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CHARACTERIZATION OF VALINE TRANSPORT IN SEA URCHIN EGGS

DENIS ALLEMAND, GUY DE RENZIS, BRIGITTE CIAPA, JEAN-PIERRE GIRARD and PATRICK PAYAN

Laboratoire de Physiologie Cellulaire et Comparée, Université de Nice, Parc Valrose, 06034 Nice Cédex (France)

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In unfertilized eggs, the mechanism of valine uptake can be summarized as follows. It is saturable over the external concentration of valine and insensitive to the presence of external sodium, depletion of cellular energy supplies and intracellular acidosis. The activation energy for the transport reaction (16.3 kcal/mol) is within the range of values reported for active transport of small molecules. In fertilized eggs, the total rate of valine uptake can be divided into two components: (i) a Na^+-insensitive uptake which accounts for about 7% of total absorption as shown by studies in Na^+-free medium seems to possess the same characteristics as in unfertilized eggs, (ii) a Na^+-dependent transport of valine which constitutes the main entry is formed about 5 min after fertilization. It follows Michaelis-Menten kinetics characterized by 15-fold increase in V_{max} with no change in K_m. These two mechanisms have characteristics in common, such as their insensitivity to metabolic energy supply, their energy of activation and their ability to concentrate valine. The relationship between the establishment of the Na^+-dependent valine uptake and the ionic events triggered by fertilization is discussed.

Introduction

Extensive review articles have been published in the past few years covering the various aspects of cell membrane permeability to amino acids (see review by Guidotti et al. [1]). Most of these studies were carried out on somatic cells where several mediating transport systems have been identified on the basis of their kinetic properties and specificity toward the classes of amino acids. Three main systems known as A, ASC and L participate in the transport of neutral amino acids [2]. The main conclusion which can be drawn from these researchs is that, with a few exceptions, the amino acid transport system subject to regulation by environmental conditions, proliferative stimuli and hormonal messages, corresponds to System A or to a Na^+-dependent agency closely ressembling System A.

Since Stephens and Schinske [3] it is known that specialized cells of marine invertebrates may absorb dissolved organic matter from external medium containing extremely low concentration of substrate. In particular, gametes of sea urchin will take up amino acids from the surrounding sea water containing micromolar concentration [4,5]. A most interesting physiological aspect of studying absorption of amino acid by sea urchin egg lies in the fact that Epel [4] showed that two different mechanisms for amino acid transport were involved, a Na^+-independent one in the unfertilized egg and a Na^+-dependent one in the fertilized egg.

Recent studies performed on sea urchin eggs [6,7] have shown that ionic exchanges completely reorganized the intracellular ionic balance at fertilization. First a transitory Na^+-H^+ exchange, lasting 4–5 min, increases by about 30% the Na^+ content of the egg and by 0.3 unit the intracellular pH [8]. Simultaneously, the stimulation of Na^+-K^+ exchange triggers an increase of 10% in K^+ content coupled to a further 50% decrease in Na^+ content. Using radioactive tracers of Na^+, Payan

et al. [6] showed that at fertilization permeability to Na^+ increased to reach an equilibrium just before the first division.

The present study concerns the characteristics of valine absorption in unfertilized and fertilized eggs until the first two cells stage in *Paracentrotus lividus*. Particular attention has been drawn on the effect of sodium gradient in fertilized eggs on the rate of valine uptake. Our data show that the mechanisms of valine uptake by the eggs are similar to the well described transport systems in somatic cells of mammals. It is suggested that in unfertilized eggs valine absorption occurs by means of an L System while fertilization activates a Na^+-dependent system closely ressembling the ASC system.

Materials and Methods

Biological materials. Gametes of *Paracentrotus lividus* were obtained from the bay of Villefranche/Mer and handled by procedures previously described [9]. Eggs concentrations were adjusted to 2% on a volume basis and maintained in suspension at 20 °C by stirring with a three-blade propeller.

Measurement of amino acid transport. Two techniques were used to measure the transport of amino acid: (1) cumulative uptake measurements involved exposure of eggs to the isotope for 2 h and determination of the uptake at set intervals during this incubation. Intracellularly accumulated radioactivity was measured on 2-ml sample of eggs suspension after elimination of external contamination by the rapid filtration technique previously described [6], and disruption of the eggs with a sonicator. The disappearance of radioactivity from the external medium was simultaneously followed. At the indicated times 200 µl samples were removed, rapidly centrifugated in an Eppendorf centrifuge (3200) and the radioactivity of 100 µl of supernatant was counted. From the uptake of radioactivity by the eggs and the disappearance of isotope from the external medium the distribution ratio, R, was determined. R was referred to as the ratio of intracellular to extracellular concentration of the isotope. Water space of the eggs (5.8 µl/mg protein) was assumed to be 70% of the total cellular volume (calculated from a egg diameter of about 90 µm and 20000 eggs per mg of protein [6]).

(2) Pulse experiments. This procedure was used to determine the rate of uptake of amino acid during short-time incubation. Preliminary experiments performed with various times of incubation (from 20 s to 2 min) in 0.5 µM of valine showed that uptake of [^{14}C]valine by fertilized eggs was linear for 1 min 30 s. Therefore, pulse experiments consisted in egg incubation during 1 min in order to determine the initial rate of valine absorption. Measurement was started by adding 1.5 ml of the egg suspension to a test-tube containing 0.5 ml of the incubating medium. The eggs suspension was gently agitated for 1 min. Radioactive influx was stopped by isotopic dilution (addition of 100 µl of unlabelled valine, 2 g/l). After rapid filtration, the eggs were disrupted and protein content and radioactivity were determined. During the incubation, a 100 µl aliquot of egg suspension was sampled and the total radioactivity was counted to determine the specific radioactivity of the external medium.

Incorporation into protein. Unfertilized eggs were preloaded with [^{3}H]valine for 2 h then rinsed by five successive centrifugations with unlabelled sea water. To determine the amount of radioactive incorporation into the trichloroacetic acid-precipitable protein, 0.75 ml of the egg suspension was added to 0.25 ml of a solution containing bovine serum albumin (4 g/l) and valine (4 g/l). Eggs were quickly disrupted and proteins precipited with 20% trichloroacetic acid/2 $g \cdot l^{-1}$ valine. Samples were kept in ice for 20 min, then centrifuged. The pellet was washed twice with 20% trichloroacetic acid/2 $g \cdot l^{-1}$ valine, dissolved with 1 ml 1 M NaOH and counted in 4 ml Aquassure.

Materials. All experiments were performed using artificial sea-water (ASW containing 550 mM NaCl/29 mM $MgSO_4$/27 mM $MgCl_2$/10 mM $NaHCO_3$/10 mM KCL/10 mM $CaCl_2$, pH adjusted to 8.0 with NaOH), in order to avoid any contamination by exogenous amino acids, abolishing trans-effect in this way.

Na^+-free artificial sea water (0Na^+-ASW) was made using choline chloride as substitute for NaCl, and $KHCO_3$ as substitute for $NaHCO_3$. In K^+-free artificial sea water, KCl was replaced by NaCl. Transfer in artificial sea water was performed by

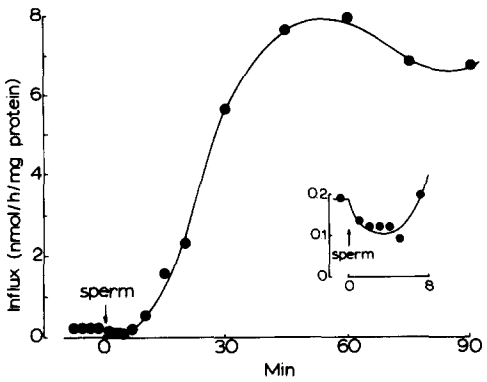

Fig. 1. Time-course of valine uptake during the first 90 min after fertilization for an external concentration of 0.5 μM of valine. Inset: Detailed analysis of valine uptake during 10 min following fertilization.

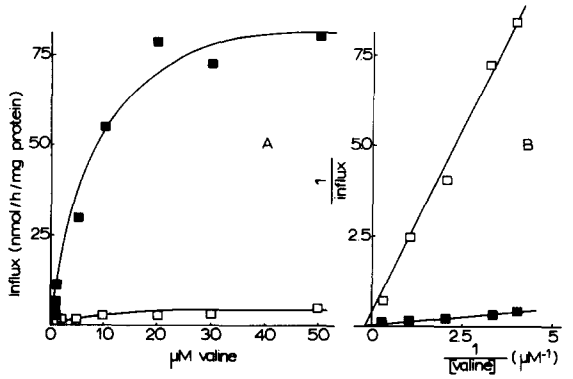

Fig. 2. (A) Concentration dependence of valine uptake in unfertilized eggs (□) and in fertilized eggs (■). (B) Lineweaver-Burk plots of the data from (A). Unfertilized eggs: K_m 8 μM, V_{max} 4 nmol/h/mg protein; fertilized eggs: K_m 6 μM, V_{max} 82 nmol/h/mg protein.

five successive centrifugations.

The labelled precursor was [^{3}H]valine (40 Ci/mmol) and [^{14}C]valine (300 mCi/mmol) obtained from CEA, Saclay. Unlabelled valine was purchased from Sigma. Measurement of sodium and potassium was made by flame photometry (Eppendorf). Proteins are measured by the method of Lowry with the help of a Technicon auto-analyzer.

Results

Time-course of valine uptake at fertilization

Fig. 1 shows that fertilization dramatically increases valine uptake by the eggs. For an external concentration of 0.5 μM of valine, the influx increases from 0.19 ± 0.012 nmol/h per mg protein ($n = 31$) in unfertilized eggs to 5.15 ± 0.350 nmol/h per mg protein ($n = 27$) when measured 45 min after fertilization. At this time valine uptake reaches a plateau about 15 min before the first cleavage. 60 min after fertilization we observed a progressive decrease of valine uptake suggesting that the amino acid entry follows a cyclic variation synchronous with the mitotic cycle as previously described [10]. Indeed, in the present work we studied the characteristics of valine uptake during the steady-state period, i.e., 40 to 50 min after fertilization.

A detailed analysis of valine uptake during 10 min immediately following the addition of sperm shows a net decrease of the amino acid influx (Fig. 1, inset). 2 min after sperm contact, valine uptake is significantly reduced by about 30% compared with the unfertilized level. The rise of valine uptake is really effective 5 min after fertilization. One can postulate that this transient decrease corresponds to the morphological re-arrangement occurring in the plasma membrane of the eggs. In particular, it could be associated with the elevation

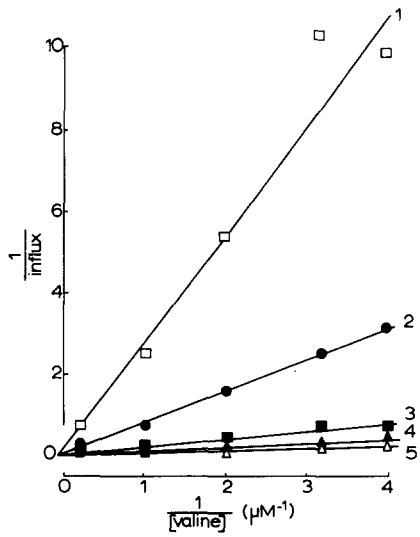

Fig. 3. Lineweaver-Burk plots showing the time-course of kinetic parameters of valine uptake at various times after fertilization. 1, unfertilized; 2, 15 min; 3, 30 min; 4, 45 min; 5, 60 min after fertilization.

of the fertilization membrane which lasts about 2 min [11].

Influx of valine as a function of external concentration of amino acid

Fig. 2A shows the influx of valine measured in the presence of external concentrations ranging from 0.2 to 50 μM in unfertilized and in 40 min post-fertilization eggs. The absorption of valine presents a saturable kinetic with a maximum at about 20 μM both in fertilized and unfertilized eggs. Above 20 μM the valine uptake does not markedly vary. These results support the view that a transport system for amino acid exists in the plasma membrane of the eggs and that no diffusional entry of valine occurs in both unfertilized and fertilized eggs. The Lineweaver-Burk plot (Fig. 2B) of the valine influx can be fitted with a single straight line which allows for calculation of the apparent affinity of the carrier for valine (K_m) and the maximal rate of absorption (V_{max}). 40 min after fertilization the mechanism of valine absorption is characterized by a 15-fold increase in V_{max} while the K_m remains unchanged.

The kinetic characteristics (V_{max} and K_m) of valine absorption were determined at different times following fertilization (Fig. 3). This figure shows that maximal flux is reached 40 min after the sperm contact. This stimulation corresponds to a progressive increase of the capacity of the transporting system without any modification of the affinity of the carrier for valine.

Concentrative capacity of the valine transporting mechanism

In a series of experiments, the disappearance of external radioactivity was simultaneously followed with the appearance of [^{14}C]valine into the eggs. Fig. 4 illustrates the results obtained in unfertilized eggs with various concentrations of valine.

For concentration of 0.5 and 5 μM the internal accumulation of [^{14}C]valine reached a plateau within 2 h (Fig. 4A). This was not due to equilibrium distribution but to complete removal of valine from the external medium (Fig. 4B). In these conditions of low external concentration of valine, the eggs were able to completely absorb the total amount of valine present in the outer medium and to set up a concentrative ratio, R, which increased towards infinity.

At 50 μM and 2 h after addition of valine, the eggs had absorbed half of the initial amount of amino acid. When the concentration of valine was high (500 μM) its disappearance from the external medium was not significant. Its accumulation into the eggs reached a plateau which was due to the saturation of the transporting system (Fig. 4C). In this case the distribution ratio was about 2. Therefore, the value of R depends on both the initial external concentration and the time length of absorption. The amount of valine absorbed by eggs may be slightly overestimated as part of its may be incorporated into proteins. However, we showed that only 8% of the total radioactive valine that entered into the eggs was incorporated into proteins after 2-h incubation. In conclusion, whatever the external concentration of valine (between 0.5 and 500 μM), these results clearly show that a concentration process is involved in unfertilized eggs.

Similar experiments were performed with 40 min post-fertilization eggs in the presence of two different concentrations of valine in sea water. In the presence of 0.5 μM valine, total removal of the amino acid from the external medium was effective within 20 min, at which time the uptake reached a plateau (Fig. 5A). This is comparable to what we obtained with unfertilized eggs. When the concentration of valine was raised to 500 μM the disappearance of the amino acid from the sea water and its accumulation into the eggs did not reach a plateau even after 2 h. The amount of radioactive valine incorporated into protein did not modify the shape of valine absorption (Fig. 5B).

Two hypotheses may be proposed to explain the difference in kinetics of valine transport in unfertilized and fertilized eggs. First, it is conceivable that in fertilized eggs the intracellular pool of valine may be expanded by exogenously added valine [12]. This would lead to an absorption of valine quite linear until the four-cell stage in spite of an increased rate of valine uptake ($\times 20$). The second hypothesis arises from the work of Epel [13] who suggested that like leucine, valine may be metabolized into another compound when eggs are fertilized. This latter hypothesis is not in agreement with our results: a silica gel chro-

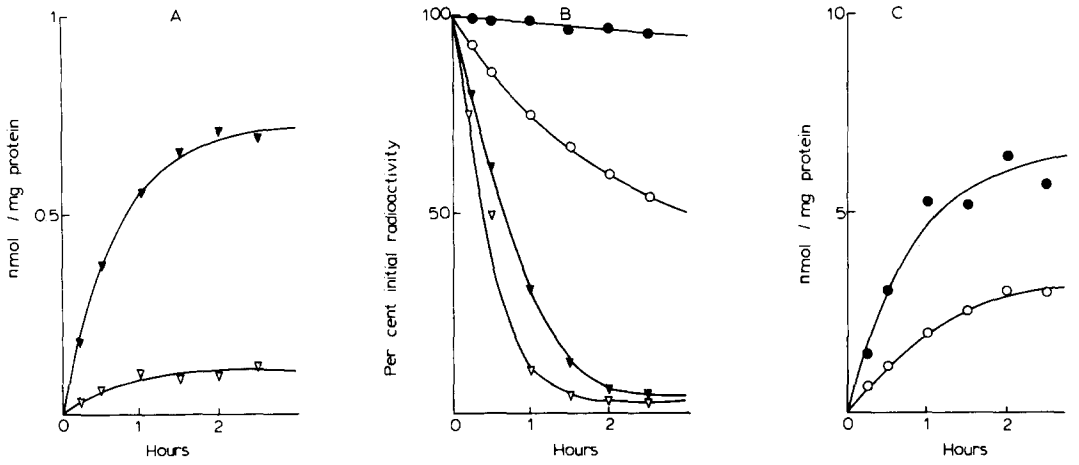

Fig. 4. Internal accumulation (A and C) and corresponding external disappearance (B) of [^{14}C]valine at various concentrations of valine in unfertilized eggs. Eggs were transferred in medium containing 0.5 μM (▽), 5 μM (▼), 50 μM (○) and 500 μM (●) [^{14}C]valine. Radioactivity was followed simultaneously in the medium (B) and in the eggs (A and C).

matography of the trichloroacetic acid-soluble fraction of eggs preloaded with [^{14}C]valine did not show any conversion of valine into substances other than proteins. Hence, the fact that fertilization enhances the intracellular pool of valine in the presence of high external valine concentration remains an attractive possibility. In any case, it is obvious that a concentrating process is also involved in the mechanism of valine absorption in fertilized eggs. However, although it seems difficult to calculate the concentration ratio as long as a steady state is not reached, the present results demonstrate that fertilized eggs accumulate much more than unfertilized eggs [14].

Sodium dependence of amino acid transport

In most cells studied, thus for substrates such as glucose, lactate, amino acids and chloride are cotransported with sodium. As sea urchin eggs live in a NaCl-rich medium, we carried out a study of the relationships which may exist between the external sodium concentration and valine uptake.

Fig. 6 shows the uptake of valine as a function of its external concentration in unfertilized and 40 min post-fertilized eggs rapidly transferred to Na^+-free artificial sea water. The absence of sodium in the external medium did not change the kinetic parameters of valine absorption by unfertilized eggs as shown by the Lineweaver-Burk plot (compare Figs. 2B and 6B); in fertilized eggs maximal absorption of valine markedly reduced to the same level as in unfertilized eggs without any modification of the apparent affinity (Figs. 6 and 2). These results suggest that the transport of valine in fertilized eggs is mediated by two mechanisms: (1) a Na^+-dependent one, which accounts for 95% of valine uptake and (2) a Na^+-independent one, which possesses the same kinetic char-

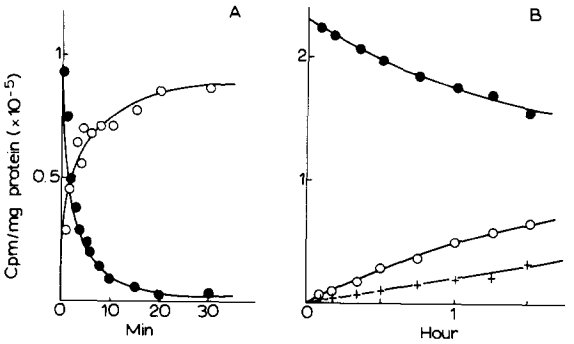

Fig. 5. Uptake of [^{14}C]valine simultaneously measured by external disapperance (●) and internal accumulation (○) in eggs 50 min post fertilization at external valine concentrations of 0.5 μM (A) and 500 μM (B). Changes in the trichloroacetic acid-insoluble fraction are also shown (+) for 500 μM valine.

acteristics as in unfertilized eggs. It is noteworthy that fertilization triggers the development of a Na^+-dependent transporting system as in certain somatic cells, where hormones or growth factors stimulates an 'A system' characterized by its Na^+-dependency.

These results prompted us to study further the valine absorption at various external sodium concentrations ranging from 0 to 550 mM. Fertilized eggs were rapidly transferred to media containing different Na^+ concentrations. The experiments have been carried out in the presence of 0.5 μM and 2.5 μM valine. For both valine concentrations the amino acid uptake plotted as a function of the external sodium concentration followed a sigmoid curve (Fig. 7). A detailed analysis of the initial part of the curve indicates that the stimulation of valine influx is effective from about 15 to 20 mequivalents of external sodium per litre.

In order to test the effect of substitution of Na^+ by Li^+, fertilized eggs were transferred into Na^+-free artificial sea water and resuspended in 100 mM NaCl artificial sea water or 100 mM LiCl artificial sea water. The influx of valine measured in these media was 1.17 and 0.17 mmol/h per mg protein, respectively. In the presence of LiCl, valine influx is thus reduced to the same level as in fertilized eggs transferred into Na^+-free artificial sea water or in unfertilized eggs. This clearly demonstrates the strickly Na^+-dependence of the valine transporting mechanism in fertilized eggs.

Competition experiments

The interaction between structural analogues has been often used to discriminate between several transport systems in a given cell type [2]. Table I shows the inhibition of the initial rate of valine uptake by different neutral amino acid. In unfertilized eggs only leucine and phenylalanine widely compete with valine, suggesting that a L system is involved. In fertilized eggs, the most stricking result is the absence of competition with the analog *N*-methylaminoisobutyric acid which is considered as the system A specific substrate. On the other hand, amino acids preferentially transported by ASC or L systems widely inhibit the valine influx.

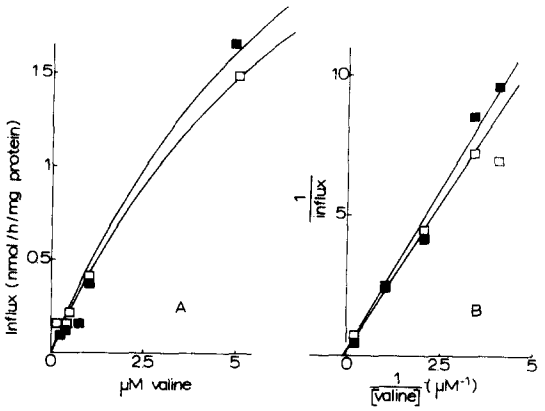

Fig. 6. (A) Concentration dependence of valine uptake in unfertilized (□) and fertilized [■] eggs rapidly transferred in $0Na^+$-ASW (Na^+-free artificial sea water). (B) Lineweaver-Burk plot of the data from (A). Unfertilized eggs: K_m 9 μM, V_{max} 4 nmol/h/mg protein; fertilized eggs: K_m 6 μM, V_{max} 3 nmol/h/mg protein.

Effect of temperature on valine influx

Valine absorption has been studied in unfertilized and fertilized eggs at five different temperatures. Eggs were transferred from room temperature to appropriate temperature and equilibrated for 3 min. The influx of valine was determinated

TABLE I

EFFECT OF DIFFERENT NEUTRAL AMINO ACIDS ON VALINE UPTAKE

Results are expressed as percent of control. Test amino acids were present at a concentration 50-times higher than that of valine, i.e. 1 mM. In brackets, the number of determinations. NME-AIB, *N*-methylaminoisobutyric acid; AIB, aminoisobutyric acid.

	NMe-AIB	AIB	Gly	Ala	Ser	Leu	Phe
Unfertilized	100	77	90	59	77	21	19
	(2)	(2)	(2)	(6)	(2)	(6)	(4)
Fertilized	100	73	31	20	26	10	26
	(6)	(4)	(4)	(6)	(4)	(8)	(2)

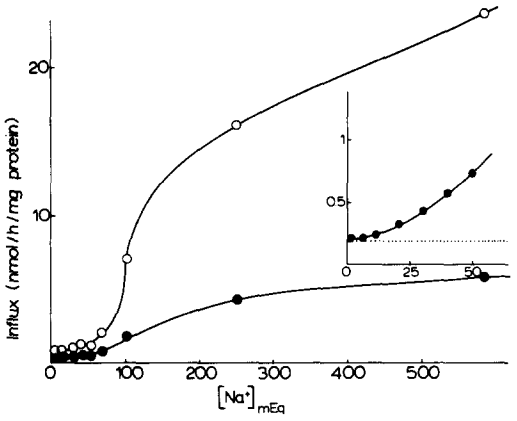

Fig. 7. Influence of external sodium on valine uptake in fertilized eggs at two concentrations of valine. O——O, 2.5 μM; ●——●, 0.5 μM. Inset: Detailed analysis of the initial part of the curve for $[Val]_{ext} = 0.5$ μM.

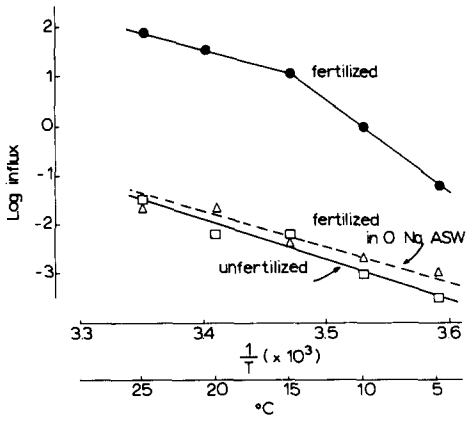

Fig. 8. Influence of temperature on valine transport: Arrhenius plot. 0Na-ASW, Na^+-free artificial sea water.

by the pulse technique, in the presence of 0.5 μM valine.

For unfertilized eggs, the Arrhenius plot is linear while for fertilized eggs the temperature dependence of valine uptake is characterized by a break at 15 °C (Fig. 8). The activation energy calculated from the slope of the curves was of the same order of magnitude in unfertilized eggs between 5 and 25 °C (16.3 kcal/mol) and in fertilized eggs between 15 and 25 °C (14.2 kcal/mol). These values are within the range usually found for a carrier-mediated active transport process [15] and typical enzyme-catalysed reactions [16]. Between 15 and 5 °C in fertilized eggs the energetic barrier is 2-fold higher (37 kcal/mol), a value very close to that reported for transport systems which carry out facilitated diffusion. In fertilized eggs, the composite Arrhenius plot suggests that lowering of the temperature induces a fall in lipid solubility which leads to a decrease in transporting activity of the membrane.

In fertilized eggs transferred to Na^+-free artificial sea water the relationship between temperature and valine uptake was linear between 5 and 25 °C and led to a calculated activation energy of 12.3 kcal/mol. This value is close to that found in unfertilized eggs in Na^+-free artificial sea water (15.3 kcal/mol, Fig. 8). This is in agreement with the existence of a Na^+-independent transport of valine in fertilized eggs comparable to that characterized in unfertilized eggs.

Effect of metabolic inhibitor on valine influx

The dependence of the amino acid uptake on cellular supply of energy was studied using cyanide (1 mM KCN). Cyanide did not modify the uptake of valine by unfertilized eggs (control: 0.21 ± 0.031; KCN: 0.22 ± 0.036 nmol/h per mg protein, $n = 5$) even when eggs were maintained during 2 h in the presence of the inhibitor.

Addition of 1 mM KCN 30 min after fertilization did not significantly modify the valine uptake measured 10 min later (control: 2.90 ± 0.282; KCN: 2.50 ± 0.367 nmol/h per mg protein, $n = 4$).

If KCN was added to eggs 1 min before sperm, the abrupt fall in valine absorption occurring immediately after sperm contact persisted but was not followed by the sharp increase of valine influx noted in the control eggs. 40 min after fertilization in the presence of cyanide, valine uptake was stimulated only by a factor 2, instead of 40 in controls (Fig. 9).

These data are consistent with the interpretation that metabolic energy is necessary for the enhancement of the amino acid uptake at fertilization but is not required further for the maintenance of such mechanism.

Effect of intracellular acidosis

When unfertilized eggs are incubated during 2 h in Na^+-free artificial sea water the intracellular pH decreases from 7.4 to 7.10 [9]. Under similar conditions we found the rate of valine uptake by unfertilized eggs in 0.5 μM valine to be not significantly different from the control value (0.247 ±

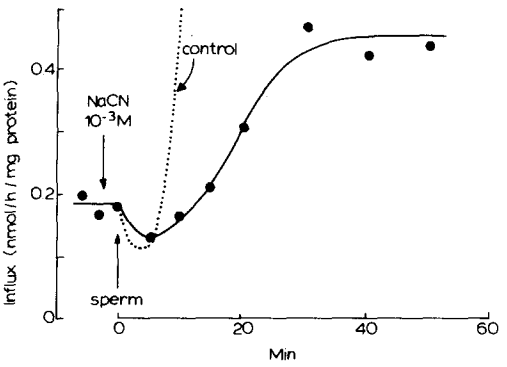

Fig. 9. Effect of the addition of cyanide (10^{-3} M) 1 min before fertilization on the establishment of the transport of valine. A control experiment is shown by the dotted line.

0.027 nmol/h per mg protein, $n = 5$). This results suggest that internal acidosis has no effect on the rate of valine absorption in unfertilized eggs.

Discussion

The results reported in this paper confirm and largely extend earlier data on the activation and the properties of amino acid transport in sea urchin embryos [17,4].

We propose that unfertilized eggs accumulate valine by means of a carrier-mediated system which is not dependent upon the external Na^+ nor cellular energy and is unaffected by intracellular acidosis. The activation energy of this uptake is characteristic of an active transport. Fertilization superposes on the mechanism described above a considerable Na^+-dependent uptake of valine which accumulates amino acid into eggs. This sperm-provoked entry of valine is insensitive to cyanide but needs cellular energy for its initiation at fertilization.

Energizing of valine transport in sea urchin eggs

In unfertilized eggs the uptake of valine appears to be highly concentrative, as it represents amino acid absorption from an external concentration of nanomolar range. This process is not driven by the chemical gradient of Na^+, thus raising the question of the energy supply allowing for valine absorption by unfertilized eggs. In preliminary experiments, valine absorption was not modified when unfertilized eggs were transferred to artificial sea water in which chloride, bicarbonate and magnesium were substituted (655 mM sodium gluconate/10 mM $CaNO_3$, $p_{osm} = 1100$ mosM (pH 8.0)). The lack of effect of mineral ions seems to be correlated with very low permeability of unfertilized eggs to these ions (Whitaker and Steinhardt [18]). According to Jaffe and Robinson [19] the plasmic membrane of unfertilized eggs behaves as a K^+ electrode. In other preliminary experiments, we found that neither hyperpolarization (0.5 mequiv./l of K^+ in external medium) nor depolarization (100 mequiv./l of K^+) changed the influx of valine suggesting that the membrane potential is not involved in amino acid transport. At the present time, no hypothesis may be proposed concerning the nature of the driving force responsible for valine absorption by unfertilized eggs.

In 40 min post-fertilized eggs the Na^+-dependent mechanism of valine uptake initiated by fertilization is fully effective. At this time, when the external Na^+ varies from 550 to 0 mequiv./l the valine influx plotted against the Na^+ concentration is characterized by a sigmoid curve (Fig. 7). This curve shows that the valine absorption is significantly stimulated by an external Na^+ of about 20 mequiv./l. Intracellular Na^+ content measured 40 min post-fertilization equals 0.18 ± 0.01 μmol/mg protein ($n = 4$). This value corresponds to an intracellular Na^+ activity of about 15 mequiv./l assuming a water space of 70% and an activity coefficient of 0.5 [20]. We may conclude that under these conditions the valine influx seems to be stimulated when the chemical gradient for sodium between external and internal medium becomes positive. The sigmoid shape suggests possible cooperativity of Na^+ in valine transport. Binding of Na^+ to sites on plasma membrane would be required for activation of the mechanism directly involved in the absorption of valine. For external sodium concentration of up to 30–50 mequiv./l the Na^+-dependence of valine uptake follows a saturable kinetics with an apparent K_m of 250 mequivalents. Thus, in physiological conditions, i.e., in the presence of 550 mequiv./l of external Na^+, the transporting mechanism operates under optimal conditions.

Initiation of the Na^+-dependent transport of valine: role of intracellular pH

As previously stated by Epel [4] the appearance of Na^+-dependent transport would be a 'late' fertilization response. This point is confirmed by the detailed analysis of valine influx following fertilization which showed an abrupt increase from 6 to 7 min after sperm contact (Fig. 1). This coincides with the end of the increased intracellular pH observed at fertilization [8]. However, maintenance of an intracellular alkalosis does not seem to be a necessary condition to initiate the development of a Na^+-sensitive valine uptake. Unfertilized eggs transferred in Na^+-free artificial sea water develop an acidosis, external protons being exchanged against cellular Na^+ [9]. When these eggs are fertilized in 10 mM Na^+ artificial sea water the initial alkalosis triggered by sperm is only transitory and the internal pH is reestablished to the unfertilized level within 20 min [9]. Surprisingly, we noted that in these acidotic eggs, the Na^+-dependent valine uptake was potentially initiated by fertilization, since 40 min later eggs replaced in normal sea water absorbed valine at rate similar to that in the controls (5.25 ± 0.55 nmol/h per mg protein ($n = 3$) in 0.5 μM valine).

It is therefore conceivable that sperm induced alkalinization is a prerequisite step to establish the Na^+-dependent valine uptake but is not necessary for the maintenance of such a mechanism.

Development of a Na^+-dependent valine transport in relation to increased ionic permeabilities at fertilization

Using a radioisotope, Payan et al. [6] have recently shown that Na^+ permeability was strongly increased about 5 min after fertilization to reach a maximum within 30 min. Strikingly we noted that the increase of Na^+ permeability and of valine uptake followed the same relation to time. Furthermore, both show cyclic variations synchronous with the cell cycle [10,6]. However, in sea urchin eggs, even when the influx of valine is maximal (0.05 μmol/h per mg protein), it does not exceed 5% of the Na^+ influx (1 μequiv./h per mg protein). This is an important difference with other cell types which transport amino acids and sodium at similar rates.

Comparison of these results on increased Na^+ and amino acid permeability at fertilization with those of Steinhardt et al. [21] on the formation of K^+-sensitive membrane potential indicate that these phenomena may be closely linked.

Comparison with somatic cells

The results presented in this paper suggest that mechanisms of amino acid transport in sea urchin embryos possess some similarities to the well-described transport systems present in mammalian cells (see review by Guidotti et al. [1], Shotwell et al. [22]). In unfertilized eggs, the mechanism of valine transport resembles the L system which operates in most non-stimulated somatic cells. At fertilization, a Na^+-sensitive mechanism of valine absorption is initiated which seems to share common characteristics with the ASC system: intolerance to *N*-methylaminoisobutyric acid, Na^+-dependency, competition by substrates such as serine and alanine and impossibility to substitute Li^+ for Na^+ [24]. It is worth noting that in all types of cell studied, when the amino acid transport system is subject to regulation (developmental transition, hormonal messages), this system corresponds to a Na^+-dependent transport closely ressembling system A or ASC.

However, we were not able to demonstrate that Na^+ is cotransported with valine as the maximal rate of valine absorption does not exceed more than 5% of total Na^+ influx. Furthermore, significant differences exist between somatic cells and gametes. In eggs, the Na^+-dependent system seems to be insensitive to metabolic inhibitors while it depends on cellular energy in mammalian cells. From competition experiments we observed that leucine (system L specific substrate) inhibits valine uptake to a greater extend than serine (system ASC specific substrate). Furthermore, the apparent affinity is much smaller by a factor of 1000 in eggs than in mammalian cells. This high affinity is in agreement with that found for methionine transport in sea urchin sperm [5]. The latter point might be correlated with their respective environmental conditions: valine concentration in sea water is very small (50 nM; [25]) in comparison to mammalian plasma (0.30 mM [26]).

In conclusion, although the present work demonstrated surprising similarities in amino acid transport between invertebrate gametes and mam-

malian somatic cells, it stressed important differences which did not permit to strictly define amino acid transport mechanisms in eggs as was established by L and ASC systems in mammalian cells.

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