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AMINO-ACID-DEPENDENT MODULATION OF AMINO ACID TRANSPORT IN *XENOPUS LAEVIS* OOCYTES

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Summary

We have measured rates of uptake of arginine, glutamine, glutamate, serine, phenylalanine and glycine in *Xenopus laevis* oocytes cultured for periods of up to 24 h in saline in the presence or absence of a mixture of 20 amino acids at concentrations approximating those in *Xenopus* plasma. Amino acid supplementation increased the total intracellular amino acid concentration from 8.2 to 18.4 nmol per oocyte. Specific Na^+-dependent amino acid transporters (systems $B^{0,+}$, X_{ag}^-) exhibit 'adaptive regulation' (up-regulation during amino acid deprivation and down-regulation during amino acid supplementation). Na^+-independent transporters of glutamate, glutamine and glycine (including system asc) display an opposite modulation in activity, which may help to combat amino-acid-induced oxidative stress by increasing the supply of glutathione precursors. Single amino acids at physiological plasma concentrations (0.47 mmol l^{-1} L-alanine, 0.08 mmol l^{-1} L-glutamate) mimicked at least some effects of the amino acid mixture. The mechanisms of transport modulation do not appear to include *trans*-amino acid or membrane potential effects and, in the case of Na^+-independent transport, are independent of protein or mRNA synthesis. Furthermore, activation of protein kinase C by phorbol 12-myristate 13-acetate did not significantly affect endogenous glutamine and glutamate transport. The *Xenopus* oocyte appears to possess endogenous signalling mechanisms for selectively modulating the activity of amino acid transport proteins expressed in its surface membranes, a factor for consideration when using oocytes as an expression system for structure–function studies of cloned amino acid transporters.

Key words: amino acid, membrane transport, transporter regulation, *Xenopus laevis*.

Introduction

Oocytes from the toad *Xenopus laevis* have been widely used to study membrane phenomena because of their large size (1.2–1.3 mm) and thus relative ease of handling. Isolated *Xenopus* oocytes also provide a good expression system for mRNA from other cell types (Colman, 1984). These features of the oocyte have facilitated expression cloning of a wide range of membrane-bound proteins, including transport proteins for amino acids (e.g. Kanai and Hediger, 1992; Closs *et al.* 1993; Arriza *et al.* 1993).

A complicating factor when using *Xenopus* oocytes as an expression system for studies of cloned amino acid transporters is that these oocytes possess significant endogenous amino acid transport activities. Amino acid transport activity in many cell types changes in response to alterations in the composition of the incubation medium (Christensen, 1990; see Guidotti and Gazzola, 1992, for a review), and we decided to identify and characterise any such responses in *Xenopus* oocytes to see whether they could be manipulated to minimise endogenous amino acid transport 'noise' when studying overexpressed transporter activities from clonal cRNA or tissue mRNAs. Up-regulation of amino acid transport has been reported to occur in response to amino acid starvation of cells and down-regulation in response to amino acid supplementation; this phenomenon is termed 'adaptive regulation', and it has been suggested (Shotwell *et al.* 1983) that amino acids mediate their effect by promoting the synthesis of regulatory proteins affecting transporter activity either directly at the carrier site or at an intermediate stage of its emergence.

Adaptive regulation has been described for the Na^+-dependent transport systems A and N/N^m (Shotwell *et al.* 1983; Low *et al.* 1994), but these transporters are not expressed by *Xenopus* oocytes in culture (Taylor *et al*. 1989; Mackenzie *et al.* 1994). Nevertheless, *Xenopus* oocytes express a range of Na^+-dependent and Na^+-independent amino acid transport activities and they are generally cultured in saline (e.g. Barth's medium) lacking amino acids, a condition which might cause up-regulation of amino acid transport. The purpose of the present study was therefore to investigate possible mechanisms by which exogenous amino acids modulate endogenous amino acid transport systems in cultured *Xenopus* oocytes. We have investigated the transport of five amino acids (serine, a small neutral molecule; glutamine, a large neutral molecule;

glutamate, an anionic molecule; arginine, a cationic molecule; and phenylalanine, an aromatic molecule) representing the major classes of transporter substrate.

Materials and methods

Chemicals

Unless otherwise specified, chemicals were obtained from BDH (Poole, UK) or Sigma Chemical Company (Poole, UK). Radiotracers ([^{3}H]glutamate, [^{3}H]glutamine, [^{3}H]serine, [^{3}H]arginine, [^{3}H]phenylalanine, [^{14}C]glucose, [^{14}C]methylaminoisobutyrate) were obtained from Amersham International (Aylesbury, UK) or NEN-Dupont.

Isolation and culture of oocytes

Large, female *Xenopus laevis* toads (Blades Biological, Edenbridge, UK, or South African *Xenopus* facility) were maintained in freshwater aquaria. Ovarian tissue was surgically isolated from toads (anaesthetised by immersion in 0.1 % aminoethylbenzoate; Colman, 1984), rinsed immediately in modified Barth's medium (MBM) then incubated in Collagenase A (Boehringer) solution (2 mg ml^{-1} in Ca^{2+}-free MBM) for 3 h to liberate and defolliculate oocytes as described previously (Taylor *et al.* 1989). Stage V–VI oocytes were cultured at 18 °C in MBM containing gentamycin sulphate (10 mg l^{-1}). All experiments were performed within 3 days of isolation of oocytes.

Experimental preincubations of oocytes

Groups of cultured oocytes were preincubated in 10 ml of MBM + gentamycin either lacking amino acids (amino-acid-deprived) or containing an amino acid mixture (amino-acid-supplemented); preincubation was carried out for timed periods of up to 24 h prior to transport assay using the methods described below. The composition of the amino acid mixture (based on standard human plasma amino acid concentrations; Jefferson and Korner, 1969) is similar to that found in *Xenopus* plasma (see Table 1).

In certain experiments, the protein synthesis inhibitor cycloheximide (100 μg ml^{-1}) or the mRNA synthesis inhibitor actinomycin D (50 μg ml^{-1}) was added to the preincubation medium; the actions of these inhibitors on amino acid uptake were compared with control preincubations in the absence of inhibitor. The inhibitor concentrations used are known to inhibit protein and mRNA synthesis in oocytes (Colman, 1984).

In order to assess the influence of individual amino acids on amino acid transport, groups of oocytes were also preincubated for 24 h in 10 ml of MBM containing L-glutamate, L-phenylalanine or L-alanine at the same concentration as in the amino acid mixture (see Table 1).

Transport assay

A radiotracer technique (Taylor *et al.* 1989) was used to measure the rate of amino acid uptake by oocytes (9–11 oocytes for each experimental manoeuvre). The initial rate (at 20 or 30 min) of ^{3}H- or ^{14}C-labelled solute uptake was measured in transport medium [containing 100 mmol l^{-1} NaCl or tetramethylammonium chloride (TMACl), 2 mmol l^{-1} KCl, 1 mmol l^{-1} $CaCl_2$, 1 mmol l^{-1} $MgCl_2$, 10 mmol l^{-1} Hepes, adjusted to pH 7.5 with Tris]; uptake was terminated by aspiration of transport medium and rinsing of the oocytes in distilled water. Oocytes were individually transferred to plastic scintillation vials and processed for liquid scintillation counting using methods described previously (Taylor *et al.* 1989; Ahmed *et al.* 1995).

The effects of different amino acids on tracer amino acid uptake were assessed by the inclusion of unlabelled amino acid (at a concentration of 5 mmol l^{-1}) in the transport medium.

In experiments designed to assess the effects of increased intracellular amino acid concentrations on amino acid transport, oocytes were injected with distilled water (50 nl nominal) containing 8 pmol of amino acids in amino acid mixture ratios (see Table 1); control oocytes were injected with the same volume of distilled water only. Oocytes were allowed to recover for 15 min from the injection procedure prior to measurement of tracer amino acid uptake.

Additional experiments were performed to investigate the effect of protein kinase C (PKC) activation on amino acid transport. Oocytes were injected with 50 nl of 10 % dimethyl sulphoxide (DMSO) solution containing the PKC activator phorbol 12-myristate 13-acetate (PMA) or the inactive homologue 4α-phorbol 12,13-didecanoate (4αPDD) to give a final intra-oocyte concentration of 0.1 μmol l^{-1}, assuming an oocyte volume of 0.5 μl (Corey *et al.* 1994). Control oocytes were injected with an equivalent volume of 10 % DMSO only. Measurement of tracer amino acid uptake was commenced 30 min post-injection.

Measurement of amino acid 'free pools' within oocytes

An acid precipitation method with sulphosalicylic acid (SSA) was used to extract amino acids from oocyte homogenate and toad plasma. Groups of five oocytes were initially homogenised in 0.2 ml of distilled water; 50 μl of 400 μmol l^{-1} Norvaline in 10 % SSA was added to the homogenate prior to cooling for 30 min at 4 °C. The mixture was then centrifuged for 5 min at 14 000 ***g***; 0.2 ml of the resulting supernatant was aspirated and diluted (1:1) with lithium citrate buffer (pH 2.2) before filtration through 45 μm filters (Gelman) into Eppendorf tubes for automated amino acid analysis by ion exchange chromatography using a Biotronik LC 5000 analyser with fluorometric (*O*-phthaldehyde) detection.

Measurement of oocyte membrane potential

The membrane potential (E_m) of isolated oocytes was measured using a high-resistance electrometer (World Precision Instruments) attached to a chart recorder. A glass micropipette filled with 1 mol l^{-1} potassium chloride solution mounted on a movable head stage was used for oocyte impalement; the resistance of the microelectrodes was approximately 10 MΩ. Oocytes were superfused with MBM (at 5 ml min^{-1}) and E_m was measured over a 10 min period, to allow for stabilisation after impalement, after denoted preincubation protocols. In certain experiments, E_m was also

monitored immediately after the addition of the amino acid mixture to the superfusate.

Data presentation and evaluation

Except where stated, all data are presented as mean values ± S.E.M. Data were evaluated for significance using Student's *t*-test or the paired *t*-test for the comparison of two means, where $P<0.05$ was taken as a significant difference.

Results

Amino acid concentrations in Xenopus *oocytes and plasma*

Amino acid concentrations in *Xenopus* plasma were broadly similar to those of the amino acid mixture used to supplement Barth's medium (Table 1). Amino acid concentrations in oocytes were generally 2–4 times higher in amino-acid-supplemented cultures than in amino-acid-deprived cultures, notable exceptions being aspartate, glutamine and lysine, concentrations of which were similar in the two cell types. Glutamate made a large contribution (about 40–45 %) to the amino acid pool. A stage VI oocyte has a cell volume of approximately 0.5 μl (Colman, 1984); intra-oocyte concentrations of glutamate and total amino acids are therefore approximately 7.2/14.7 mmol l^{-1} and 16.4/36.8 mmol l^{-1}, respectively, in amino-acid-depleted/amino-acid-supplemented oocytes.

Effects of amino acid supplementation on Na^+-dependent amino acid uptake in oocytes

Xenopus oocytes cultured for 24 h in MBM supplemented with a mixture of 20 amino acids at plasma concentrations showed a reduced rate of Na^+-dependent transport of glutamine, arginine and glutamate compared with oocytes cultured in amino-acid-free medium (Fig. 1). Such differences in glutamine transport rate appeared to reflect the operation of a reversible modulatory process which was active within 4 h of transfer from one culture medium to the other (Fig. 2). Na^+-dependent rates of transport of serine and phenylalanine were not significantly affected by amino acid supplementation of the culture medium (Fig. 1), indicating selective modulation of transport activity. We were unable to detect Na^+-dependent transport of the system A substrate methylaminoisobutyric acid in either amino-acid-deprived or amino-acid-supplemented oocytes (data not shown).

Effects of amino acid supplementation on Na^+-independent amino acid uptake in oocytes

In contrast to the results with Na^+-dependent uptake, oocytes

Table 1. *Amino acid concentrations in* Xenopus laevis *plasma and in oocytes cultured in amino-acid-supplemented or amino-acid-deprived (control) media*

	Amino acid mixture (μmol l^{-1})	*Xenopus* plasma (μmol l^{-1})	*Xenopus* oocytes (nmol per oocyte)	
			Control	Amino-acid-supplemented
Aspartate	38	50±8	0.43±0.17	0.37±0.13
Threonine	270	186±36	0.20±0.07	0.58±0.19
Serine	243	156±17	0.48±0.23	1.30±0.56
Asparagine	63	ND	ND	ND
Glutamine	667	228±38	0.16±0.05	0.18±0.09
Proline	186	ND	ND	ND
Glutamate	76	112±23	3.60±0.50	7.35±3.20
Glycine	408	155±33	0.41±0.15	0.80±0.30
Alanine	471	449±56	0.64±0.50	2.40±1.30
Valine	173	178±16	0.20±0.04	0.72±0.26
Methionine	46	ND	ND	ND
Isoleucine	90	81±7	0.07±0.04	0.33±0.20
Leucine	161	151±11	0.19±0.07	0.62±0.20
Tyrosine	83	83±23	0.09±0.03	0.18±0.08
Phenylalanine	54	72±8	0.13±0.03	0.30±0.15
Lysine	418	292±29	1.13±0.20	1.43±0.60
Histidine	68	132±20	0.20±0.07	0.47±0.23
Arginine	132	78±29	0.35±0.15	1.21±0.50
Cysteine	38	ND	ND	ND
Tryptophan	70	25±5	ND	ND
Total			8.2±1.45	18.4±6.7

All values are means ± S.E.M. for five toads or 4-5 oocyte preparations.
ND, not determined.

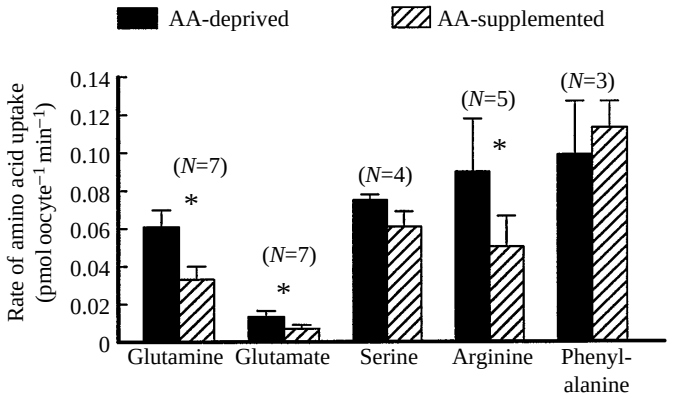

Fig. 1. Effect of 24 h of amino acid deprivation or supplementation on Na^+-dependent amino acid transport in *Xenopus laevis* oocytes. Values are mean + S.E.M. of the difference between rates of uptake of 5 μmol l^{-1} amino acid in NaCl and in tetramethylammonium chloride (TMACl) medium; *N* denotes the number of cell preparations. * denotes a significant difference between the amino-acid-deprived and amino-acid-supplemented value (P<0.05 by paired-sample *t*-test). AA, amino acid.

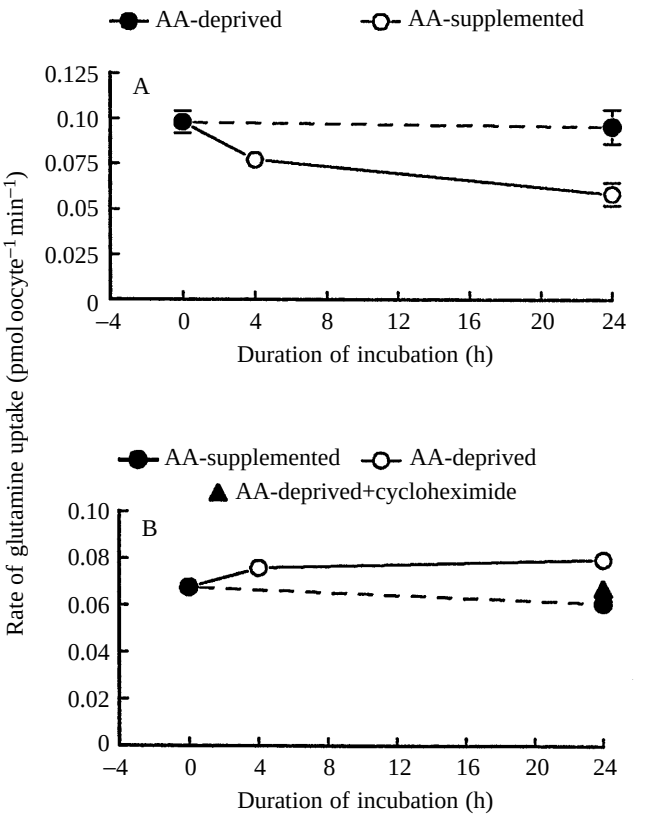

Fig. 2. Time course of changes in Na^+-dependent glutamine transport in oocytes after (A) transfer from amino-acid-deprived to amino-acid-supplemented culture medium and (B) transfer to amino-acid-deprived medium ± cycloheximide after 24 h of preincubation in amino-acid-supplemented medium. Values are mean ± S.E.M. of the difference between rates of uptake of 5 μmol l^{-1} amino acid in NaCl and in TMACl medium for 9–11 oocytes from a single cell preparation. Error bars not shown lie within symbols. The dotted line indicates transport in control (i.e. non-transferred) oocytes. From B, it can be seen that the protein synthesis inhibitor cycloheximide appeared to prevent the activation of glutamine transport resulting from amino acid deprivation.

cultured for 24 h in MBM supplemented with amino acids showed enhanced rates of Na^+-independent uptake of glutamine and glutamate in comparison with oocytes cultured in amino-acid-free medium, although Na^+-independent rates of uptake of arginine, serine and phenylalanine were not significantly affected (Fig. 3). Amino acid supplementation therefore had opposing effects on the expression of Na^+-dependent and Na^+-independent transport of glutamine and glutamate in oocytes. Amino acid supplementation had no significant effect on Na^+-independent transport of 50 μmol l^{-1} glucose (from 0.019 pmol oocyte^{-1} min^{-1}) or 5 μmol l^{-1} MeAIB (from 0.001 pmol oocyte^{-1} min^{-1}). We observed considerable differences in basal Na^+-independent amino acid uptake rate between different batches of oocytes (which ranged, for example, between 0.001–0.02 and 0.0005–0.048 pmol oocyte^{-1} min^{-1} for 5 μmol l^{-1} glutamine or glutamate uptake respectively), but consistent effects of amino acid supplementation were observed throughout the study. The enhancements of both glutamine and glutamate uptake rates were evident within 4 h of amino acid supplementation (Fig. 4; with glutamate appearing to show the more rapid response), were saturable (Table 2) and were sensitive to inhibition by cysteine (Table 3) but not arginine (Table 2). The enhanced glutamine uptake was partially inhibited by the system L substrate 2-amino-2-norbomane carboxylic acid (BCH) (Table 2) and glutamate uptake was inhibitable by L-aspartate (data not shown). Na^+-independent glycine uptake rate also appeared to increase after amino acid supplementation of oocytes (Table 3).

Culture of oocytes in MBM supplemented with a single amino acid (L-alanine or L-glutamate) at a physiological concentration (0.47 or 0.08 mmol l^{-1} respectively) was sufficient to enhance rates of Na^+-independent uptake of glutamate and glutamine to levels achieved by the amino acid

Table 2. *Effect of amino acids on Na^+-independent uptake of glutamine and glutamate in amino-acid-supplemented* Xenopus laevis *oocytes*

Inhibitory amino acid	Percentage of control rate of amino acid uptake	
	L-[^{3}H]glutamate	L-[^{3}H]glutamine
L-Glutamate	31.25±8.3	106.25±31.25
L-Glutamine	58.3±18.75	22.9±5.2
L-Arginine	90.6±6.25	70.8±13.5
BCH	91.7±14.6	54.2±10.4

Inhibitory amino acids were added at 5 mmol l^{-1}.

Results are expressed as a percentage of uptake in the absence of inhibitor; values are the mean ± S.E.M. for 3–5 preparations.

Uptake of 50 μmol l^{-1} amino acid was measured in TMACl medium.

BCH, 2-amino-2-norbomane carboxylic acid.

Table 3. *Effect of cysteine and cystine on Na^+-independent uptake of amino acids in amino-acid-deprived and amino-acid-supplemented* Xenopus laevis *oocytes*

Amino acid	Incubation conditions	Rate of uptake of 5 μmol l^{-1} amino acid (fmol oocyte^{-1} min^{-1}) Control	+5 mmol l^{-1} cysteine	+0.35 mmol l^{-1} cystine*
Glutamine	AA-deprived	1.89±0.14	0.49±0.05 (26 %)	0.51±0.07 (27 %)
	AA-supplemented	8.06±0.65	0.43±0.06 (5.3 %)	6.81±0.37 (85 %)
Glutamate	AA-deprived	2.57±0.25	0.31±0.03 (12 %)	2.05±0.38 (80 %)
	AA-supplemented	12.3±2.5	0.98±0.14 (8 %)	20.2±1.8 (164 %)
Glycine	AA-deprived	1.08±0.19	0.71±0.10 (66 %)	0.89±0.07 (82 %)
	AA-supplemented	2.98±0.50	0.40±0.11 (13 %)	3.91±0.50 (131 %)

Data are presented as mean uptake ± S.E.M. for 8–10 oocytes from a single preparation.
Values in parentheses represent the rate of uptake in the presence of inhibitor as a percentage of the respective control uptake rate.
*Inhibitor concentration limited by solubility of cystine in aqueous solution.
AA, amino acid.

Table 4. *Effect of supplementation with individual amino acids (L-alanine, L-glutamate or L-phenylalanine) or a mixture of 20 amino acids on Na^+-independent glutamine and glutamate transport in* Xenopus laevis *oocytes*

Supplementation conditions	Percentage increase over control rate of amino acid uptake: Glutamine	Glutamate
Amino acid mixture	94±27*	94±27*
L-Alanine	105±30*	96±22*
L-Glutamate	100±23*	113±42*
L-Phenylalanine	4±10	41±15*

All amino acids used were at concentrations appropriate for *Xenopus laevis* plasma (see Table 1 for values).

Values are the mean ± S.E.M. of the percentage increase over the average uptake in control (amino-acid-deprived) oocytes (9–11 oocytes from a single preparation); *percentage increase is significantly different from zero; $P<0.05$.

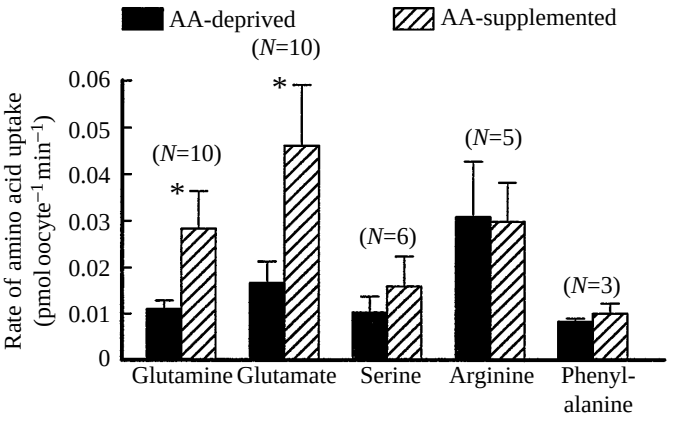

Fig. 3. Effect of 24 h of amino acid deprivation or supplementation on Na^+-independent amino acid transport in *Xenopus laevis* oocytes. Values are mean + S.E.M. of the rates of uptake of 5 μmol l^{-1} amino acid in TMACl medium for 3–10 separate oocyte preparations as detailed in the figure. * denotes a significant difference between the amino-acid-deprived and amino-acid-supplemented value ($P<0.05$ by paired-sample *t*-test).

mixture (Table 4), although phenylalanine appeared to be significantly less effective in this role (Table 4).

Investigation of possible mechanisms for amino-acid-dependent modulation of glutamine and glutamate transport in oocytes

Possible mechanisms by which transporter activity could be modulated by changes in external amino acid availability include (i) *trans*-effects on amino acid uptake induced by loading or depleting oocytes with amino acids, (ii) changes in transport protein number induced *via* altered rates of protein or mRNA turnover, (iii) changes in oocyte membrane potential affecting rheogenic amino acid transport and/or (iv) changes in transport activity induced by alteration of phosphorylation status by, for example, activation of protein kinase C (Corey *et al.* 1994). We investigated these possibilities in the following experiments.

(i) Injection of oocytes with amino acids (approximately doubling the total intracellular amino acid concentration) did not significantly affect glutamine and glutamate uptake in oocytes (Table 5), indicating that the transport mechanisms involved are not acutely sensitive to *trans*-stimulation or *trans*-inhibition.

(ii) Cycloheximide had no significant independent effect on glutamine or glutamate uptake in amino-acid-deprived oocytes (see Figs 5, 6), although it abolished the modulatory effects on Na^+-dependent glutamine transport of transfers between amino-acid-deprived and amino-acid-supplemented media (Figs 2B, 5). Cycloheximide actually appeared to increase the enhancement of Na^+-independent glutamine uptake rate observed in certain amino-acid-supplemented cultures; however, this phenomenon was not observed in all experiments and overall the added enhancement was not statistically significant (Fig. 6). Cycloheximide did not affect the stimulatory effects of amino acid supplementation on Na^+-

Table 5. *Effect of amino acid injection on glutamine and glutamate transport in* Xenopus laevis *oocytes*

Amino acid (5μmol l^{-1}) and experimental conditions	Rate of amino acid uptake (pmol oocyte^{-1} min^{-1})	
	Water-injected oocytes	Amino-acid-injected oocytes
Glutamine		
NaCl	0.1144±0.0057	0.1265±0.0073
TMACl	0.0104±0.0009	0.0104±0.0006
Glutamate		
TMACl	0.0076±0.0005	0.0062±0.0008

All rates of uptake are means ± S.E.M. for 10 oocytes from a single preparation.

Similar results were obtained using oocytes from two other preparations.

The quantity of amino acid mixture injected was equivalent to approximately 8 nmol per oocyte.

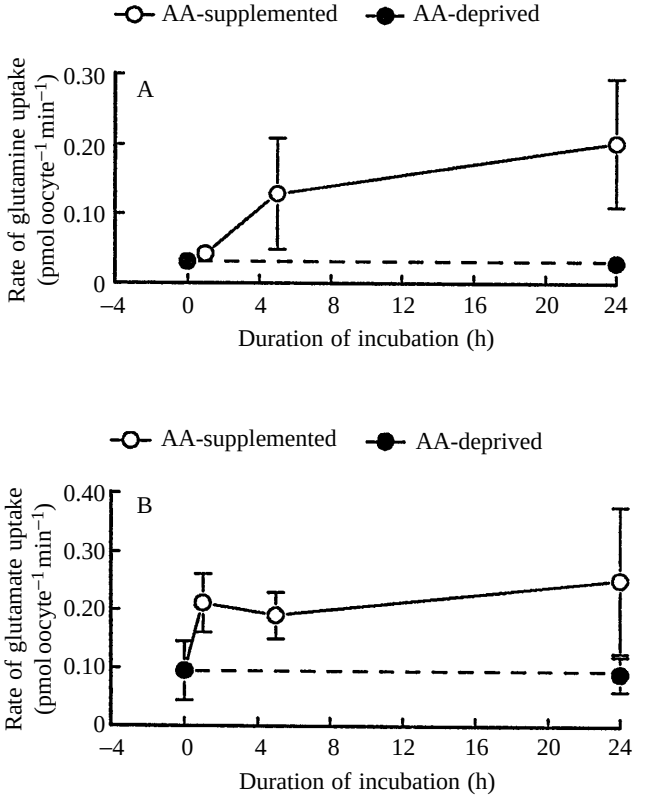

Fig. 4. Time course of changes in Na^+-independent transport of (A) glutamine and (B) glutamate in oocytes after transfer from amino-acid-deprived to amino-acid-supplemented culture medium. Values are mean ± S.E.M. of the rate of uptake of 50 μmol l^{-1} amino acid in Na^+-free medium from three separate oocyte preparations.

independent glutamate transport in oocytes (Fig. 6). Actinomycin D had an overall inhibitory effect on amino acid transport in oocytes, but the percentage stimulation of Na^+-independent glutamine and glutamate uptake rate by amino acid supplementation was similar in the presence or absence of

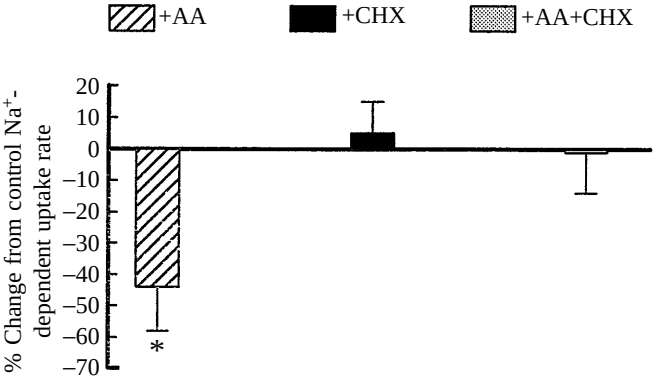

Fig. 5. Effect of cycloheximide (CHX) on modulation of Na^+-dependent glutamine (5 μmol l^{-1}) transport in oocytes after amino acid supplementation. Values are mean (+ S.E.M.) percentage change from the control (amino-acid-deprived) Na^+-dependent rate of glutamine uptake for four oocyte preparations (* indicates that the percentage change is significantly different from zero; $P<0.05$).

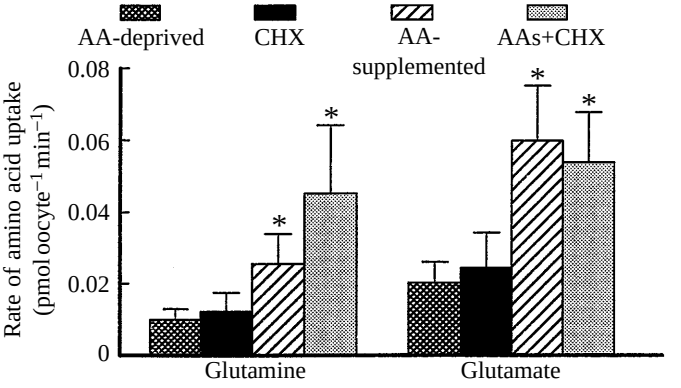

Fig. 6. Effect of the protein synthesis inhibitor cycloheximide (CHX) on Na^+-independent transport of glutamine and glutamate (both at 5 μmol l^{-1}) in amino-acid-deprived and amino-acid-supplemented *Xenopus laevis* oocytes. Oocytes were incubated for 24 h under the conditions described before transport measurements were performed. Values are mean + S.E.M. for six oocyte preparations. * indicates that the amino-acid-supplemented value is significantly greater than the corresponding amino-acid-deprived value ($P<0.05$ by paired-sample *t*-test).

actinomycin D (data not shown). The results indicate that amino-acid-dependent modulation of Na^+-dependent, but not of Na^+-independent, amino acid transport in oocytes involves mechanisms requiring protein (and possibly mRNA) synthesis.

(iii) The E_m of oocytes showed a small (4.80±0.44 mV; N=4 oocytes), transient depolarisation immediately after amino acid supplementation, which persisted for less than 30 min before returning to the original resting value (−59 mV; Table 6). In the longer term, the resting E_m (measured in MBM) of oocytes supplemented with amino acids for 24 h showed a small but significant positive shift (6.5 mV) relative to that of amino-acid-deprived oocytes (Table 6), possibly reflecting a depolarising effect of net Na^+ accumulation into oocytes by Na^+/amino acid symport. Cycloheximide had an independent depolarising effect on oocyte E_m (Table 6; this appeared to be

Table 6. *Comparison of oocyte membrane potential (E_m) after 24 h of pre-incubation in amino-acid-depleted or amino-acid-supplemented medium*

Number of oocytes	Preincubation medium	E_m in MBM (mV)
16	MBM	–58.8±1.8
16	MBM + amino acids	–52.3±2*
7	MBM + cycloheximide	–51.5±3.2*
7	MBM + amino acids + cycloheximide	–41.7±5.1*

*Significant difference from value for control amino-acid-deprived cells (MBM); where $P<0.05$ using Student's *t*-test.
MBM, modified Barth's medium.

additive to the effect of amino acid supplementation), but the observation that cycloheximide had no significant independent effect on amino acid transport (see ii above) argues against a role for these relatively small changes in E_m in the observed modulations of amino acid transport activity.

(iv) Injection of the protein kinase C activator PMA into oocytes had no significant effect on either Na^+-dependent or Na^+-independent uptake rates of glutamine or glutamate (Fig. 7) at a PMA concentration (0.1 μmol l^{-1}) recently shown (Corey *et al.* 1994) to be maximally effective in oocytes for modulation of the activity of overexpressed γ-aminobutyric acid (GABA) transporters by endogenous protein kinase C.

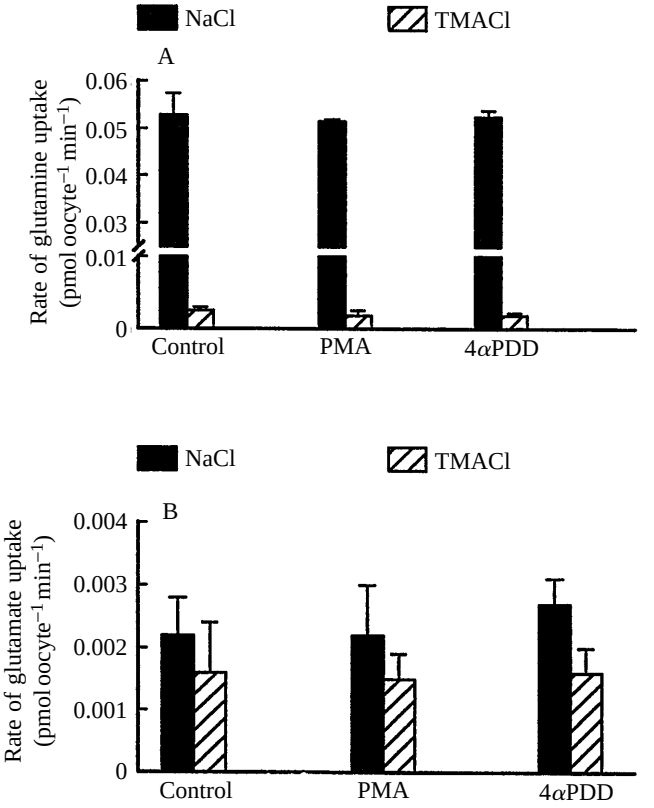

Fig. 7. Effect of the protein kinase activator PMA and the inactive analogue 4αPDD (0.1 μmol l^{-1} final intra-oocyte concentration) on the Na^+-dependent (filled columns) and Na^+-independent (hatched columns) rates of uptake of (A) glutamine and (B) glutamate in amino-acid-deprived ooctyes; both amino acids at 5 μmol l^{-1}. Uptakes measurements were performed 30 min after injection, control oocytes were injected with vehicle (10 % DMSO) only. Values are mean + S.E.M. for three oocyte preparations.

Discussion

The present results show that amino acid transport in *Xenopus* oocytes is modulated with respect to the external amino acid supply. Na^+-dependent transport of several amino acids (notably glutamine, arginine and glutamate) exhibits up-regulation during amino acid deprivation and down-regulation during amino acid supplementation, properties consistent with the phenomenon of 'adaptive regulation' described for Na^+-dependent amino acid transporters in other cell types (Guidotti and Gazzola, 1992; Low *et al.* 1994). In contrast, certain Na^+-independent transport processes show the opposite effect; i.e. increased activity during amino acid supplementation and *vice versa*. The relatively high intracellular amino acid concentrations found in amino-acid-supplemented oocytes (noted previously by Bravo *et al.* 1976) presumably reflect the net accumulation of amino acids by the Na^+-coupled amino acid transporters. The fact that amino-acid-deprived oocytes maintain intracellular amino acid concentrations in the millimolar range is likely to be due to a combination of a relatively low efflux of amino acids (Ahmed *et al.* 1995) and an endogenous source of amino acid supply, presumably from net breakdown of ovalbumin and other oocyte proteins. Glutamine concentration (approximately 0.17 nmol per oocyte; 0.3 mmol l^{-1}) in oocytes is lower than that in toad plasma (where glutamine is the most abundant free amino acid) and there was no appreciable difference in glutamine concentration between amino-acid-supplemented and amino-acid-deprived oocytes. This is surprising, given the relatively high Na^+-dependent glutamine transport rate in oocytes, and may reflect a high glutaminase activity in oocytes (which would also help to explain the high intracellular glutamate concentration despite a relatively low activity of Na^+/glutamate transport).

Several kinetically distinct transport systems have been identified in *Xenopus* oocytes; Na^+-dependent systems include $B^{0,+}$ for neutral and cationic amino acids (Taylor *et al.* 1989), ASC for small neutral amino acids (Campa and Kilberg, 1989) and X^-_{ag} or anionic amino acids (Steffgen *et al.* 1991). Less information is available for Na^+-independent systems, but transport activities resembling known systems asc, L, y^+, $b^{0,+}$ and x^- have been reported (Taylor *et al.* 1989, 1992; Campa and Kilberg, 1989; Ishii *et al.* 1991; Magagnin *et al.* 1992). Our results clearly demonstrate selective amino-acid-dependent modulation of transport activities rather than a generalized effect on transmembrane solute fluxes. The elevated intracellular amino acid concentrations in amino-acid-supplemented oocytes (approximately double the concentrations in amino-acid-deprived oocytes) are unlikely to exert significant *trans*-stimulatory or *trans*-inhibitory effects on amino acid uptake mechanisms because no such effects occur after doubling the intracellular amino acid

concentrations of amino-acid-deprived oocytes by injection; *trans* effects are therefore unlikely to account for the observed modulation of transport activities. Furthermore, a major effect of membrane potential on amino-acid-dependent modulation of transport can be excluded because (i) cycloheximide has significant effects on E_m but not on glutamine transport and (ii) the small differences in E_m between amino-acid-supplemented and amino-acid-deprived oocytes would affect Na^+-coupled amino acid transport by less than 10 % (Mackenzie *et al.* 1994), but the observed effects are considerably greater than this value. Possible mechanisms by which specific amino acid transport activities may be modulated are considered below.

System $B^{0,+}$ is quantitatively the most important Na^+-dependent transporter of neutral and cationic amino acids in cultured oocytes (Taylor *et al.* 1989). Glutamine and arginine are major substrates of this transporter; therefore, it appears likely from the present results that system $B^{0,+}$ exhibits adaptive regulation in oocytes. This would also account both for the relatively minor amino-acid-dependent effect on serine uptake, which includes a major system ASC component characteristically lacking the capacity for adaptive regulation, and for the lack of any effect on phenylalanine uptake, which is not mediated by system $B^{0,+}$ (Taylor *et al.* 1989). The glutamate-transporting system X^-_{ag} also appears to undergo adaptive regulation in *Xenopus* oocytes. Adaptive regulation of systems A and N (neither of which is expressed in the oocyte membrane) and system X^-_{ag} have all been reported previously (Christensen, 1990; Guidotti and Gazzola, 1992; Low *et al.* 1994), but to our knowledge this is the first report of adaptive regulation of system $B^{0,+}$, although it does show developmental regulation in oocytes and early conceptuses (Richter *et al.* 1984; Van Winkle, 1988; Lewis and Kaye, 1992). Cycloheximide inhibited the amino-acid-dependent modulation of Na^+-dependent glutamine transport, and therefore amino acids may produce their effect in part by modulating the synthesis of system $B^{0,+}$ transporters or proteins regulating their activity (or possibly synthesis of their respective mRNAs), as appears to occur in adaptive regulation of other amino acid transport activities (e.g. Plakidou-Dymock and McGivan, 1993; Low *et al.* 1994).

Na^+-independent uptakes of arginine and phenylalanine (substrates of systems $b^{0,+}/y^+$ and $b^{0,+}$/L, respectively, in oocytes) are unaffected by external amino acid supply, indicating a lack of significant amino-acid-dependent regulation of their transporters. In contrast, 24 h of amino acid supplementation enhances the Na^+-independent uptake rates of glutamine and glutamate. The transport processes involved for the two amino acids are not identical because the two amino acids do not show mutual inhibition of transport and only glutamine transport has cystine- and BCH-sensitive components (Tables 2, 3), the latter observation suggestive of minor involvement of an L-like system. In addition, the time courses for induction of glutamine and glutamate transport appear to be dissimilar (Fig. 4). Nevertheless, Na^+-independent uptakes of glutamine, glutamate and glycine are inhibited by cysteine (Table 3) in both amino-acid-deprived and amino-acid-supplemented oocytes, indicating the possible involvement of a transporter resembling system asc (which accepts neutral and protonated anionic amino acids as substrates; Christensen, 1990). The observed small increase in Na^+-independent serine uptake is also consistent with stimulation of system asc activity. An additional anionic amino acid or dicarboxylate transporter may also contribute to the enhanced glutamate uptake, but system x^-_c is excluded because aspartate but not cystine acts as an inhibitor (Guidotti and Gazzola, 1992).

The up-regulation of Na^+-independent amino acid transport activities cannot be accounted for by *trans*-stimulation effects and is not blocked by either cycloheximide or actinomycin D; it may therefore involve direct effects on pre-existing transport or regulatory proteins, conceivably by influencing their subcellular distribution or phosphorylation status. Indeed protein kinase C (Corey *et al.* 1994) in oocytes has been shown to modulate the activity of heterologously expressed GABA transporters, although this particular kinase does not appear to be capable of directly modulating endogenous glutamine and glutamate transport in oocytes. The specific mechanism by which amino acids may modulate amino acid transport processes in oocytes is not clear. One possibility is that appropriate signalling pathways are directly affected by changes in intracellular concentrations of specific amino acids or by associated changes in ion (e.g. Na^+, Cl^-, Ca^{2+}) concentrations resulting either from cotransport with amino acid or from altered membrane permeability due, for example, to the effect of transient amino-acid-evoked depolarisation of E_m on voltage-gated ion channels. An alternative possibility is that oxidative or osmotic stress (due to the respective metabolic and osmotic effects of increased cell amino acid concentrations) activates membrane transporters, as has been reported to occur in hepatocytes (Häussinger *et al.* 1994). Osmotic stress effects seem unlikely given that oocytes injected with a solution increasing their fluid volume by about 10 % (see Table 5) exhibited unchanged transport activities, but the fact that metabolically important amino acids such as glutamate and alanine can produce modulatory effects in isolation may indicate a role for an increased rate of intra-oocyte amino acid oxidation (and resultant oxidative stress) in initiating transporter regulation. Other cell types appear to up-regulate glutamate uptake by system x^-_c as part of a mechanism to resist oxidative stress (e.g. Van Winkle *et al.* 1992); this mechanism involves synthesis of glutathione. An analogous mechanism may operate in *Xenopus* oocytes (which contain glutathione; Fernández-Checa *et al.* 1993) because the relatively specific increases in transport activity observed in the present study would enhance the supply of substrates (glutamate/glutamine, glycine and cysteine/cystine) necessary for glutathione synthesis. Elevated intra-oocyte amino acid concentrations may also inhibit the breakdown of the proteins (Mortimore *et al.* 1989) involved in stimulating transport activities. The effective down-regulation of dissipative (Na^+-independent) amino acid transport systems during amino acid deprivation will help minimise net amino acid losses to the extracellular environment.

In summary, we have shown that specific Na^+-dependent amino acid transporters (systems $B^{0,+}$, X^-_{ag}) in *Xenopus* oocytes

undergo adaptive regulation and that certain Na^+-independent activities (apparently including system asc) display an 'opposing' regulation, which may help to combat amino-acid-induced oxidative stress by increasing the supply of glutathione precursors. The magnitude of the regulatory responses varies between oocyte batches (as does the efficiency of exogenous mRNA/cRNA expression after injection into oocytes; Colman, 1984), and it would therefore be difficult to utilise them for control of endogenous transport 'noise' during studies of exogenous amino acid transporter expression. The *Xenopus* oocyte appears to possess endogenous signalling mechanisms for selectively modulating the activity of amino acid transport proteins expressed in its surface membranes; this factor should be taken into consideration when oocytes are used as an expression system for structure–function studies of cloned transporter proteins.

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