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The Physiological Model of Na^+-Dependent Transporters for Glucose and Amino Acids in Rat and Turtle

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Abstract

Conditions in rat and turtle small intestine tissue where glucose and glycine transport is inhibited while glucose-induced Na^+ transport is preserved are described. The generally accepted model for the Na^+-dependent transporter (a single channel for the Na^+ and nutrient) does not account for the data obtained from the analysis of the interaction between the transport of glucose, glycine, and Na^+ at different temperatures and the effect of inhibitors on these processes. The phenomenon of temperature uncoupling of Na^+ and nutrient transport can best be described by a two-pathway model with a gate mechanism. According to this model, the Na^+-dependent transporter has at least two pathways: one for Na^+ and another for nutrients. The model provides for the passage of Na^+ in both directions along a channel opened by glucose. Experiments are carried out using the addition of glucose and glycine on backgrounds of glycine and glucose, respectively. It has been hypothesized that when all three transporters (for Na^+, glucose and glycine) are unite in a single structure, then there should be "competitive relations" between short-circuit current changes on glycine and glucose for sodium ions passing through its transporter.

Keywords

Small Intestine, Short-Circuit Current, Nutrient Absorption, Molecular Mechanisms, Na^+ Dependent Glycine Transport, Na^+ Dependent Glucose Transport

1. Introduction

According to current thinking, Na^+-coupled transport occurs via a single special channel, with probably two Na^+ providing for the transfer of one molecule of glucose or other nutrients through the same pathway [1]-[3]. In

previous experiments [4], we have shown that at low temperatures glucose transport is inhibited but Na^+ transport is preserved. The existence in the membrane of two channels with different temperature sensitivities might be the simplest explanation of this phenomenon. As early as 1983, we proposed a two-channel model with a gate "regulator" [4]. In this paper, we report our studies on glucose transport in rats and turtles and amino acid transport in rats at different temperatures. The results are in good agreement with published data on the primary structure and topography of the Na^+ glucose [5] and Na^+ galacose cotransporter [6].

A common transporter model predicts that by stopping any way of transport of one substrate (for example, glucose), transport of another substrate (sodium) should also stop. As this occurs, three cases are possible. 1) The stage of glucose adsorption on the transporter is inhibited; hence, the formation of a ternary complex in all models does not occur and therefore transition to a following stage—the translocation of a complex through a membrane—proves to be impossible. 2) Glucose on the transporter is adsorbed, but the stage of translocation through a membrane is sharply inhibited. Formation of a ternary complex is possible, but as its translocation across a membrane is complicated, glucose on the internal side of a membrane does not appear. 3) Stages of adsorption and translocation are possible, but glucose does not dissociate in intracellular space. In one-channel models, transition of the transporter in an initial state and also the following cycles becomes impossible. For carrier models it means that the carrier will fulfill one full cycle; in so doing, sodium should be transferred into intracellular space. Glucose will travel on a carrier to an internal surface of a membrane and back. In this case, the stage of dissociation of glucose in an external solution also becomes impossible or will be slowed. In actuality, it is difficult to imagine conditions when dissociation of glucose from the transporter on one side of a membrane became impossible, and on another one to be unchanged. But the occurrence of such circulation of glucose in a membrane is unequivocally discriminated in the experiment. In this case, the stimulating glucose effect will be maintained indefinitely (or, at least, for a long time) after its removal from a mucosal solution. Any attempts to observe such an effect have not met with success. Thus, irrespective of the reason, the termination of the coupled glucose transport should result in the termination of the coupled sodium transport. Such inference of common serial transporter models is supported by experiments using a specific inhibitor of the coupled glucose transport—phlorizin. According to data [3], in the presence of 0.1 mM of phlorizin in a mucosal solution, active glucose transport stops almost completely and the stimulating glucose effect on the active sodium transport measured by the SCC technique is offset. In this article, the data are gathered for the existence of two-, or even multipathway transporter for Na^+, glucose and glycine.

2. Materials and Methods

2.1. Experimental Animals

The experiments were carried out on isolated segments of the proximal small intestine of white rats (Wistar, male, weighing 180 - 220 g) which had been deprived of food but with free access to water for 16 - 18 h. After decapitation of the rats the abdominal cavity was opened by midline incision and the removed intestine was rinsed two or three times with cold (1˚C - 3˚C) Ringer's solution (145.3 mM NaCl, 5.6 mM KCl, 2.2 mM $CaC1_2$, 2.05 mM $NaHCO_3$, pH 7.4). The intestine was separated from the mesenteric fat in the cold, with the aid of a magnifying glass. Some experiments were performed on the small intestine of the turt1e *Testudo gargaria*. In this case, the small intestine was removed through a hole in the shell over the right hind leg. These preparations were used for the study of Na^+, glucose, and glycine transport.

2.2. Na^+ Transport

Active Na^+ transport from the mucosa to the serosa was monitored by measuring the short-circuit current (SCC) in a thermostated Ussing chamber modified as described in [3]. The temperature of the mucosal and serosal solutions was maintained at a temperature selected from the range 7˚C - 37˚C (see **Figure 1**) by means of an ultrathermostat and controlled in close proximity to the preparation by a graduated micro thermistor. The preparations we used consisted of intestinal segments 1.5 cm in length, cut along the contramesenteric line [3] [4]. To obtain preparations in which respiration and glycolysis were inhibited we substituted oxygenation of the bathing solution with nitrogenation and added NaF at a concentration of 1 $mg \cdot ml^{-1}$ to both mucosal and serosal solutions (for 40 min) or to only the mucosal solution (for 60 min). The experiment was started when the basal SCC and the responses of the SCC to glucose or glycine had dropped to zero.

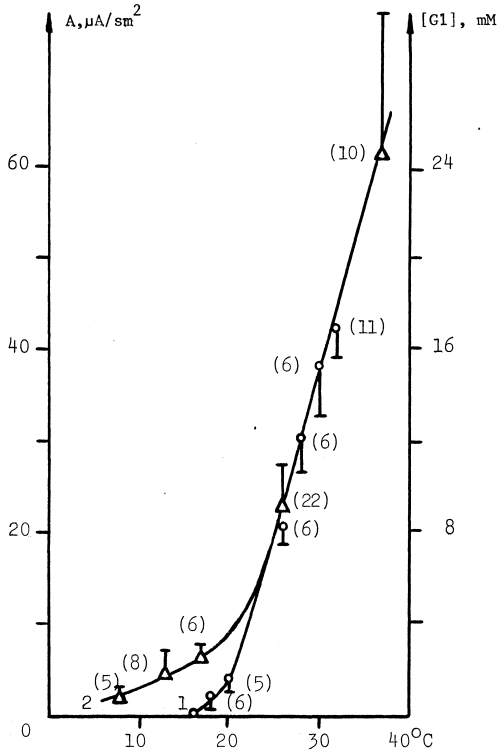

Figure 1. Temperature dependence of the stimulatory effect of glucose on active Na^+ transport (as measured by the short-circuit current, SCC) through the rat small intestine (curve 2) and of active glucose accumulation in segments of rat small intestine (curve 1). Results are given as means and half the SEM. The number of determinations is given in parentheses.

In the electrophysiological experiments, we used modified Ringer's solution where the NaCl and in some experiments the KCl were substituted with 80 mM Na_2S0_4 and 2.8 mM K_2SO_4, respectively. It is known that active Na^+ transport does not change under these conditions [7] and the SCC corresponds to the flow of actively transported Na^+ [8]. In all experiments, glucose and glycine instead of equimolar mannitol were added to the mucosal fluid. Only the serosal fluid was oxygenated.

2.3. Glucose and Glycine Transport

Two approaches that provide similar results were used to study the active transport of glucose and glycine in the small intestine mucosa of rats *in vitro*: 1) the accumulating mucosal preparation technique [9], which allows study of the substances transported from the mucosal fluid into enterocytes; and 2) the everted sac technique, which allows not only glucose accumulation in the mucosa to be monitored but also its transfer from the mucosal to the serosal fluid. In both cases incubation was carried out in Ringer's solution for 60 min in the presence of 10 mM glucose or glycine in the mucosal fluid. The tissue concentrations of the nutrients used were determined by the methods developed by [10] and [11] for glucose, and by the method of [12] for glycine. The temperature of the incubation medium was kept constant by the ultrathermostat.

3. Results

3.1. Stimulating Effects of Glycine and Glucose on the SCC

Over a wide temperature range (7˚C - 37˚C), we examined three parameters: 1) tissue glucose and glycine accumulation against a concentration gradient; 2) basal SCC in the absence of nutrients; and 3) the effects of added glucose and glycine on the SCC. At the beginning of the experiments at a temperature of 26˚C the preparation resistance was 28.8 ± 4.0 $\Omega \cdot cm^{-2}$, with a spontaneous potential difference of −2.2 ± 0.4 mV. The magnitude of the SCC response to 10 mM glucose was 27.0 ± 5.1 $\mu A \cdot cm^{-2}$. This response could be abolished by re-

moving glucose from the solution or by the addition of 0.1 mM phlorizin, a competitive inhibitor that binds at the glucose site of the Na^+-dependent glucose cotransporter. Elsewhere [4], we have shown that the effect of temperature on passive glucose accumulation in tissues is insignificant and therefore the concentration of glucose that passively accumulated in the tissues at 16˚C could be calculated by dividing the value obtained at 26˚C by 1.2 (the temperature coefficient of passive glucose transport [13]. In accordance with a generally accepted approach the active component of glucose accumulation (**Figure 1**, curve 1) was estimated [10] as the difference between the overall accumulation measured under aerobic conditions and the passive accumulation measured under anaerobic conditions. As can be seen in **Figure 1**, glucose active transport fell to zero as the temperature decreased from 32˚C to 16˚C. However, rather unexpectedly, it was found that although the stimulatory effect of glucose on active Na^+ transport (**Figure 1**, curve 2) fell 10-fold as the temperature decreased from 37˚C to 16˚C, it still remained rather strong. Since the period of incubation used in measurement of sugar accumulation was 60 min whereas SCC responses developed in 5 - 7 min, it was decided that comparison of these two measurements might be more useful if they were recorded over the same time scale. In special experiments we showed that increases in SCC seen after the addition of 10 mM glucose stay at the new level for over 40 - 50 min.

Based on the concept that glucose and Na^+ transport processes are not separable we chose the ordinate scales used in **Figure 1** so that curves 1 and 2 would coincide at high temperatures. It was important that glucose stimulation of active Na^+ transport could be recognized even at 7˚C.

A similar phenomenon was observed for glycine, and we were able to record SCC stimulation by glycine in the whole temperature range studied right down to 10˚C (**Figure 2**).

However, active glycine accumulation in the enterocytes was abolished at 20˚C (**Figure 3**).

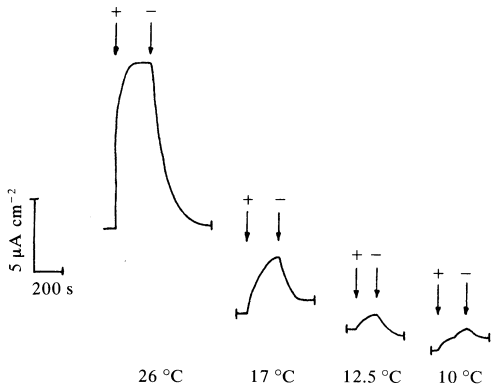

Figure 2. Effect of 10 mM glycine on active Na^+ transport (as measured by the short-circuit current, SCC) through the rat small intestine at different temperatures. Duration of experiment was 60 min.

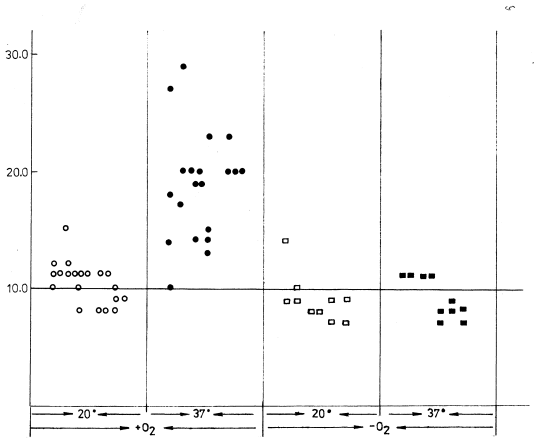

Figure 3. Glycine accumulation in rat accumulating mucosal preparations at 37˚C and 20˚C under aerobic (oxygenation) and anaerobic (nitrogenation) conditions. Each point represents the results for one preparation. Incubation was carried for 60 min. Glycine concentration in the solution was 10 mM.

The results from our preparations in which respiration and glycolysis were inhibited (**Figure 4**) resemble those of [14] with the following differences: a) in our experiments incubation with nutrients was started after 60 min incubation with inhibitors, whereas the experiments of Rose and Schultz [14] were already finished by this time; b) instead of 1 mM iodoacetate we used 24 mM NaF (an inhibitor of two enzymes involved in glycolysis); c) Rose and Schultz [14] found a 1.5-fold increase in electrical resistance after treatment, whereas we found a 1.5-fold decrease in the electrical resistance of preparations after treatment.

If after metabolic poisoning of the mucosa the potential difference across the preparation was held close to 0 mV, the SCC responses to glycine (**Figure 4(a)**, middle curve) or to glucose (**Figure 4(b)**, middle curve) were small if present at all. Phlorizin added after glucose was not effective. With a positive potential difference across the preparation (minus on serosa) a current flowed across it in the same direction as the SCC at the beginning of the experiment. The current flowing across the preparation increased when glycine (**Figure 4(a)**) or glucose (**Figure 4(b)**) were added. The relative magnitude of these responses was rather small (~1%) but they were specific because phlorizin lowered the increased current to close to its initial value (**Figure 4(b)**, upper curve). With a negative potential difference (minus on mucosa) on the gut wall the glucose and glycine responses were again seen but they were in the opposite direction (**Figure 4(a)** and **Figure 4(b)**, lower curves). These glucose responses were sensitive to phlorizin (**Figure 4(b)**, lower curve).

Similar experiments were carried out on the turtle small intestine. According to our data, the Ktfor glucose in turtles (1.2 mM) was approximately 4 times lower than that for glucose in rats (4.5 mM). Therefore positive and negative responses in this case were determined by the addition of 1 mM glucose to the mucosal solution. Such responses, dependent on the direction of the current, could be recorded several times from the same gut preparations from rats or turtles.

It should be noted that the responses observed in **Figure 4** could not be ascribed to nutrient-induced streaming potentials because the effects recorded had different signs despite nutrient addition to the same mucosal solution.

It is known that the voltage-current relationships of epithelial tissues are linear (**Figure 5**). Hence, to reconstruct this relationship it is sufficient to plot only two points. At a potential difference of 5 mV the magnitude of the current response to glucose was 20 times lower than the SCC response to glucose with the intact preparation at the beginning of the experiment. The slope of the voltage-current relationship of the effect (**Figure 5**) at positive and negative potentials was the same, namely 2 $k\Omega \cdot cm^{-2}$.

Thus, we have shown that after treatment of our preparations with NaF under anoxic conditions the absolute value of the current flowing across the preparations in response to glucose or glycine was increased irrespective of the sign of the potential difference. These responses were specific because for glucose they could be abolished by the highly specific inhibitor phlorizin.

3.2. Link between Stimulating Effects of Glycine and Glucose on the SCC

The SCC increases in response to the addition of glucose or glycine to a mucosal solution [3] [14] [15]. Thus, by adding various nutrients to a mucosal solution and calculating the value of SCC change, one can estimate the

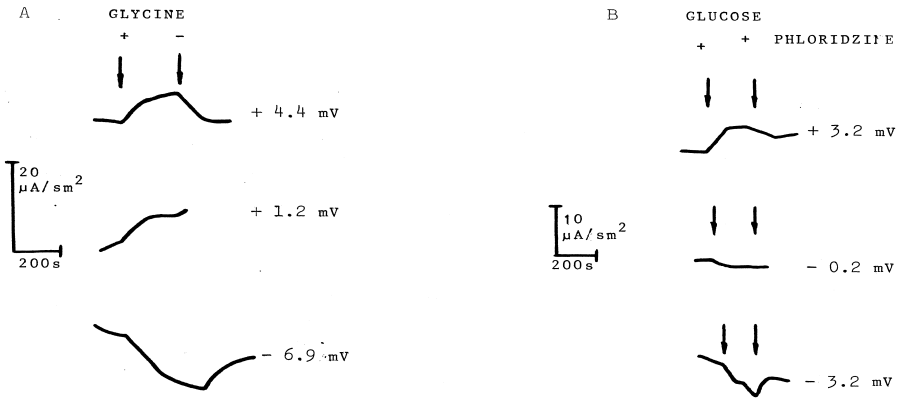

Figure 4. Depicts the data obtained with our preparation.

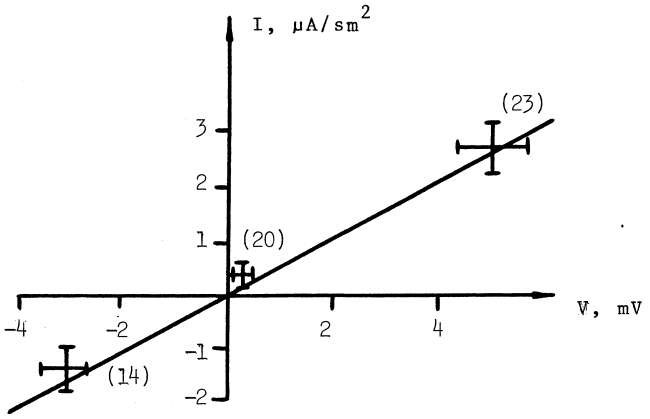

Figure 5. Dependence of current response (I) to the addition of 10 mM glucose at fixed potential differences (V) in preparations of rat small intestine treated with nitrogen and NaF. Mean voltage-current relationship for a number of experiments. The voltage is positive on the mucosal side of the preparation. Vertical and horizontal bars denote standard errors of the mean for the current and potential difference respectively. A number of determinations are given in parentheses.

quantity of coupled transporters for a corresponding nutrient [3]. In studies [15], a preparation was tested by recording on it the pairs of SCC responses to 10 mM of glucose and 40 mM of glycine. Such responses varied in magnitude by more than a factor of ten, although a good correlation (r = 0.995) between SCC changes in response to the addition of glucose and glycine was observed, and the equation of linear regression looks like $Y = -1.1 + 0.68x$.

In the following series of experiments (**Table 1**) [15], the total value of SCC response to 10 mM of glucose + 10 mM of glycine was recorded. The registration of the total response began at the addition of glycine or glucose. The value of total SCC change in the beginning increases and decreases slightly by the end of the experiment. The relative value of stimulating effects of glycine and glucose (in comparison with value of total effect) remains constant, (*i.e.*, the percentage composition of the total response during the experiment does not change).

The value of SCC changes on the total addition of two nutrients does not depend on the order in which they are added [15]); on 10 mM of glucose it is equal to 58.5 μA/cm^2, and on 10 mM of glycinein the presence of glucose result in an additional increase by 6.7 μA/cm^2. The value of SCC changes upon the addition of 10 mM of glycineequals 14.7 μA/cm^2, and the addition of 10 mM of glucose in the presence of this amino acid result in an additional increase in SCC of 47.0 μA/cm^2. Hence, the value of the effect of glycine on the background of glucose decreases by a factor of two, and the value of SCC change on glucose in the presence of glycine decreases by 19% **Table 1**.

Hence, there is a link between SCC changes on glucose and glycine. It is of interest to find out just how strong is this link and whether there are any conditions under which it could be broken.

In experiments *in situ*, it has been established [3] [15] **Table 2** that in preparations from controlsegments (preincubation *in situ* without nutrients) of an intestine, the value of effects of glycine and glucose are equal to 13.8 and 23.7 μA/cm^2 (ratio 0.58), respectively. In the neighboring segments preincubated with glycine, distinctions between these effects tend to increase 15.7 and 36.5 μA/cm^2, respectively (ratio 0.42). On the contrary, for the preparations preincubated with glucose, the value of the effects of glycine and glucose become almost identical—24.3 and 21.3 μA/cm^2, respectively (ratio 1.14). The ratio of stimulating effects of glycine and glucose in control preparations is taken as a unit; then after the treatment of preparations by glycine this ratio decreases by a factor of 1.4; and after treatment by glucose it increases by a factor of two. It is significant that in both cases the change in this ratio is achieved mainly due to an increase in SCC response to addition of the nutrient which was initially absent in a preincubation medium [3].

4. Discussion

Cellular glucose concentration is a function of three parameters-rates of influx, efflux, and metabolism. The temperature effects shown in **Figure 1** were not due to changes in glucose efflux because the two experimental

Table 1. Changes of short-circuit current on glucose and glycine additions across rat small intestine measured in the presence of glycine and glucose in mucosal solution, respectively [3].

1st addition	2nd addition	Changes of SCC responses on nutrient addition, μA/cm²	Changes of SCC responses on both additions, μA/cm²
Glucose, 10 mM		58.5 ± 10.4 (16)	69.9 ± 11.7 (16)
	Glycine, 10 mM	6.7 ± 1.5 (16)	
Glycine, 10 mM		14.7 ± 3.2 (15)	62.2 ± 12.8 (15)
	Glucose, 10 mM	47.0 ± 3.2 (15)	

Table 2. Effect of preincubation (30 minutes) of rat small intestinal segments *in situ* in the presence of 1.5% glycine and 5% of glucose on responses of short-circuit current through preparations isolated from the corresponding intestinal segments on addition of 10 mM glucose and 10 mM glycine [3].

Nutrient and its concentration (%) in the intestinal preincubation solution *in situ*	0 (control)		Glycine, 1.5%		Glucose, 5%	
Addition to the experimental using chamber	Glucose, 10 mM	Glycine, 10 mM	Glucose, 10 mM	Glycine, 10 mM	Glucose, 10 mM	Glycine, 10 mM
SCC response, μA/cm²	23.7 ± 8.4 (3)	13.8 ± 3.9 (3)	37.7 ± 13.7 (3)	15.7 ± 8.3 (3)	21.3 ± 10.8 (3)	24.3 ± 11.1 (3)
Ratio of SCC responses to glycine and glucose	0.58		0.42		1.14	
Change of ratio of SCC responses relatively to control	1.0		0.72		2.0	

approaches used gave similar resu1ts; namely, the traditional everted sac technique and the accumulating mucosal preparation technique, where the glucose efflux stage is absent. The difference between the curves for SCC and glucose accumulation below 26˚C **Figure 1** may have been due to a relative acceleration in absorbed glucose metabolism. Enterocyte glucose is metabolized irrespective of its origin, *i.e.* whether transported actively or passively. Hence, acceleration of metabolism should be observed from the temperature dependence of passive glucose accumulation. But we [4] and [13] have failed to observe this effect.

Moreover, active glucose accumulation across the apical membrane was calculated by subtracting passive glucose uptake measured under anaerobic conditions from total glucose uptake, and in both cases cell glucose was metabolized. Thus, in the calculation of active glucose accumulation, the effect of metabolized glucose should be partly cancelled. The smaller the amount of active glucose accumulation (*i.e.* at lower temperatures), the more precise is the cancellation of the effect of metabolized glucose. Finally, we have studied active glucose accumulation in tissues by the method of [11], which permits both the glucose and the fructose content of tissues to be determined. These data also indicate a switching off of active glucose uptake at around 16˚C - 18˚C.

So, the difference between the temperature curves for SCC and glucose accumulation was not due to glucose efflux or metabolism. The question arises whether any additional transport mechanism exists to explain this difference. Hajjar *et al.* [16] have described a mechanism of nutrient uptake by metabolically poisoned cells where the nutrient enters the cell as a result of a Na^+ concentration gradient. It is therefore quite possible that we underestimated glucose uptake. However, it should be noted that a) for this mechanism to operate, the Na^+ concentration gradient should be directed into the cells and that under our conditions this gradient was probably absent; and b) the Na^+ concentration difference once generated on the brush border could probably maintain additional nutrient transport for only 15 min, whereas we used 60 min periods of incubation. The differences between curves 1 and 2 in **Figure 1** might arise if at lower temperatures such a mechanism brought about the efflux of nutrients from the enterocytes. But according to [4] [16] the efflux of nutrients and sodium under these circumstances remains coupled and hence both SCC and active glucose accumulation would be underestimated to the same degree. Hence, the glucose accumulation curve in **Figure 1** (curve 1) can be considered to reflect active glucose transport. On the other hand, the SCC responses (**Figure 1**, curve 2) were not overestimated because of artefacts such as streaming potentials set up by the asymmetric glucose concentration, because all additions to the mucosal fluid were isoosmotic.

4.1. The Two-Pathway Transporter

The generally accepted models for cotransportation of Na^+ and nutrients [1] [3] [14] [17] [18] preclude the possibility of uncoupling of Na^+ and nutrient transport, and this point has experimental support. According to this theory, transport is associated with the formation of a ternary complex and there is coupled transfer of glucose and Na^+ by a common transporter to the internal surface of the plasma membrane. The coupling of Na^+ and nutrient transport is considered not separable and therefore if one of these processes, for example glucose transport, is inhibited there will also be inhibition of Na^+ transport by the common transporter. This prediction was tested by blocking the glucose binding site with phlorizin. It was hypothesized that elimination of glucose transport on membrane cooling should be accompanied by elimination of the glucose-induced stimulatory effect on the SCC (*i.e.* Na^+ transport). This phenomenon was not seen in our experiments. We did, however, show that uncoupling of Na^+ and glucose transport took place at temperatures below 16˚C **Figure 1**.

Apart from this, uncoupling of glucose transport from the stimulatory effect of glucose on Na^+ transport was found under the same conditions. Attempts to explain the two types of uncoupling discovered in this study in terms of generally accepted models run into serious difficulties. The model for a Na^+-glucose cotransporter presented in **Figure 6** offers a more adequate explanation. It consists of two parallel interacting pathways, one for Na^+ and one for the nutrient (e.g. glucose or glycine), as well as a surface gating mechanism for binding nutrient at the entrance to the transport system. The transport cycle in our model is characterized by the following states: a) nutrient and Na^+ pathways are not initially active; b) the Na^+ pathway is activated by the binding of nutrient at the allosteric site on the gating mechanism (this stage is similar to that seen in the control of Na^+ permeability by acetylcholine) and Na^+ moves from the extracellular to the intracellular fluid; c) on Na^+ transfer along the pathway the nutrient pathway is allosterically activated; d) the nutrient molecule initially bound to the gating mechanism is transported through the activated nutrient pathway; e) dissociation of nutrient from the gate mechanism is followed by inactivation of the Na^+ pathway. Reactivation of the Na^+ pathway occurs when another nutrient molecule binds at the allosteric site. At temperatures below 16˚C - 20˚C the nutrient pathway is inactivated but the Na^+ pathway is operational.

Moreover, our two-pathway model can account for various phenomena that cannot be satisfactorily explained by other models [1] [2] [17]. The two-pathway transporter has the ability to separate the opening of the Na^+ pathway after nutrient adsorbtion onto the gate mechanism from the energization stage of nutrient transfer along the nutrient pathway. Hence, our two-pathway model predicts that when coupled nutrient transport is blocked, the addition of glucose or glycine to the mucosal fluid will be followed by opening of the Na^+ pathway. According to current views, spontaneous active Na^+ transport and the stimulatory effects of glucose and glycine on Na^+ transport disappear after inhibition of enterocyte respiration and glycolysis. Active transport processes in such cells are therefore blocked and the equivalent electric circuit for a preparation treated in this way can be represented as shown in **Figure 7**. It is clear that zero current through the preparation does not change after the

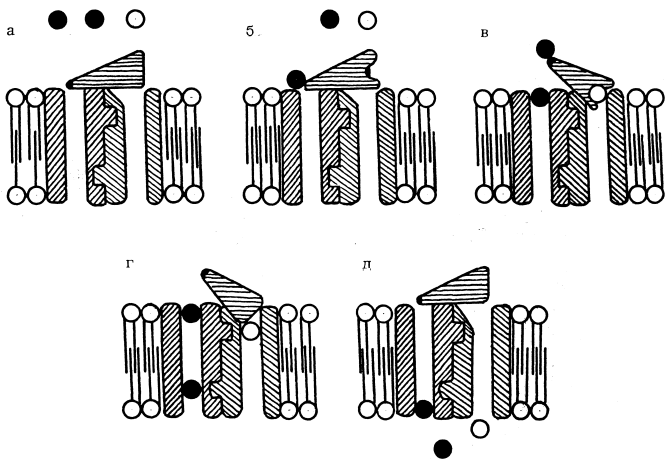

Figure 6. Theoretical physiological model for a two-pathway transporter for Na^+ and some nutrients. Horizontal hatching depicts the gate mechanism, diagonal hatching the pathways.

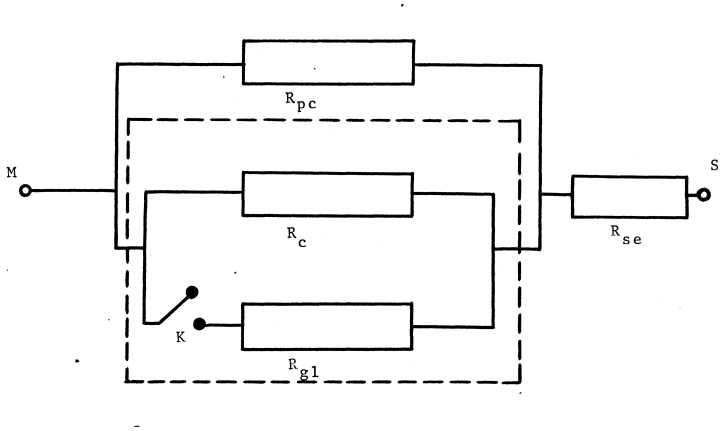

Figure 7. An equivalent direct-current electric circuit for a small intestine preparation treated with 1 mg·ml^{-1} NaF under anoxic conditions. Rpcparacellular pathway resistance; Rc, transcellular resistance; Rgl, glucose-or glycine-sensitive pathway resistances; Rse, subepithelial tissue resistance. Key "K" is switched on after nutrient addition and off after nutrient removal or after addition of a specific inhibitor (e.g. ph1orizin) to the solution. "M" and "S" refer to solutions bathing the mucosa and serosa, respectively.

glucose-or glycine-sensitive pathway resistance (Rgl) is switched on. The specific responses of the preparation to glucose or glycine can nevertheless be recorded while maintaining a positive or negative potential difference across it. Some of the current flowing through the preparation (~1%) is due to Na^+ transport across the absorbing mucosal cells. While maintaining any potential difference between the mucosal and serosal bathing solutions, other than zero, switching on resistance Rgl due to opening of the glucose-or glycine-sensitive pathway in the apical membrane would cause an increase in the absolute value of the current. If the resistance of the apical membrane is higher than that of the basolateral membrane, then the voltage-current relationship shown in **Figure 6** exactly reflects the properties of the apical membrane. The resistance 2 kΩ·cm^{-2} due to the glucose-sensitive Na^+ pathways agrees well with the resistance of the galactose-sensitive Na^+ channels (1.7 kΩ·cm^{-2}) in the apical membrane measured on intact small intestine [19].

This agreement of our results with published data, as well as our results on the inhibition of glucose-sensitive Na^+ conductivity by phlorizin, indicate that current responses to nutrients **Figure 4** are determined by Na^+ transfer through the cotransporter. It is of interest that Na^+ can be transported in both directions with the same efficiency-from the mucosal fluid to enterocytes and from enterocytes into the mucosal fluid.

Thus, our data, particularly those on Na^+ transport from enterocytes to the mucosal fluid induced by the addition of glucose or glycine to the mucosal fluid, indicate that a two pathway model for a coupled cotransporter is most probable. The existence of Na^+-coupled nutrient transport is very common in nature. The widespread occurrence of Na^+ coupled transport, its high specificity, and its variations may be explained easily in terms of our two pathway model. Recently, the protein topography of the glucose transporter was established [5]. This protein crosses the membrane eleven times. We consider that both pathways in our two pathway model are either separate regions of this protein or parts within the quaternary structure of the active enzyme oligomer, since the glucose carriers in the small intestine occur as tetramers (B Stevens 1989, personal communication). The gate or regulatory mechanism is probably a special region of the protein that binds glucose and phlorizin on the outer surface of the membrane.

4.2. The Multipathway Transporter

On the other hand, such a parallel two-pathway cotransporter model finds an unexpected strong support [6]. In this study (**Figure 1**), where the structure of hSGLT1 viewed in the membrane plane and from the intracellular side is presented, one can clearly see that bounded galactose and Na^+ in the middle of the cotransporter are separated from one another by 2 - 3 helices! Then, authors note that structural alignment with LeuT reveals a possible Na^+-binding site at the intersection of TM2 and TM9, ~10 Å away from the substrate binding site. This is quite a large distance. According to [20], the cross-section of the sodium channel is 3 Å × 5 Å. Hence, at a distance of 10 Å, two channels like sodium one can be placed.

Authors [6] suggest that the intracellular exit pathway appears as a large hydrophilic cavity blocked by the intracellular gate residue (Y263 on TM7E). The last **Figure 4** in [6] shows that the studied cotransporter works according to a one-pathway model (see above), which is not consistent with **Figure 1**, where it is clearly visible that Na^+ and glucose in the center of the core are spatially separated. In our view, there are direct evidence in favor of the two-pathway parallel model, such as Na^+ and galactose entering into a large hydrophilic cavity together then being transferred via two differ rent pathway.

The feature of the offered cotransporter is the separation of a stage of opening of a sodium transporter upon binding of a glucose molecule with a gate mechanism from a stage of energization of a glucose molecule transfer along its transporter. From this feature, it follows the attribute which distinguishes it from models discussed earlier, which have a common carrier and transporter. The multipathway model predicts what even under such states of a cell when the coupled glucose and sodium transport stops, addition of glucose in a mucosal solution will result in opening of a sodium transporter. For the purpose of verification of this prediction, experiments were carried out on preparations in which respiration and glycolysis were suppressed [3].

Finishing his fundamental review on amino acid transport across mammalian intestinal and renal epithelia and discussing the issue about how glycine, proline, and the β-amino acids across the basolateral membrane, the author [21] raises some very interesting high-priority and reasonable issues without the solving of which it is difficult to understand this matter: “Do epithelial transporters form complexes in the membrane? Are they held in place by scaffolding proteins?”

Scaffoldings are an important family of scaffolding proteins that assemble a variety of cellulases into the so-called cellulosome, a microbial extracellular nanomachine for cellulose adhesion and degradation. These proteins anchor the microbial cell to cellulose substrates, which makes their connecting region likely to be subjected to mechanical stress. Scaffoldings are noncatalytic structural proteins of the cellulosome, a multienzyme, cell-surface complex required for adhesion and degradation of crystalline cellulose, a particularly recalcitrant substrate. Scaffolding proteins act as a molecular Lego, binding a number of cellulases through its type I cohesin (cohesin I) modules to spatiotemporally regulate the efficiency of the entire enzymatic cascade [22].

Therefore, we consider the issues raised above [21] to be very accurate and timely. And in turn, we can confirm there importance by the following facts and considerations.

The presence of a strong link between SCC changes on glycine and glucose in each preparation points to the fact that glucose and glycine transporters may be incorporated in one quaternary structure (perhaps with the help of scaffolding-like or tetraspanin-like proteins) with the same type of sodium transporters. This is rather surprising because the transport system for glycine (as for other amino acids) is pretty much duplicated. So, there are four different transporters for glycine, proline/hydroxyproline, namely, 1) a common transporter for all three amino acids in the kidney; 2) a common transporter for all three amino acids in the intestine; 3) a specialized transporter for glycine; and 4) a specialized transporter for proline/hydroxyproline.

It should be mentioned that a significant fraction of proline and glycine transport in both kidney and intestine is mediated by the neutral amino acid transporter B^0AT1 (SLC6A19). As a result, five transporters contribute to the transport of these three amino acids. The common transporter for all three amino acids in the intestine is the proton amino acid transporter PAT1 [21]. By taking into account the existence of a strong relationship between Na^+-dependent transport of glycine and glucose (see above), one can offer two types of such transporters: two separate two-pathway transporters (sodium-glucose and sodium-glycine). There are direct evidence in favor of the two-pathway parallel model, such as Na^+ and galactose entering into a large hydrophilic cavity together then being transferred via two differ rent pathway [6] or the three-pathway transporter (sodium-glucose-glycine). Because the idea of spatiotemporally complexes of transporters (used in [9] and discussed so far [21] has received strong support in the form of detection of scaffoldings, hypothesis of the existence of quaternary structures of transporters does not seem quite incredible. Therefore, the scaffolding protein PDZK1 provides spatiotemporal coupling of cAMP transporter to CFTR chloride channel function in the gut epithelia [23].

The latter, three-transporter type of transporters seems more probable.

If all three transporters (for Na^+, glucose and glycine) can unite in a single structure, then there should be “competitive relations” between SCC changes on glycine and glucose for sodium ions passing through its transporter. That conclusion is supported by experiments using the addition of glucose and glycine on backgrounds of glycine and glucose, respectively [3] **Table 1**. On a background of glucose a response to glycine decreases by 8.0 $\mu A/cm^2$, and on a background of glycine a response to glucose decreases by a similar value (11.5 $\mu A/cm^2$); but, at the same time, the first responses to 10 mM of glycine are less by a factor of four than res-

ponses to 10 mM of glucose! It is not likely that development of the response to the second addition is inhibited because of a depolarization of a brush-border membrane followed by the first one, since the depolarization of a membrane in this case is insignificant. It is also unlikely that the work of sodium transporters of the glycine transporter (Kt = 40 mM) with the rate 20% (10mM/ (10 mM + 40 mM)) of the maximal one can cause the same changes of a membrane potential or of a chemical gradient of sodium as switching on of sodium transporters of the glucose transporter (Kt = 5 mM) for the near maximal rate (70%) (10 mM/(10 mM + 5 mM)). However, switching on of the smaller part of sodium transporters of the glycine transporter (20%) and the greater part of sodium transporters of the glucose transporter (70%) followed by decline the SCC response to the addition, respectively, of glucose and glycine, respectively, with a close efficiency. Hence, the competition between glucose and glycine occurs for sodium passing through the common transporter or for interaction with the sodium transporter.

The above viewpoint is in agreement with the universally accepted one that the competition between amino acids and sugars in their transport occurs because of a competition for a gradient of electrochemical potential of sodium on a brush-border membrane or even for a membrane potential only [14] [24]. In actuality, sodium transport through its transporter will depend on the value of its gradient of electrochemical potential.

The time-dependences of SCC responses to the addition of glucose and glycine are the same: they are increased and decreased simultaneously [3]. It is difficult to explain these data if we suppose that the induction of additional sodium transport in the presence of glycine or glucose occurs due to two separate two-transporter molecular machines. However, if the work of glucose, glycine, and sodium transporters is probably coupled (maybe due to scaffolding-like protein) as is the case of coupling of cAMP transporter to CFTR chloride channel function in the gut epithelia [19], the above data are easily explainable [3].

In actuality, if permeability of the common sodium transporter for some reason increases or decreases with time, it will result in a corresponding simultaneous increase or decrease in SCC changes on glycine and glucose. It should be mentioned that a significant fraction of proline and glycine transport in intestine is mediated by the neutral amino acid transporter B^0AT1 (SLC6A19) [25]. Na^+-dependent transport of glucose in intestine is mediated by SGLT1. Therefore we must assume that both the transporter B^0AT1 and SGLT1 during their operation in the small intestine must somehow interact (may be due to scaffoldings-like protein?). It would not be surprising if it turns out that the enzymes formed a quaternary structure with scaffoldings can influence each other's work. This is a risky assumption. It is contrary to some known facts. But as it was pointed above some transporters may be united in such spatiotemporal complexes **Figure 8**.

If we admit that this assumption is correct, we can assume that in the case of prolonged transportation of one nutrient, transport of another one can be stimulated. This prediction is supported by experiments *in situ* [3] [15]. The preincubation of intestine segments with glycine result in substantial growth of SCC changes (through a preparation obtained from that intestine segment) on glucose. Similarly, upon preincubation of intestine segments with glucose, SCC responses to glycine **Table 2** are considerably increased. These results can be explained in the following way [3] [15]. Cycling of transporterproteins between intracellular storages and a plasmatic membrane seems to be a widespread process [26]-[28]. This way the transport of glucose transporters from a Golgi complex on the adipocyte membrane transition under the action of insulin is revealed [29] [30].

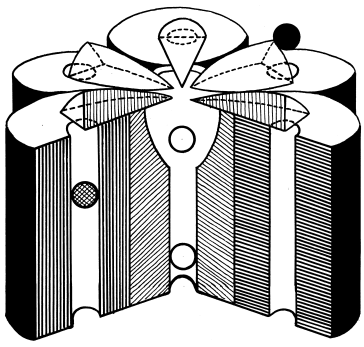

Figure 8. Theoretical physiological model for a multipathway transporter for Na^+ (white circle) and two nutrients-glucose (black circle) and glycine (gray circle).

Additional sodium transporters can appear in an apical membrane of enterocytes under the action of some agents such as the antidiuretic hormone [31].

One is inclined to think that such a cycle occurs for all transporters of a multipathway cotransporter—for glucose, glycine, and sodium. In actuality, it is a well-known phenomenon of up-regulation of glucose transport in response to the increased loading by carbohydrates [32]. Upon preincubation *in situ* with glycine, part of the glycine transporter degrades [3] [15]; the "damaged" glycine transporter can remain in the structure of the multi-pathway transporter or dissociate from it. The glucose transporter of the transporter meanwhile is not used, and therefore remains intact. With use of such "damaged" glycine transporters, a constant rate of glycine transport is maintained due to delivery of new transporters to a brush-border membrane. Owing to fusion with a brush-border membrane of additional transporters some time after the beginning of preincubation with glycine, the SCC response to glucose increases.

Apparently, *in vitro* the nutrient-dependent sodium transport can be "damaged" in the process of use [3] [15]. When in a mucosal solution, the part of the multi-pathway transporter carrying out transport of glucose constantly works, and the part carrying out transport of glycine remains inactive. Testing the state of these multi-pathway transporter components by adding an additional quantity of glucose or glycine to a solution has revealed that, in actuality, the value of an additional stimulating glucose effect relative to a stimulating effect of glycine gradually decreases down to zero. It is significant that the disappearance of such relative effect of glucose is not caused by an exhaustion of power resources of a cell, since responses to glycine at this time are maintained.

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