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Na^+-DEPENDENT CO-TRANSPORT OF α-METHYL D-GLUCOSIDE ACROSS THE MUCOSAL BORDER OF RABBIT DESCENDING COLON

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(1) The uptake and bidirectional fluxes of 1-α-methyl D-glucoside were studied in isolated rabbit colonic mucosa. (2) The uptake of α-methyl D-glucoside was linear over the first 30 min and reached maximum after 1 h; was a saturable function of sugar concentration and was Na^+-dependent. (3) An increase in sugar uptake across the mucosal border and net transepithelial sugar flux across sheets of colon was observed in the presence of 10^{-4} M amiloride. (4) Phlorizin (10^{-4} M) inhibited sugar uptake into the tissue water and abolished net sugar flux. Amiloride-stimulated sugar uptake was also abolished by 10^{-4} M phlorizin. (5) Ouabain (10^{-4} M) prevented the effect of amiloride on sugar uptake and inhibited sugar uptake into the tissue. (6) These results corroborate the findings of Henriques de Jesus et al. (Henriques de Jesus, C., Da Gracia Emilio, M. and Santos, M.A. Gastroenterol. Clin. Biol. 3, 172–173) who found a sugar-dependent increase in short-circuit current in colonic mucosa exposed to amiloride.

Introduction

Sugar absorption has been reported to be absent from the colon [1]. However, Robinson et al. [2] showed that dog colonic mucosa absorbs sugars and that the process exhibited Na^+-dependence, energy dependence and saturation kinetics. These characteristics are usually attributed to the Na^+-linked sugar transport.

More recently, Henriques de Jesus et al. [3] have shown that, in the presence of amiloride and 3-*O*-methyl D-glucose there was an Na^+-dependent increase in short circuit current across rabbit colonic mucosa, which was abolished by phlorizin. The sugar-induced increase in short-circuit current was not significantly different from zero in the absence of amiloride. Henriques de Jesus et al. [3] concluded that the increase in short-circuit current induced by sugar could indicate the presence of a sugar transport mechanism. However, no direct measurements of sugar transport were reported. The experiments reported here were designed to determine whether Na^+-linked sugar transport could be demonstrated in isolated rabbit colonic mucosa.

Materials and Methods

Animals, incubation solutions. Male white New Zealand rabbits weighing 2–3 kg were killed by intravenous injection of sodium pentobarbital. A segment of colon was rapidly removed and washed free of faeces with ice-cold Ringer's solution. The tissue was then stripped of its serosal and external muscle layers using the method of Frizzell et al. [4].

The Ringer's solution contained, in mM: 140 NaCl, 10 $KHCO_3$, 0.4 KH_2PO_4, 2.4 K_2HPO_4, 1.2 $CaCl_2$ and 1.2 $MgCl_2$ and was continuously bubbled with 95% O_2/5% CO_2. In some experiments the Na^+ in the Ringer's solution was replaced by choline.

Sugar uptake measurements. Pieces of colonic mucosa stripped of muscle, weighing about 25 mg, were incubated at 37°C in Ringer's solution contain-

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ing α-methyl glucose labelled with ^{14}C for 30 min unless stated otherwise. At the end of the experiment the tissues were washed for 2 min with gentle shaking in ice-cold Ringer's solution and blotted carefully on both sides to remove excess moisture. The tissue was weighed wet and extracted by shaking for 15 h in 1 ml of 0.1 M HNO_3. 0.1 ml samples were taken from the bathing solutions and from the extracts of the tissues for radioactivity counting. The extracted tissues were dried for 24 h at 80°C and the dry : wet weight ratio determined. Tissue water was calculated as the difference between wet and dry weights.

The inhibitors were added to the incubation solutions at the beginning of the incubation period.

Extracellular space determination. The extracellular space measurements of rabbit colon were determined in order to accurately estimate the true intracellular concentration of the accumulated sugar. Pieces of isolated mucosa were incubated in Ringer's solution at 37°C containing 2–3 mμCi/ml ^{3}H-labelled poly(ethylene glycol) (mol. wt. 4000, NEN) for 30 min, the pieces of mucosa were then blotted gently on filter paper and weighed, then extracted in 1.0 ml 0.1 M HNO_3 overnight. 0.2 ml aliquots of the extract were then counted together with 0.2 ml aliquots of the bathing solution. Following extraction, the tissue was dried at 80°C for 24 h then reweighed.

The poly(ethylene glycol) space (extracellular space) and wet/dry weight ratios of the mucosa in control and amiloride conditions are shown in Table I. It can be seen that amiloride (0.1 mM) causes a significant decrease in cell water (total water – extracellular water)/total weight, this effect is almost certainly due to amiloride-dependent inhibition of Na^+ uptake into the mucosa; the effect is not observed in ouabain-treated tissues. When this change in volume is used to correct the estimates of intracellular sugar concentration, the estimated concentration is raised further in the amiloride-treated tissue. Although this correction has been applied throughout, it has also been shown that the maximal effects of amiloride on α-methyl glucoside accumulation are statistically above control values even without the correction for volume change.

Transepithelial flux measurements. The stripped mucosa was mounted as a flat sheet in Ussing-type chambers. The bathing solutions on the mucosal and serosal surfaces of the tissues were maintained at 37°C using a circulating water-bath as described previously [5]. Both solutions contained 0.1 mM α-methyl D-glucoside. Mucosal to serosal and serosal to mucosal sugar fluxes were measured by placing the ^{14}C-labelled α-methyl glucoside either in the mucosal or serosal side, respectively. 0.5 ml samples were removed from the cold side at 45-min intervals for 135 min. One sample only was taken for counting from the hot side, although 0.5 ml aliquots were removed from the hot side when the cold side was sampled to keep the hydrostatic pressure difference across the tissue close to zero.

At the end of the experiment both mucosal and serosal chambers were washed rapidly with ice-cold Ringer's solution with the tissue still in place. The chambers were then opened and the exposed circle (1.76 cm^2) of tissue was cut out, then the tissue was extracted as described above. Samples of the radioac-

TABLE I

THE EFFECT OF AMILORIDE AND OUABAIN ON THE EXTRACELLULAR (POLY(ETHYLENE GLYCOL)) SPACE

Values are means ±S.E. (in brackets the number of independent estimates of the mean).

	Control	Amiloride (0.1 mM)	Ouabain (0.1 mM)	Ouabain and amiloride
Extracellular space	0.22 ± 0.018(11)	0.33 ± 0.018(12) [a]	0.24 ± 0.014(10)	0.22 ±0.08(11)
Tissue water fraction wet – dry weight / wet weight	0.90 ± 0.006(11)	0.91 ± 0.008(11)	0.90 ± 0.006(9)	0.90 ± 0.005(10)
Cell water fraction	0.68 ± 0.02	0.58 ± 0.02 [a]	0.66 ± 0.015	0.68 ± 0.01

[a] $P < 0.0025$, Student's t-test.

tive solution were counted using a Packard Tricarb liquid scintillation counter. Unidirectional fluxes across the mucosal and serosal borders of the tissues were calculated from J_{ms}, J_{sm} and R the ratio of radioisotope present in tissue coming from mucosal and serosal solutions, respectively, as follows [6]:

$$J_{12} = J_{sm} \cdot R + J_{ms}; J_{21} = J_{sm}(1 + R);$$

$$J_{23} = J_{ms}(1 + (1/R); J_{32} = J_{sm} + (J_{ms}/R)$$

1, 2, 3 refer to mucosal, tissue and serosal compartments, respectively.

Results

1. Concentration dependent effect of amiloride on α-methyl glucoside uptake into stripped rabbit colon

In control conditions the uptake of 1 mM α-methyl D-glucoside into tissue water was found to be 2.2 μmol/g tissue wet wt. per h (see Fig. 1). Amiloride increased sugar uptake over the entire range of amiloride concentrations tested, reaching its maximal effect at 10^{-4} M ($P < 0.01$) (1.4-fold increase). Ouabain (10^{-4} M) decreased sugar uptake in control tissues and prevented the effect of amiloride ($P < 0.01$).

2. Time course of α-methyl glucose uptake into colon

The results shown in Fig. 2 reveal that the entry of sugar into the tissue water was linear over the first 30 min of incubation and reached maximum after one hour, regardless of whether or not amiloride (10^{-4} M) was present in the Ringer's solution. The uptake of sugar within the tissues exposed to amiloride was observed to be significantly higher than in control tissues ($P < 0.01$). 10^{-4} M phlorizin inhibited sugar uptake and prevented the effect of amiloride on α-methyl D-glucoside uptake ($P < 0.01$).

3. The effect of varying concentrations of Na^+ on α-methyl glucoside uptake into rabbit colonic mucosa

By substitution of choline chloride for NaCl in the

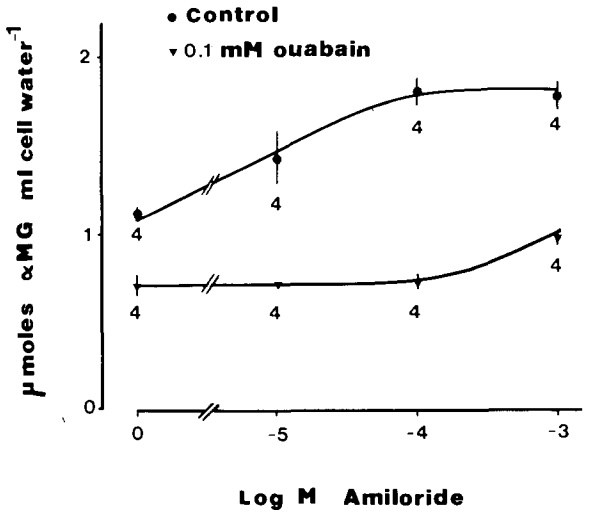

Fig. 1. Effect of varying concentrations of amiloride on 1-α-methyl D-glucoside uptake into stripped rabbit colonic mucosa, in the presence and absence of 10^{-4} M ouabain. The concentration of sugar in the incubation solutions is 1 mM. The errors bars represent standard errors of the mean; the numbers beside each point are the numbers of independent observations. At amiloride concentrations of 10^{-4} M and above, sugar accumulation is significantly raised above control ($P < 0.01$). Ouabain inhibits sugar accumulation at all levels of amiloride ($P < 0.01$).

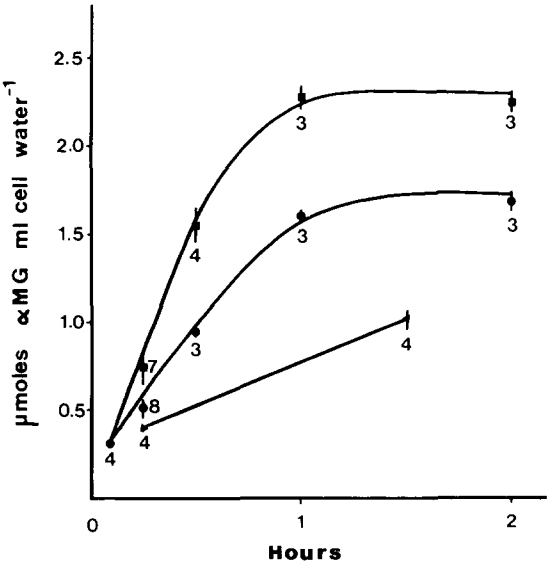

Fig. 2. The time dependent changes in α-methyl glucoside uptake into cell water of rabbit colon. •——•, control; ▪——▪, 10^{-4} M amiloride; ▲——▲, amiloride (10^{-4} M) + phlorizin (10^{-4} M). The concentration of sugar in the external of sugar in the external solution is 1 mM. The data are plotted as means ±S.E. The numbers beside each point are the number of independent observations.

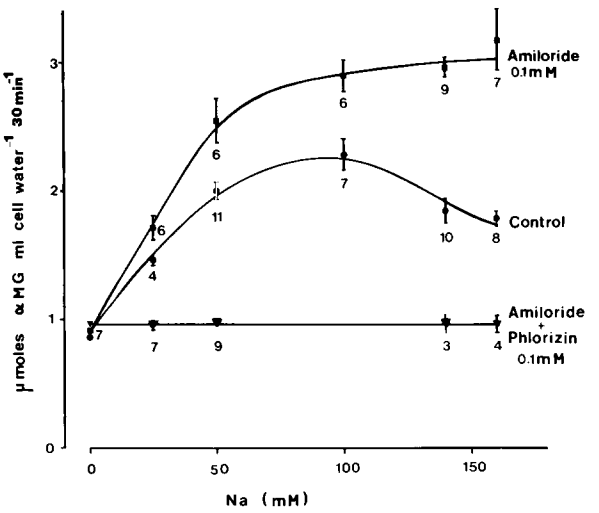

Fig. 3. Effect of replacement of Na^+ in Ringer's solution with choline on the tissue α-methyl glucoside uptake, ●——●, control; ■——■, 10^{-4} M amiloride; ▲——▲, 10^{-4} M amiloride + phlorizin (10^{-4} M). The concentration of sugar in the bathing solution is 1 mM. Phlorizin inhibits accumulation of sugar at all levels of Na^+ above zero ($P < 0.001$). Amiloride increases sugar accumulation significantly when Ringer Na^+ is 50 mM or above ($P < 0.001$). Sugar uptake in control tissue falls when Ringer Na^+ is raised above 100 mM ($P < 0.01$).

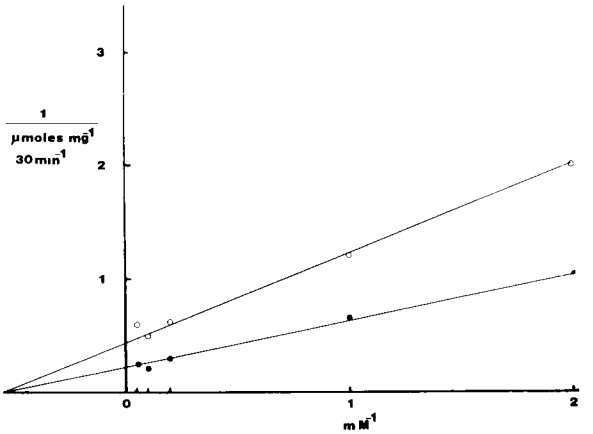

Fig. 4. Lineweaver-Burk plot of the rate of active uptake of a α-methyl glucoside during 30 min into strips of isolated colonic mucosa. Active uptake = rate of uptake into cell water (control or amiloride-treated) – rate of uptake into cell water exposed to 0.1 mM phlorizin. Each point is the mean of at least four separate observations. Derived parameters: Control: K_m 1.5 ± 0.1 mM; V 1.7 ± 0.15 μmol · g^{-1} cell water in 30 min. Amiloride: K_m 1.50 ± 0.1 mM; V 2.80 ± 0.22 μmol · g^{-1} cell water in 30 min. Amiloride increases V ($P < 0.001$), but does not affect K_m. Lines were determined by least-square analysis of the data.

Ringer's solution it is possible to vary the steady-state extracellular [Na_o^+] and intracellular [Na_i]. The effect of raising Ringer [Na_o^+] from 0 to 160 mequiv. on sugar uptake is displayed in Fig. 3. In control conditions sugar uptake increased with Ringer [Na^+] ($P < 0.001$). Maximal sugar uptake occurred when the external [Na^+] was 100 mequiv. and decreased when [Na^+] was raised above 100 mequiv. ($P < 0.01$). With amiloride (0.01 mM) present, raising Ringer [Na^+] increased sugar uptake; above Ringer [Na^+] = 50 mM, sugar uptake was increased significantly above control levels ($P < 0.001$) and no reduction in sugar uptake below maximum was seen when Ringer Na^+ was raised above 100 mM. 10^{-4} M phlorizin had no effect on sugar uptake with zero Na^+ in the incubation solutions. However with the Na^+ present, phlorizin inhibited sugar uptake, in both the presence and absence of amiloride ($P < 0.001$).

4. The effect of varying the concentration of α-methyl D-*glucoside on the rate of sugar uptake into colonic mucosa*

The effect of varying the concentration of α-methyl glucoside on active sugar uptake rates (uptake rate – uptake in phlorizin-treated tissue) is displayed in Fig. 4.

These results are consistent with the view that amiloride raises the V of the active transport system for sugar without affecting its K_m, or the passive entry of sugar into the tissue.

5. Study of transepithelial sugar fluxes across rabbit colon

To gain more insight into the mechanisms that might be involved in sugar absorption across rabbit colon, we measured α-methyl glucoside (0.1 mM) fluxes across the stripped colonic mucosa (see Materials and Methods). Amiloride (10^{-4} M) and phlorizin (10^{-4} M) were added to the mucosal solution at the beginning of the incubation period. The results summarized in Table II reveal that the presence of phlorizin (10^{-4} M) or amiloride (10^{-4} M) in the mucosal bathing solution, produced opposite effects on the mucosal to serosal sugar flux, without significantly affecting serosal to mucosal sugar flux. Phlorizin reduced the mucosal-serosal sugar flux ($P < 0.005$), thereby abolishing net transepithelial

TABLE II

THE EFFECT OF AMILORIDE AND PHLORIZIN ON THE BIDIRECTIONAL TRANSEPITHELIAL FLUXES, NET FLUXES, UNIDIRECTIONAL FLUXES AND THE SPECIFIC ACTIVITY RATIO (R) OF 0.1 mM 1-α-METHYL GLUCOSE

	1-α-Methyl glucose (nmol · cm^{-2} · h^{-1})		
	No addition	Amiloride 10^{-4} M	Phlorizin 10^{-4} M
J_{13}	2.91 ± 0.27(6)	5.50 ± 0.31(8) [a]	1.74 ± 0.22(2)
J_{31}	1.54 ± 0.20(8)	1.82 ± 0.16(8)	1.45 ± 0.11(4)
J_{net}	1.37 ± 0.35	3.68 ± 0.37 [b]	0.29 ± 0.31
J_{12}	5.48 ± 0.98	9.74 ± 1.40 [b]	2.80 ± 0.40
J_{21}	4.11 ± 0.63	6.06 ± 0.82	2.51 ± 0.33
J_{23}	4.55 ± 0.60	7.86 ± 0.92	4.10 ± 0.51
J_{32}	3.28 ± 0.58	4.18 ± 0.60	3.83 ± 0.48
R	1.67 ± 0.16(4)	2.33 ± 0.24(3) [c]	0.73 ± 0.01(3)

[a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$; Student's t-test.

sugar flux. In the presence of amiloride, a significant increase in mucosal-serosal α-methyl glucoside flux was observed ($P < 0.001$), increasing net sugar absorption. A view concerning the effects of amiloride on the movements of sugar across the cell boundaries can be obtained by examining the unidirectional entry and exit fluxes of α-methyl glucoside. Unidirectional fluxes are calculated according to the formulae derived previously [6], utilizing the bidirectional transmural fluxes and the specific activity ratio R (labelled sugar within the tissue originating from the mucosal and serosal bathing solutions, respectively). The results obtained (see Table II) show that phlorizin produced nearly a 2-fold decrease in the unidirectional sugar fluxes across the brush border. With amiloride present there was an increase in both unidirectional sugar fluxes across the mucosal boundary. However, the rise in exit flux of α-methyl glucoside across the mucosal and serosal borders is entirely accounted for by the increase in concentration of sugar within the tissue fluid.

These results indicate that amiloride affects the rate of active sugar uptake across the mucosal border without significantly affecting the passive fluxes across the mucosal and serosal borders.

Discussion

It is well established that there is Na^+-linked transport of many organic solutes in the small intestine. However, the existence of a similar mechanism in the large intestine has been disputed [7–9]. Exceptionally it was shown unequivocally that dog colon possesses a vigorous active sugar and amino acid transport mechanism [2], neonatal pig colon has Na^+-dependent-cotransport systems for amino acids which are lost within the first week of life [7,10] and adult hen fed on high Na^+ diet also have a vigorous Na^+-dependent sugar transport system [11]. In contrast, Henriques de Jesus et al. [3] found that following exposure of rabbit colon to amiloride in vitro, there is a sugar-dependent increase in short circuit current which is sensitive to phlorizin. This finding indicates that there is an Na^+-dependent sugar transport system in rabbit colon and represents a significant departure from the accepted view that the colon is incapable of active sugar transport.

To confirm the findings of Henriques de Jesus et al. we have examined the uptake of α-methyl D-glucoside into rabbit colonic mucosa in vitro and also transepithelial transport of this to determine whether the sugar flux is affected by the presence of amiloride. We chose α-methyl glucoside as the transport sugar because it is not appreciably metabolised and it is transported well by the Na^+-dependent process in the small intestine [12].

Our results clearly indicate that rabbit colonic epithelium is capable of active sugar accumulation into the tissue fluid and able to support a small, but significant, net absorption of sugar across the epithelium, and that amiloride elicits an increase in both sugar accumulation and net transepithelial flux.

The presence of an Na^+-dependent specific sugar transport process across the mucosal border of rabbit colon is confirmed by the fact that the active accumulation is Na^+-dependent, inhibited by phlorizin and is a saturable function of α-methyl glucoside concentration, K_m 1–2 mM. Amiloride blocks the majority of Na^+ conductance channels across the mucosal border of rabbit colon thereby reducing the short circuit current across the tissue to almost zero, it also reduces intracellular [Na^+] from 25 mM (control) to 12 mM (amiloride) and the electrical potential difference across the mucosal border is decreased from −30

mV so that an overall change in electrochemical potential is from −80 mV equivalents to −112 mV equivalents [13]. Thus the amiloride enhanced entry of α-methyl glucoside is consistent with the hypothesis that there is an Na^+-coupled sugar movement via amiloride-insensitive channels present within the mucosal membrane. Other evidence which is consistent with the view that the energy derived from the electrochemical potential gradient of Na^+ across the mucosal border drives uphill sugar accumulation in rabbit colon is that phlorizin-sensitive sugar accumulation is only observed when Na^+ is present in Ringer and that exposure to ouabain which inhibits the Na^+-pump and thereby abolishes the Na^+ gradient, also inhibits sugar accumulation.

With control tissue, raising Ringer $[Na^+]$ above 100 mM decreases sugar uptake (Fig. 3). This decrease may result from an increase in intracellular $[Na^+]$ due to partial saturation of the Na^+-pump. With amiloride present, Na^+ uptake across the mucosal border is reduced, so the pump will be less saturated at high Ringer $[Na^+]$, hence no decrease in electrochemical potential gradient will occur when Ringer $[Na^+]$ is raised above 100 mM. The raised sugar accumulation at Ringer $[Na^+] \geqslant 100$ mM seen when amiloride is present suggests that the amiloride-sensitive cells are part of the same population which accumulate sugar and is inconsistent with the view that the sugar accumulating cells are a discrete sub-population of amiloride insensitive cells.

Comparison of sugar transport in rabbit colon and ileum

Transport of β-methyl D-glucoside has been investigated in rabbit ileum using similar methods to those described here [14]. There is little difference in the transport properties of the α and β anomers of methyl glucoside [12], so that any difference between colon and ileum in transport of these two sugars reflects a difference in the tissue transport properties. The mucosal-serosal flux is approx. 10-fold larger across ileum than colon; the serosal-mucosal flux across colon is 3-times larger than in ileum. Comparison of sugar influx across the mucosal and serosal borders is more instructive. It can be seen from Table III that sugar uptake across the mucosal border of rabbit ileum is approx. 8-fold larger than across that of colon. This low rate of sugar transport across colonic mucosal membrane could either be due to a relative paucity of Na^+-dependent sugar transport channels across colonic mucosal membranes or an increase in the sugar : Na^+-coupling ratio in colon.

Serosal transport of α-methyl glucoside

It can be seen in Table III that the rate of entry and exit of α-methyl glucoside across the serosal border of rabbit colon is similarly to entry and exit of β-methyl glucoside across the serosal border of rabbit ileum, indicating that the primary difference in sugar transport between colon and ileum is between their mucosal membrane transport system.

Recently Scharrer and Amann [15] have shown that there is a saturable transport system for both 2-deoxy-D-glucose and for 3-*O*-methyl D-glucose across the basolateral border of lamb colonic mucosa. These results lend support to the view that the major difference between the colon and ileum is at the mucosal border, the basolateral borders of both tissues appear to have identical sugar transport properties.

TABLE III

COMPARISON OF TRANSPORT OF α- AND β-METHYL d-GLUCOSIDE BY RABBIT COLON AND ILEUM, RESPECTIVELY

Control conditions colon data normalized to flux at 1 mM. J_{ij} is presented as nmol · cm^{-2} · h^{-1}. $P_{ij} = J_{ij}/c_i$ cm · h^{-1}, where i = 1, 2 or 3 and j = 1, 2 or 3. 1, 2, 3 refer to the mucosal, cell and serosal compartments, respectively.

Condition	J_{13}	J_{31}	R	Accumulation (mM)	J_{12}	P_{21}	P_{23}	J_{32}
Ileum (Holman and Naftalin [14])								
1 mM β-methyl glucoside	340	5.6	15.1	11.3	430	8	32	28
Colon (0.1 mM) normalized to								
1 mM α-methyl glucoside	39	15.0	1.7	2.3	55	18	21	32

We conclude from these results that an Na^+-dependent sugar transport system exists within the mucosal border of rabbit colon, which can be stimulated by the presence of amiloride. These findings corroborate the data of Henriques de Jesus et al. [3].

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