REVISTA ESPAÑOLA DE FISIOLOGIA, 31, 271-276. 1975

Sodium Dependence of Intestinal Active Transport of Sugars in Snail (*Cryptomphalus hortensis* Müller)

A. Barber, R. Jordana and F. Ponz

Departamento de Investigaciones Fisiológicas, C.S.I.C.
Universidad de Navarra
Pamplona (España)

(Received on 25 March, 1975)

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Active transport of sugars (D-galactose, D-glucose, 3-0-methylglucose and L-arabinose) by sacs of everted intestine of snail *(Cryptomphalus hortensis)* was strongly inhibited, but not abolished, when all Na from the bathing solutions was substituted by K, Tris, Mg or Ca. Absence of Na produced also a marked inhibition of O_2 consumption by the tissue.

Omission of other cations (K, Ca, Mg), substituted by Tris, did not affect sugar transport or O_2 uptake. Sodium seems to play a specific and important but not indispensable rôle in sugar active transport by snail intestine. Since anaerobiosis did not affect sugar transport, this Na role is independent of its effect on O_2 uptake.

Active transport of sugars across the intestine very frequently depends on the presence of sodium (15, 16). Experiments on this subject were conducted mainly with common laboratory mammals and, to a lesser degree, with other vertebrate and invertebrate (6, 7, 16) species. Usually, sugar active transport disappears in the absence of sodium (4).

Two main hypotheses have been advanced to explain this sodium dependence, viz. sugar and Na are simultaneously transported on different sites, of the same carrier molecule (5); or sodium affects the transport energy supplying system, i.e. the Na-K-ATPase activity (12).

In the scarce literature on intestinal absorption in mollusca, references on sodium dependence are lacking. After verifying the existence of an active intestinal transport of sugars in snail (2) and its relation to different metabolic alterations (3), this paper studies the influence of the ionic environment on this process. It shows that intestinal transport of sugar in snail is only partially dependent on sodium.

Materials and Methods

Adult specimens of *Cryptomphalus hortensis* Müller, a common snail in this country, weighing approximately 10 g shell included, were used. They were

raised on fresh lettuce and kept under proper humidity control before the experiments.

Sacs of everted intestine were prepared according to the WILSON and WISEMAN method (20) for mammals, after appropriate modifications described elsewhere (2).

MENG's solution (13) was used adequately modified with Tris-ClH buffer (2) for control experiments. Other solutions, lacking in sodium, potassium, calcium or magnesium were obtained by omitting the corresponding chloride compounds from the control solution, and substituting them to maintain pH, and osmolarity as required.

At the beginning of each experiment the sacs were filled and suspended in the same solution. For this reason the initial ionic composition and sugar concentration were identical in the mucosal and serosal compartments. Incubation was carried out in small flasks (7 ml), shaked in Warburg apparatus at 100 oscillations per minute, 3 cm amplitude, under oxygen atmosphere, at 30° C±0.01° C, for 60 minutes. The weights of the sacs, both empty and filled, were recorded before and after incubation. Aliquots from the serosal and mucosal solutions were taken for sugar evaluation at the end of each experiment.

Oxygen consumption was measured by direct Warburg method (19) as described by UMBREIT *et al.* (18). D-galactose, 3-0-methylglucose, and L-arabinose were determined according to NELSON-SOMOGYI method (14, 17); D-glucose, by a glucose-oxidase enzymatic method (11).

Active transport rate is expressed as the gradient developed at the end of the experiment, i.e. the Sf/Mf ratio, where Sf and Mf represent sugar concentration in the final solution in the serosal and mucosal compartments.

Results

Omission of sodium. The effects of the absence of sodium in both serosal and mucosal solutions are shown in table I.

Sodium free solutions were obtained on omitting NaCl from the control solution and dissolving as substitutes Tris-HCl,

Table I. *Effects of the absence of sodium on sugar active transport and oxygen consumption by intestine of snail* (Cryptomphalus hortensis *Müller*).
Sacs incubated in solutions with or without Na^+, in the presence of sugar, for 60 minutes. Mean values of final serosal/mucosal sugar gradients (Sf/Mf) and of oxygen consumption are given with their standard errors. Number of experiments in parenthesis. Statistical significance after t Student's test.

Na^+ Sustitute	Sf/Mf		µl O_2/100 mg w.w.	
5 mM D-galactose				
Control	1.61±0.03	(118)	55.33±1.62	(147)
Tris	0.95±0.02	(19)	38.29±3.33	(18)
	$p < 0.001$		$p < 0.001$	
K^+	0.95±0.04	(10)	33.09±5.40	(9)
	$p < 0.001$		$p < 0.001$	
Mg^{++}	0.98±0.04	(8)	34.82±3.61	(7)
	$p < 0.001$		$p < 0.001$	
Ca^{++}	1.32±0.07	(35)	30.55±2.15	(36)
	$p < 0.001$		$p < 0.001$	
1 mM D-galactose				
Control	3.48±0.32	(10)	58.26±2.92	(11)
Tris	1.95±0.29	(8)	23.69±2.26	(8)
	$p < 0.01$		$p < 0.001$	
1 mM D-glucose				
Control	4.42±0.44	(12)	48.96±2.64	(12)
Tris	1.41±0.13	(19)	24.30±2.55	(19)
	$p < 0.001$		$p < 0.001$	
2.5 mM 3-0-methyl-glucose				
Control	2.54±0.15	(8)	56.12±2.72	(16)
Tris	1.62±0.10	(14)	29.32±1.70	(15)
	$p < 0.001$		$p < 0.001$	
1 mM L-arabinose				
Control	3.39±0.27	(10)	56.15±3.32	(10)
Tris	1.99±0.17	(14)	27.38±2.10	(14)
	$p < 0.001$		$p < 0.001$	

KCl, $MgCl_2$ of $CaCl_2$ to maintain constant osmolarity and pH. These four Na-free solutions plus the control one were used in the experiments with 5 mM galactose. The rest of the experiments were carried out only with the control Na-containing solution and the Tris Na-free solution, since the results obtained with the other substitutes, except calcium, were very similar.

With 5 mM galactose in the control solution the gradient developed was 1.61, evincing active transport. No gradient was developed and galactose was not actively transported, however, when sodium was substituted by Tris, potassium or magnesium. With calcium as substitute, a small gradient was observed, though lower than that obtained when Na was present. Nevertheless, in this case a clear decrease in sac weight and serosal water was produced, whereas in all the other cases the serosal volume did not practically change throughout the experiments. This difference was probably caused by strong intestinal contractions in the presence of calcium. In fact, when preparations of isolated snail intestine are suspended in control solution at 30° C, a rhythmic spontaneous motor activity can be recorded, but this activity changes to a strong persistent contraction when the control solution is substituted by the Na-free, calcium containing, solution. If the control is used again, contracture ceases and rhythmic activity returns. This intestine contracture presses against the serosal solution and produces a net flux of fluid to the mucosal compartment. But since water permeability is higher than that for sugar, the result may be an increase in sugar concentration in the serosal compartment, that generates a S/M gradient unrelated to sugar active transport.

With low initial concentrations of sugars (1 mM galactose, 1 mM glucose, 2.5 mM 3-0-methylglucose and 1 mM L-arabinose), high Sf/Mf ratios were obtained in the control experiments. But on using Tris instead of Na^+, the corresponding final gradients diminished considerably although active transport was still produced.

Oxygen consumption by snail intestine did not appreciably change by the presence of various sugars at different concentrations (2, 3). A strong inhibition was observed, however, amounting from 30 to 50 per cent, when sodium was omitted from the bathing solutions independently of the nature of the substitute used. These inbihitions were of clear statistical significance.

Omission of potassium, calcium or magnesium. The salts of the omitted cations were substituted in the solutions by the necessary quantities of Tris-HCl to maintain osmolarity and pH. The other compounds retained the same concentration as the control solution.

As table II shows, the lack of potassium, calcium or magnesium had no significant effect on active transport of ga-

Table II. *Effects of the absence of potassium, calcium or magnesium on sugar active transport and oxygen consumption by Intestine of snail* (Cryptomphalus hortesis *Müller*). Sacs incubated in control solution or in solutions lacking in K^+, Ca^{++} or Mg^{++}, in the presence of an initial 5 mM galactose concentration, for 60 minutes. Mean values of final serosal/mucosal sugar gradients (Sf/Mf) and of oxygen consumption are given with their standard errors. Number of experiments in parenthesis. Statistical significance after t Student's test.

Solutions	Sf/Mf	$\mu l\ O_2$/100 mg w.w.
Control	1.61±0.03 (118)	55.33±1.62 (147)
K^+-free	1.48±0.05 (14) $p < 0.2$ *	63.52±4.60 (16) $p < 0.1$ *
Ca^{++}-free	1.66±0.08 (13) $p < 0.7$ *	49.81±3.04 (13) $p < 0.3$ *
Mg^{++}-free	1.62±0.05 (13) $p < 0.9$ *	65.09±5.05 (13) $p < 0.1$ *

* Not significant

lactose (5 mM) when enough sodium was present. Oxygen uptake was also unaffected by omission of the same ions.

Discussion

The findings here reported reveal that sodium plays also an important rôle in sugar active transport across the snail intestine. Lack of sodium clearly inhibits this process, no matter what the substitute used: Tris, potassium, magnesium or calcium. This fact reflects the specific rôle of sodium in sugar transport.

However, sodium does not appear as a quite essential agent for up-hill transfer of sugars. When the initial concentration of galactose was 5 mM, the absence of Na impeded the development of any serosal/mucosal gradient. An active transport of sugar, however, took place and final gradient (Sf/Mf ratio) of 1.4 to approximately 2.0 developed, in the absence of sodium, when the concentration was of 1 mM galactose or 1 mM glucose, 1 mM L-arabinose or 2.5 3-0-methylglucose. Much higher gradients developed, however, with Sf/Mf ratios going from 2.5 to 4.4, when sodium was present in these solutions.

Therefore, the presence of Na favours, but it is not indispensable for active transport of sugars across the intestinal epithelium of snail. Sodium dependence in this species does not seem to be so strict as in many others (15). On the other hand, lack of sodium, as already reported for other animals (1, 9), exerts a clear inhibition (30-50 %) in oxygen consumption by intestinal tissues of snail. This effect may be attributed to a functional suppression of the Na^+-pump as assumed for some other tissues, or to a disturbance of the cell respiratory systems, as has been deduced from experiments with some mammal tissues (10).

At any rate the inhibitory action from the lack of sodium on sugar active transport cannot be related to similar effects on O_2 uptake, as explained by a deficit of metabolic energy yielded from aerobic respiratory processe. Since it has been previously shown (3) that anaerobiosis does not modify the ability of snail intestine to transport sugars against a gradient, this suggests that snail intestine may easely change its metabolic pathways to supply from anaerobic reactions the required energy for that process.

Contrary to the absence of sodium, the omission of potassium, magnesium or calcium, all the other cations present in the control solutions, had no effect on sugar transport or on oxygen consumption. The ionic environment requirements of snail intestine, at least under the adopted experimental conditions, excepting the sodium ion, do not seem to be very strict, since the preparation could sustain a normal respiration rate and sugar active transport activity on a broad range of ionic composition. This result cannot surprise if we take into account the ecological characteristics of snails, with their great adaptation capacity to survive under great changes in body fluid composition (8).

The preceding data are not enough to formulate a plausible interpretation of the sodium rôle in sugar transport across snail intestine. Both cotransport and Na-K-ATPase influence may be consistent with these results. Under assumption of cotransport of both sugar and sodium bound to the same carrier molecule, this mechanism would be here considered as non-essential, but merely coadjuvant, as sugars can still enter and cross over the epithelium toward serosal against a concentration gradient in the absence of sodium. If the sodium function has to be related to a membrane Na-K-ATPase delivering energy for active transport (12), this enzyme activity would be less sensitive to the lack of sodium.

In any case, it seems evident that sugar active transport systems of snail intestine

show very interesting peculiarities with respect to substrate specificity, oxygen requirements and sodium dependence.

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