Biochimica et Biophysica Acta, 597 (1980) 411—417

BBA 78683

TRANSPORT OF SUGARS AND AMINO ACIDS ACROSS GUINEA PIG SMALL INTESTINE

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(Received June 29th, 1979)
(Revised manuscript received September 25th, 1979)

Key words: Intestinal absorption; Sugar transport; Amino acid transport; Galactose; Na^+ gradient hypothesis; (Guinea pig)

Summary

Using guinea pig small intestine for measurements of influx across the brush border membrane (J_{mc}), unidirectional transmural fluxes (J_{ms},J_{sm}), and steady-state epithelial uptake $[A]_c$, the characteristics of the mutual inhibition were examined. The mutual inhibition of J_{mc} was in the range of 9—16%, and appeared to be sodium dependent, J_{ms} and $[A]_c$ were inhibited by at least 40%, and J_{sm} was unaffected. All the results can be explained in terms of the rheogenic version of the sodium gradient hypothesis. Whereas only the mutual inhibition of J_{mc} can also be explained in terms of allosteric interactions between the binding sites for sugars and amino acids.

Introduction

Since Newey and Smyth [1] described the inhibitory effect of galactose on amino acid transport by the rat small intestine the question of mutual inhibition between sugars and amino acids has been often and occasionally heatedly debated [2]. The differing views can be categorized as attempts to interpret the results either in terms of the sodium gradient hypothesis [3—8] or as expressions of allosteric interactions between separate binding sites for sugars and amino acids [9,10].

The use of different methods and species has contributed significantly to the apparent disagreement, and so has the absence of any attempt to interpret the clearly, partially, competitive nature [9] of the interactions in terms of the sodium gradient hypothesis. The aims of the present study were therefore, firstly to use, on the guinea-pig, methods which heretofore had been combined

only in studies of the rabbit [3] and rat [6] small intestine, secondly to examine some aspects of the role of sodium in the sugar-amino acid interactions.

Materials and Methods

Female albino guinea-pigs (body weight, 300—400 g) were used. Prior to the experiments the animals were maintained with free access to food and water. The animals were anaesthetized with intraperitoneal pentobarbital. Hereafter the abdomen was opened, the entire small intestine removed, and the animal killed by transsection of the heart. The mid 20 cm of the total small intestine were used for all types of experiments. These were performed at 37 °C in a phosphate buffer with 140 mM Na^+, 8 mM K^+, 2.6 mM Ca^{2+}, 1 mM Mg^{2+}, 140 mM Cl^-, 1 mM SO_4^{2-}, and 8 mM $(H_2PO_4^- + HPO_4^{2-})$. Glucose was not used in the media, where sugars and amino acids were present as stated. The 3 mM and 6 mM Na^+ media were prepared by substituting choline chloride for NaCl in equimolar amounts. The incubation media were aerated and stirred with 100% O_2. The chemicals were of analytical grade, and sugars and amino acids were of the highest, commercially available purity. ^{22}Na, and ^{14}C-labelled tryptophan and galactose were obtained from The Radiochemical Centre, Amersham, U.K.), ^3H-labelled poly(ethyleneglycol) (M_n 4000) was purchased from New England Nuclear Co.

Unidirectional influx across the brush border membrane (J_{mc}), unidirectional transmural fluxes (J_{ms} and J_{sm}), and steady-state mucosal uptake of galactose ($[Gal]_c$) and tryptophan ($[Try]_c$) were measured as previously described except that [^{3}H]poly(ethyleneglycol) 4000 was used as extracellular marker instead of [^{3}H]methoxyinulin [11].

Fluxes of galactose and tryptophan were calculated on the basis of measured fluxes of radioactive tracers. The radioactivity of appropriate samples was analyzed in a TriCarb fluid scintillation spectrometer, using Instagel as scintillation fluid.

The results are stated as mean values ± S.E., with the number of observations in parentheses. P values less than 0.05 according to Student's t-test are taken as indication of statistical significance.

The following equations [12] are used to calculate the unidirectional fluxes, J_{cs} and J_{sc} across the basolateral membrane and efflux (J_{mc}) across the brush border membrane:

$$J_{net} = J_{ms} - J_{sm} = J_{mc} - J_{cm} = J_{cs} - J_{sc} \quad (1)$$

$$J_{ms} = (J_{mc} \cdot J_{sc})/(J_{cm} + J_{cs}) \quad (2)$$

These equations are derived for a three compartment system in steady state. Eqn. 1 is valid for the preparations; but the use of Eqn. 2 is based on the assumption that the non-epithelial tissues constitute a well-stirred extension of the fluid bathing the serosal side of the preparation, that transport takes place across an epithelium made up by a homogeneous cell population, and that passage through paracellular shunts can be neglected.

Experiments and Results

Effects of galactose on tryptophan transport

From each animal four preparations were made for transmural flux measurements. Hereby paired measurements were made of J_{ms}^{Try} and J_{sm}^{Try} both with and without galactose. In these experiments, 1 mM tryptophan and 20 mM galactose were used. The results (Table I) demonstrate a highly significant, 41%, inhibition of J_{ms}^{Try}, but no effect on J_{sm}^{Try}.

For J_{mc} measurements each animal provided eight preparations. These were used for paired measurements of J_{mc}^{Try} at 1 mM tryptophan and 1 mM tryptophan + 20 mM galactose; 1 mM tryptophan and 1 mM tryptophan + 30 mM galactose; 5 mM tryptophan and 5 mM tryptophan + 20 mM galactose; or 5 mM tryptophan and 5 mM tryptophan + 20 mM methionine. The results (Table II) show that at 1 and 5 mM tryptophan 20 mM galactose causes significant inhibitions of 15 and 16%, respectively. In the experiments with 1 mM tryptophan and 30 mM galactose the control flux was 1.18 ± 0.06 μmol/cm² per h (n = 7) and the inhibited influx 1.03 ± 0.06 μmol/cm² per h (n = 8). Based on the control data on J_{mc}^{Try} at 1 and 5 mM tryptophan estimates are reached of a J_{max}^{Try} of 11.5 μmol/cm² per h and a K_t of 6.2 mM. Using this K_t for tryptophan the inhibitory effect of methionine on J_{mc}^{Try} indicates a K_i of 2.1 mM. These kinetic values are very close to those of the rat mid small intestine [13].

The steady-state uptake of tryptophan was measured in paired experiments at 1 mM tryptophan and 1 mM tryptophan + 20 mM galactose of 40—80 min incubation. The tissue concentration was constant throughout this period. The data (Table I) demonstrated a 77% inhibition of $[Try]_c$.

Effects of methionine on galactose transport

Using the experimental design described above J_{ms}^{Gal} and J_{sm}^{Gal} were measured at 1 mM galactose and at 1 mM galactose + 20 mM methionine. Methionine (Table I) significantly (40%) inhibited J_{ms}^{Gal}, but did not significantly affect J_{sm}^{Gal}.

J_{mc}^{Gal} was measured in paired experiments at 1 mM and 10 mM galactose with and without 20 mM methionine. At 1 mM galactose (Table I) a 9% inhibition did not reach statistical significance, but at 10 mM, a 13% inhibition was statistically significant.

In paired experiments at 1 mM galactose, 20 mM methionine reduced $[Gal]_c$ by 50%. In spite of this reduction J_{cm}^{Gal} was increased by 17%, whereas the reduction of J_{cs} corresponded well to the reduction of $[Gal]_c$.

Sodium dependence of the heteroinhibition of influx across the brush border membrane

After 30 min of preincubation at 6, respectively, 3 mM Na^+ with several renewals of the preincubation media J_{mc}^{Gal} was measured at 6 or 3 mM Na^+ in paired experiments at 10 mM galactose with or without 20 mM methionine. In two series at 6 mM Na^+ methionine did, but not statistically significantly, inhibit J_{mc}^{Gal}. When the data of these two series are normalized to the mean control values the difference between 1.00 ± 0.04 (n = 15) and 0.83 ± 0.05

TABLE I

TRANSPORT OF GALACTOSE AND TRYPTOPHAN ACROSS GUINEA PIG SMALL INTESTINE

Cross-inhibition between sugars and amino acids. J is in $\mu mol/cm^2$ per h and $[A]_c$ in mM. n.s., not significant.

	1 mM Gal		1 mM Gal + 20 mM Met	1 mM Try		1 mM Try + 20 mM Gal
J_{mc}	0.49 ± 0.03 (8)	n.s.	0.45 ± 0.03 (8)			
J_{ms}	0.215 ± 0.010 (4)	$P < 0.05$	0.129 ± 0.020 (4)	0.233 ± 0.019 (4)	$P < 0.02$	0.137 ± 0.003 (4)
J_{cm} *	0.30		0.30	1.38		1.24
J_{sm}	0.022 ± 0.001 (4)	n.s.	0.025 ± 0.002 (4)	0.027 ± 0.004 (4)	n.s.	0.032 ± 0.005 (4)
$[A]_c$	12.7 ± 0.9 (15)	$P < 0.001$	6.4 ± 0.2 (16)	21.8 ± 1.5 (16)	$P < 0.001$	5.0 ± 0.3 (16)

* Calculated using Eqns. 1 and 2.

TABLE II

INFLUX OF TRYPTOPHAN ACROSS THE BRUSH BORDER MEMBRANE IN GUINEA-PIG SMALL INTESTINE

Inhibition by galactose and methionine.

Na^+ (mM)	J_{mc}^{Try} (μmol/cm^2 per h ± S.E.)		
	1 mM Try		1 mM Try + 20 mM Gal
140	1.59 ± 0.07 (16)	$P < 0.05$	1.35 ± 0.05 (16)
6	0.29 ± 0.02 (8)		0.31 ± 0.05 (8)
	5 mM Try		5 mM Try + 20 mM Gal
140	4.76 ± 0.20 (8)	$P < 0.05$	3.99 ± 0.15 (8)
	5 mM Try		5 mM Try + 20 mM Met
140	5.54 + 0.37 (8)	$P < 0.001$	0.97 ± 0.05 (8)

TABLE III

INFLUX OF GALACTOSE ACROSS THE BRUSH BORDER MEMBRANE IN GUINEA-PIG SMALL INTESTINE

Sodium dependence of inhibitory effect of methionine. n.s., not significant.

Na^+ (mM)	J_{mc}^{Gal} (μmol/cm^2 per h ± S.E.)		
	10 mM Gal		10 mM Gal + 20 mM Met
140	3.40 ± 0.09 (16)	$P < 0.02$	2.96 ± 0.12 (16)
6	0.61 ± 0.03 (7)	$P < 0.15$	0.48 ± 0.05 (7)
6	0.88 ± 0.06 (8)	$P < 0.15$	0.76 ± 0.04 (8)
3	0.66 ± 0.07 (12)	n.s.	0.67 ± 0.08 (11)

($n = 15$) ($P < 0.01$) is statistically significant. At 3 mM Na^+ the inhibitory effect of methionine was abolished.

J_{mc}^{Try} was measured at 6 mM Na^+ in paired experiments at 1 mM tryptophan with and without 20 mM galactose. Already at 6 mM Na^+ the inhibitory effect of galactose was abolished.

J_{mc}^{Na} was measured at 6 and 3 mM Na^+ in paired experiments with and without 20 mM methionine. At 6 mM Na^+ methionine increased J_{mc}^{Na} from 1.33 ± 0.06 ($n = 8$) to 4.42 ± 0.24 μmol/cm^2 per h ($n = 8$), 20 mM galactose increased J_{mc}^{Na} from 1.45 ± 0.09 ($n = 8$) to 1.71 ± 0.03 μmol/cm^2 per h ($n = 8$). At 3 mM Na^+ the effect of 20 mM methionine was an increase from 0.70 ± 0.03 ($n = 8$) to 2.70 ± 0.08 μmol/cm^2 per h ($n = 8$).

Discussion

The results presented here, equal inhibitory effects of 20 and 30 mM galactose, and a 13% inhibition by methionine at a concentration of almost ten times K_i, confirm those reported by Alvarado [9] and by Robinson and

Alvarado [2] with which they qualitatively agree. Clearly this type of partially competitive, mutual inhibition could be caused by allosteric interactions between separate binding sites for sugars and amino acids. However, this allosteric model [2] does not account for the absence of mutual inhibition under the influence of ouabain [4]. Neither does it account for the apparently increased efflux across the brush border membrane or for the inhibition of influx across the brush border membrane being sodium dependent, which as reported here confirms data on brush border microvesicles [7]. It is therefore worthwhile to examine whether, as recently proposed [7,8], the sodium gradient hypothesis can account for these characteristics of the mutual inhibition between sugars and amino acids.

Work with microelectrodes [14—16] and on microvesicles of brush border membranes [7,17] indicates that sodium-coupled influx of sugars and amino acids across the brush border membrane is electrogenic. Therefore an estimate of the effects of the transmembrane electrical potential difference may be gained from the equation describing the unidirectional influx of a positively charged substance on an electroneutral carrier under the conditions of an inside substrate concentration of zero [18].

$$J^{A} = \frac{2J_{max}[A]\exp(-ZF\psi_{mc}/2RT)}{2K_t + [A][1 + \exp(-ZF\psi_{mc}/2RT)]} \quad (3)$$

where J^{A} is the influx of the charged compound A across the brush border membrane, [A] is the concentration on the mucosal side, ψ_{mc} is the electrical potential difference, $(\psi_c - \psi_m)$, across the brush border membrane, K_t, Z, F, R, and T have their usual meaning. According to this interpretation of the sodium gradient hypothesis sugars and amino acids augment the sodium permeability of the brush border membrane in a saturable manner. With increasing non-electrolyte concentration the depolarizing effect on the membrane potential will therefore approach a maximum. The available data indicate [14,16] that in the absence of sugars and amino acids in the mucosal fluid ψ_{mc} is in the range of —50 to —40 mV and that the maximal depolarization caused by an amino acid or a sugar is about 20 mV corresponding, according to Eqn. 3, to a maximum inhibition of 12 and 15%, respectively. This is the interval within which present and previous observations of mutual sugar-amino acid inhibition fall. Also efflux of sugars and amino acids across the brush border membrane is sodium coupled [19]. Therefore, at the same time as the influx of a sugar or an amino acid is reduced by a depolarization of the membrane the efflux will be increased. This would explain why the steady-state mucosal uptake and the transepithelial net flux are inhibited relatively more than J_{mc}, and why measurements of 2-min uptakes by everted rings [2] show slightly higher, and more consistent degrees of mutual inhibition. As outlined here the effect on ψ_{mc} of the sugar-amino acid-induced increase in sodium influx will be described by the Goldman-Hodgkin Katz equation. In this the sodium permeability appears in a logarithmic term. This alone would explain why as pointed out by Robinson and Alvarado [2] the electric effects of sugars and amino acids are not stoichiometrically additive. Thus the present results confirm those reported by Alvarado [9] and by Robinson and Alvarado [2], they agree with previous observations on rat small intestine [6] and rabbit ileum [3] in demonstrating

a more pronounced heteroinhibition of the composite steady-state transport parameters than the unidirectional influx across the brush border membrane, they confirm the observations made on microvesicles from rat small intestine brush border showing that the mutual inhibition between sugars and amino acids is sodium dependent, and whereas the observations cannot be accounted for by the allosteric interaction hypothesis they are all explicable in terms of the sodium gradient hypothesis.

Methionine and galactose do not differ significantly in efficacy as inhibitors of influx across the brush border or unidirectional transepithelial flux. It is, however, evident that with respect to steady-state mucosal uptake galactose is a more potent inhibitor than methionine. The present data suggest in accordance with previous studies on rat small intestine [6] that this greater effect may result from an increased permeability of the basolateral cell membrane. Such a mechanism is consistent with the delay in inhibitory effect of galactose observed by Saunders and Isselbacher [20], which is also apparent in observations on isolated enterocytes from the chicken small intestine [21].

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