Biochimica et Biophysica Acta, 595 (1980) 121—125

BBA 78594

ALANINE INFLUX ACROSS SEROSAL BORDER OF *TESTUDO GRAECA* INTESTINE

CAMILLE F. NASSAR, RAJA N. KHURI and JEAN-JACQUES HAJJAR

Department of Physiology, American University of Beirut, Beirut (Lebanon), and Division of Gastroenterology, University of Connecticut Health Center, Veterans Administration Medical Center, Newington, CT 06111 (U.S.A.)

(Received April 25th, 1979)

Key words: Alanine transport; Competitive inhibition; (Intestinal membrane)

Summary

The influx of alanine across the serosal membrane of *Testudo graeca* intestinal cells with preserved epithelial orientation was examined. Our results suggest that:

1. The mechanism of alanine influx across the serosal membrane of turtle intestinal cells is a carrier-mediated process that has the characteristics of facilitated diffusion.

2. Alanine influx mechanism is independent of intra- and extra-cellular changes in Na^+ and K^+ concentrations, and is not altered by reversal of Na^+ and K^+ gradients across the serosal membrane.

3. In Na^+-free media the mechanism of transport of alanine at the mucosal membrane has the same pattern of competitive inhibition by amino acids as the serosal.

Introduction

A functional polarity of the intestinal epithelial cell is postulated at characterizing the mechanism of absorption of amino acids. The mucosal and serosal cell barriers are assumed to be dissimilar since the epithelial cell accumulates amino acids while it actively translocates them transcellularly. This concept is supported by studies of the properties of the individual cell barriers. The brush border membrane being readily accessible to direct testing was studied in details [1,2]. The function of the serosal barrier is not, however, as clearly understood, because its properties cannot be tested by direct techniques as those employed for the mucosal. The reversed flux across the serosal barrier (the flux from the serosal solution into the cell) cannot be as readily assessed in

the intact epithelium. The only information about the serosal barrier is derived from studies of amino acid influx across isolated intestinal cells [3] or across their plasma membrane vesicles [4,5]. However, under the conditions of these studies, the isolated cells or their membrane vesicles lose their spatial epithelial orientation and their transepithelial transport function may thus become indistinct. The present study involves an attempt to obtain information on the influx of alanine across the serosal membrane of intestinal cells with preserved epithelial orientation.

Materials and Methods

The intestine of the fresh water turtle (*Testudo graeca*) was utilized. The turtles were pithed, their ventral shells removed and segments of their proximal intestine resected and stripped of their muscle coats by a method similar to that described by Parsons and Patterson [6]. The remaining mucosal sheet was then mounted in a Lucite chamber similar to that described by Schultz et al. [2] in such a way that a fixed area of its serosal surface was exposed to a compartment into which incubation and test solutions could be exchanged rapidly. The serosal solution was kept at room temperature (24—26° C) and was stirred by bubbling compressed air. The intestinal basolateral surface was incubated in Ringer solution (120 mM NaCl, 1.9 mM KCl, 0.45 mM $CaCl_2$, 1 mM K_2HPO_4, 0.65 mM KH_2PO_4) for a period of 30 min following which the solution was replaced by a test solution containing an appropriate concentration of L-alanine (Sigman Chemical Co., St. Louis, MO), L-[^{3}H]alanine and [^{14}C]inulin (Radiochemical Centre, Amersham, U.K.). This solution was kept in contact with the serosal surface for a period of 40—60 s. It was then withdrawn and replaced by ice-cold mannitol solution. The tissue was then removed and extracted in 0.1 N HNO_3 for 4—5 h and counted in a liquid scintillation counter for its ^{14}C and ^{3}H contents. Amino acid influx was calculated from the alanine content in tissue extracts after correction for the inulin space.

In some experiments alanine influx across the mucosal brush border membrane was measured by a similar procedure as that described above except that the mucosal surface rather than the serosal was exposed to the test solution. In other experiments where Na^+-free Ringer solution was used, NaCl was substituted by choline chloride or KCl.

Results

The method described above, assumes that the serosal surface of the stripped intestine is largely made up of the basolateral membranes of the intestinal absorptive cells. To validate this assumption and to show that the adhering submucosal cells do not significantly interfere with alanine influx across this cell barrier, the following preliminary experiments were carried out. Tissue scrapings weighing up to 30—40 mg were incubated in the presence of a 5 mM solution of alanine, [^{3}H]alanine and [^{14}C]inulin. The submucosal cell alanine concentration was estimated from the content of [^{3}H]alanine in the tissues after subtracting the ^{3}H in the extracellular space as measured by inulin. Cell alanine concentration in five determinations was 4.6 ± 0.3 mM, not different

from the alanine concentration in the incubation medium, while in strips of mucosal cells it was 34.6 ± 3.4 mM. This finding suggests that the submucosal cells do not actively transport amino acids and their uptake of amino acid during the influx measurements cannot be therefore excessive. Also the stripped intestine was mounted in the chamber with the mucosal surface lying on a filter paper that was presoaked with a phenol red solution (1 mg/ml). The serosal surface was then exposed for 2 min to a Ringer solution containing 0.08% hyaluronidase (Sigma Chemical Co.). This enzyme was then washed off three times with Ringer solution and the Lucite chamber was placed in an ultrasonic cleaner (Varian, CA) for 4 min during which the serosal surface was flushed with fresh Ringer solution. Such treatment reduced the number of submucosal cells (about 60% as judged by light microscopy) without disrupting the alignment of the epithelial cells, as judged by spectrophotometric determination of phenol red in the serosal solutions. When phenol red was detected in the washout or test solutions the tissue was discarded. Influx was measured only into non-disrupted tissues and compared with influx into tissues that were not treated with the enzyme. In the presence of a 5 mM alanine concentration, the mean influx into seven enzyme-treated tissues was estimated to be 0.54 ± 0.08 μequiv./h per cm^2, while influx into an equal number of control untreated tissues was 0.58 ± 0.06 μequiv./h per cm^2. These results suggest that the intervening submucosal cells do not pose a major complication to amino acid influx measurements across the serosal border.

In another set of preliminary experiments, alanine uptake across the serosal border was measured as a function of time of exposure to the test solution. Our results show that during the first 70 s there is a negligible loss of tracer from the tissue and therefore, the uptake rate is very likely a true measure of influx.

The effect of an increasing alanine concentration in the serosal solution on alanine influx across the serosal border is shown in Fig. 1. A hyperbolic relationship is observed which can be described by Michaelis-Menten kinetics. A Lineweaver-Burk plot of these results indicates that the points determined can be described by a straight line with a non-zero intercept. The calculated average maximal influx (J_{max}) was 5.6 μmol/h per cm^2 and apparent Michaelis constant (K_t) 42 mM. These characteristics are quite similar to those observed for the reversed flux (efflux) of alanine across this membrane in the same animal species [7]. The J_{max} for the efflux of alanine from the cell to the serosal solution was 6.1 μmol/h per cm^2 and the K_t 43 mM. These values indicate that the inward and outward fluxes of alanine at the basolateral membrane have similar kinetic characteristics, and both mechanisms are saturable mediated processes.

Alanine influx does not seem to be influenced by the concentration gradients of alkali metal ions across the serosal membrane. In Table I influx from a 5 or 20 mM concentration of alanine in the test solution was measured under conditions where intracellular and extracellular Na^+ and K^+ concentrations were varied. In conditions B and C, (Table I) Na^+ substitution with choline or K^+ in the test solution does not alter alanine influx which remains almost equal to the influx measured under control conditions in A. In conditions D, E and F, the tissues were preincubated in a 10^{-4} M concentration of ouabain. This inhibitor raises the intestinal cell Na^+ concentration to levels that

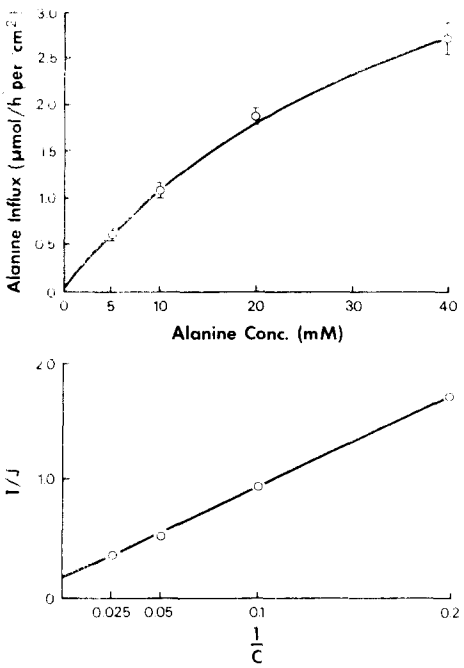

Fig. 1. Effect of alanine concentration on alanine influx across the serosal border. Each point is the mean of ten determinations. The bars represent ±1 S.E. of the mean. The curve is drawn using the characteristics: J_{max} = 5.6 μmol/h per cm^2 and K_t = 42 mM. A reciprocal plot of the data is shown in the lower figure with the line drawn by the least-square method.

are about equal to those in the incubation medium and lowers cell K^+ concentration by about 50% [7]. Under condition D (an outwardly directed Na^+ gradient), condition E (an absent Na^+ gradient) or condition F (an inwardly directed K^+ gradient), alanine influx across the serosal border was not different from control. The results show that the alanine influx process is independent of intra- and extra-cellular changes in Na^+ or K^+ concentrations, and is not altered by reversal of the Na^+ or K^+ gradients that normally exist across the serosal membrane.

TABLE I

THE EFFECT OF ALKALI METAL ION GRADIENTS ON ALANINE INFLUX ACROSS THE SEROSAL SURFACE OF TURTLE INTESTINE

Results are given as mean + S.E. of eight determinations.

Preincubation	Test	Influx (μmol/h per cm^2)	
		5 mM	20 mM
A Ringer	Ringer	0.58 ± 0.06	1.78 ± 0.13
B Ringer	Choline Ringer	0.59 ± 0.04	1.91 ± 0.15
C Ringer	K^+ Ringer	0.62 ± 0.05	
D Ringer + ouabain	Choline Ringer	0.60 ± 0.04	1.82 ± 0.14
E Ringer + ouabain	Ringer + ouabain	0.63 ± 0.08	2.20 ± 0.32
F Ringer + ouabain	K^+ Ringer	0.54 ± 0.05	1.72 ± 0.12

Alanine influx is competitively inhibited by the presence of other amino acids in the test medium. Glycine, leucine, and methionine reduced alanine influx by 35, 52 and 55%, respectively, while lysine and valine caused no significant inhibition of this influx. This pattern of inhibition was qualitatively similar to that observed when alanine influx across the mucosal membrane was examined in a Na^+-free medium. Glycine, leucine and methionine inhibited alanine influx by 45, 63 and 72%, respectively, while lysine and valine caused minimal or no inhibition. These findings suggest a similarity between the specificity of the serosal amino acid transport system and that of the Na^+-independent mucosal system.

Discussion

Alanine influx across the basolateral membrane of the muscle-stripped turtle intestine may be measured, if two assumptions are made. First, the extracellular space between the cells and the serosal solution is not exceptionally large. This is supported by alanine washout studies from the cell to the serosal solution [7]. Second, the influx into the intervening submucosal cells that remain attached to the contraluminal surface of the stripped epithelium, is small. The results reported in this manuscript show that the submucosal cells do not accumulate alanine, and therefore do not seem to possess an active accumulative alanine transport mechanism. These observations suggest that the submucosal cells do not interfere with alanine influx across the serosal surface of the stripped intestine.

Results of the experiments on alanine influx as a function of alanine concentration in the serosal solution show that the entry of this amino acid into the cell across the serosal membrane is through a mediated saturable process. These data confirm similar observations made in isolated basolateral membrane vesicles [5].

Like efflux across this membrane [7], influx is Na^+-independent and not influenced by changes in the Na^+ or K^+ concentration both in bathing medium and in the cells. This agrees with studies of amino acid transport across basolateral membrane vesicles which were also not affected by alkali metal cation gradients [4,5].

The serosal alanine influx was inhibited by some amino acids but not by others. This also suggests that the influx process is carrier mediated and displays certain specificities. The similarity of specificity of the brush border and serosal membranes suggest a similarity between the mucosal and serosal alanine carrier sites.

Acknowledgements

This investigation was supported by the American University of Beirut Research Funds and the Medical Research Service of the Veterans Administration.

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