are means of 7–13 separate determinations.

Amino acid	Uptake (μmol/ml cell water in 30 min)		
	Incubation time (h)		Difference (%)
	0	2	
α-Aminoisobutyric acid	0.078	0.124	+59
L-Proline	0.074	0.094	+27
Glycine	0.314	0.366	+17
L-Alanine	0.177	0.167	− 6
L-Serine	0.268	0.261	− 3
L-Phenylalanine	0.137	0.119	−13
L-Lysine	0.077	0.073	− 5

for naturally occurring amino acids in kidney cortex and liver slices are somewhat inaccurate since these molecules are metabolized intracellularly at different rates during the incubation period (20 min for kidney and 30 min for liver tissue). With this reservation, the results presented in Tables XIV and XV suggest that epithelial tissues from adult animals lack adaptive regulation of amino acid transport.

Experiments with crystalline lenses (Table XVI) are more accurate because the incubation period could be shortened to 10 min (thus minimizing the intracellular utilization of natural amino acids) and contamination with mesenchymal cells kept to a minimum by accurate dissection of this ectodermal tissue. Increased transport

TABLE XVI

AMINO ACID UPTAKE BY CHICK EMBRYO CRYSTALLINE LENS: CHANGES IN TRANSPORT ACTIVITY WITH TIME

Lenses were obtained from 8-day-old chick embryos (see text). The procedures of incubation and amino acid uptake measurement (on samples of four lenses corresponding to 5–6 mg, wet weight) were essentially as described in Table XIV. Duration of uptake assay was 10 min. The values are means of 9–15 separate determinations.

Amino acid	Uptake (μmol/ml cell water in 10 min)		
	Incubation time (h)		Difference (%)
	0	2	
α-Aminoisobutyric acid	0.102	0.187	+83
L-Proline	0.109	0.176	+62
Glycine	0.110	0.139	+26
L-Alanine	0.215	0.338	+57
L-Serine	0.166	0.199	+20
L-Phenylalanine	0.222	0.186	−16
L-Lysine	0.077	0.068	−12

activities with time have been detected with the analogue α-aminoisobutyric acid and with the naturally occurring amino acids L-proline, glycine, L-alanine and L-serine; moderate decrements were observed with L-phenylalanine and L-lysine. These results indicate that at least in an epithelial preparation (though represented by an embryonic tissue from avian origin) a time-dependent control mechanism of amino acid transport appears to be operative. Interestingly, the *A* mediation, which behaves as an adaptive transport system in mesenchymal cells (cf. Tables I–VII), has been identified in the crystalline lens of rabbit [38] and calf [39]. The occurrence of a time-dependent regulatory process for amino acid transport (*A* mediation) has been recently reported for kidney cortex slices of newborn rats [14] and human placenta villous tissue [13, 40]. These biological preparations, however, are less likely candidates as pure epithelial cell populations and the contribution of contaminating mesenchymal cells to the regulatory phenomena cannot be easily evaluated.

DISCUSSION

The results recounted in this paper clearly indicate that a time-dependent adaptive regulation for the transport of a number of neutral amino acids across the cell membrane is operative in a variety of tissues of avian and mammalian origin. The transport system involved, formally identified as the *A* mediation in chick embryo heart cells [9], appears to correspond to the same *A* mediation in such mesenchymal cells as fibroblasts, chondroblasts, osteoblasts and myoblasts, and in the epithelial cells of embryonal crystalline lens. However, adaptive control in macrophages and thymic lymphocytes is restricted to some amino acids (namely α-aminoisobutyric acid, L-proline and glycine) among those which are considered typical substrates of the *A* mediation [1, 30]. In these cells, the transport rate of L-alanine and L-serine does not increase under derepressive conditions, suggesting that either transport system *A* is heterogeneous (consisting of subgroups) or the latter amino acids are preferential substrates of different mediations in macrophages and thymocytes. The fact that all substrates of the *A* mediation (including L-alanine and L-serine) inhibit derepression of the restricted system and respond to insulin with increased uptake in lymphoid cells [11] and that adaptive and insulin regulations act on a common target (the *A* mediation) in muscle tissue [8, 9] are in favour of the second interpretation. Perhaps the hormone shifts the reactivity of L-alanine and L-serine toward transport system *A* in thymocytes. Adaptive regulation is absent in erythroid cells (human erythrocytes, rabbit erythrocytes and reticulocytes, avian erythrocytes) which lack the *A* mediation [1, 34, 35]. In turn the *A* mediation, whose adaptive nature requires active transcription [10] is likely to be lost during maturation of these anucleated or dormant nucleus-bearing cells. An adaptive regulation of the transport system *A* substrate L-proline has been observed in cells of rat bone marrow (results not shown).

Adaptive regulation has not been found operative in adult epithelial tissues such as kidney cortex and, perhaps, liver. (The increase in amino acid transport activity upon incubation of liver slices in Krebs-Ringer bicarbonate is likely to be due to the contaminating littoral macrophages). In a comparable investigation, Segal et al. [14] described a mechanism of adaptive regulation in kidney cortex slices from newborn but not from adult rats and suggested that this control process may be a general property of embryonic, immature or developing tissues. The occurrence of

adaptive regulation for amino acid substrates of the *A* mediation in the embryonic crystalline lens agrees with this view. On the contrary, at least two neoplastic cell lines of epithelial origin (from Ehrlich ascites carcinoma and Yoshida ascites hepatoma) endowed with a wide-range *A* system [1] are unable to regulate transport activity for amino acid substrates of this mediation [41]. However, this failure might result from a fully derepressed state of the adaptive mechanism in neoplastic cells.

The frequency of results of the type shown in the preceding Tables for many animal tissues and comparable data indicating the occurrence of repression-derepression mechanisms for adaptive regulation of amino acid transport in yeasts and fungi [42–44] emphasize the broad biological significance of these processes in eukaryotes.

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