PN 1247

The interaction between active sodium transport and active sugar transport in the isolated rabbit ileum*

Recent investigations have demonstrated that the active transport of sugar by the small intestine is dependent upon the presence of Na^+ in the bathing medium[1], that the rate of sugar transport by hamster intestine is directly dependent upon the Na^+ concentration in the bathing medium[2], and that sugar transport is inhibited by agents which inhibit active Na^+ transport[3]. Conversely, it is well recognized that active Na^+ transport by the small intestine is dependent upon the presence of glucose in the bathing medium. This dependence has been attributed to the function of glucose as a source of metabolic energy for the active-transport process[4]. The present communication is concerned with the results of recent experiments which indicate that active Na^+ transport by isolated segments of rabbit ileum is stimulated by the active transport of sugar *per se* (as distinguished from its dependence upon metabolic energy), and suggests that a reciprocal interaction exists between the mechanisms responsible for the active transport of Na^+ and sugars.

New Zealand white rabbits, weighing approx. 2.5–4 kg, were anesthetized by the intravenous administration of nembutal. After opening the abdomen, the terminal 5 cm of ileum was excised and rapidly opened by cutting along the mesenteric border. The exposed mucosal surface was then rinsed free of intestinal contents and the tissue clamped between two lucite chambers. Perfusion and aeration of each surface was accomplished by means of a water-jacketed, gas-lift circulating system employing a 95 % O_2–5 % CO_2 gas mixture. The salt composition of the bathing solution was: 137 mM NaCl, 5.0 mM KCl, 2.5 mM $CaCl_2$, 2.2 mM $MgCl_2$, 1.1 mM Na_2HPO_4, 0.2 mM KH_2PO_4, and 2.5 mM $NaHCO_3$. The solutions were maintained at 37° by means of a constant-temperature circulating pump connected to the water jackets.

The transmural potential difference and short-circuit current (I_{sc}) were determined using methods similar to those employed in the frog skin[5], frog large intestine[6], and the toad colon and guinea-pig cecum[7]. In the case of the rabbit ileum we have demonstrated[8] that, under the conditions described above, and in the presence of 11 mM glucose, the transmural potential difference is approx. 8 mV, serosa positive. We have also shown that the I_{sc} is in good agreement with the net transport of Na^+ from the mucosal to the serosal solutions both in the presence and absence of glucose.

In Fig. 1 are shown the results of an experiment in which the mucosal and serosal surfaces were initially perfused with a buffer solution from which glucose was omitted. As was expected, the I_{sc} was considerably lower than that obtained in the presence of glucose[8]. Following the addition of glucose to both the mucosal and the serosal solutions (final concentration 10 mM) there was a rapid increase in the I_{sc}. Further experiments have indicated that the increase in both the I_{sc} and the transmural potential difference commences within 5 sec after the addition of glucose (to the solutions in the reservoirs) and reaches a maximum within 1 min.

Although the direction of the response was not unexpected, the rapidity of the glucose effect suggested a function other than that of a metabolic energy source. In Table I we have summarized the results of a series of experiments designed to

* The views expressed herein are those of the authors and do not necessarily reflect the views of the U.S. Air Force or the Department of Defense.

determine whether the rapid effect on the I_{sc} is the result of either the entrance of the sugar into metabolic pathways, with the subsequent production of ATP, or the active transport of the sugar *per se*. It should be noted that a rapid increase in the I_{sc} is observed only when the added sugar is one which is actively transported by the intestine. Thus, fructose, which is metabolized by the intestine but which is not actively transported, has no effect on the I_{sc}. On the other hand, 3-*O*-methylglucose which is not metabolized but is actively transported produces a rapid rise in the I_{sc}.

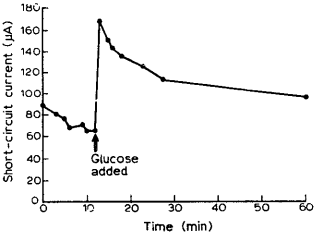

Fig. 1. The effect of glucose on the short-circuit current of an isolated segment of rabbit ileum.

Furthermore, phlorizin ($5 \cdot 10^{-4}$ M) which inhibits the active transport of sugars but does not affect the passive movements of these sugars, prevents the stimulatory effect following the addition of either glucose, 3-*O*-methylglucose or α-D-methylglucose. If phlorizin is added after the I_{sc} has been stimulated by the addition of an actively transported sugar, metabolized or not, an immediate rapid decline in the I_{sc} is observed. Finally, the addition of an actively transported sugar to the serosal side

TABLE I

EFFECT OF SUGARS ON THE SHORT-CIRCUIT CURRENT IN ISOLATED RABBIT ILEUM*

*Sugar***	*Active transport*[9]	*Metabolized*[10]	*Effect on* I_{sc}***
Glucose	Yes	Yes	+
Galactose	Yes	Yes	+
3-*O*-Methylglucose	Yes	No	+
α-D-Methylglucose	Yes	—	+
Fructose	No	Yes	o
Mannose	No	Yes	o
Ribose	No	Yes	o
6-Deoxygalactose	No	—	o
2-Deoxyglucose	No	No	o
Glucose + phlorizin§	No	—	o

* The sugars (final concentration 10 mM) were added simultaneously to the reservoirs containing the mucosal and serosal perfusing solutions.

** All chemicals used were reagent grade. The 3-*O*-methylglucose and α-D-methylglucose were demonstrated to be glucose free by means of paper chromatography[11,12].

*** A positive effect (+) on the I_{sc} indicates a rapid increase to 50% or more over the base-line value commencing within 10 sec after the addition of the sugar to the perfusing solutions; o indicates no change in the I_{sc} following the addition of the sugar.

§ Phlorizin ($5 \cdot 10^{-4}$) was added 5 min prior to the addition of the glucose.

alone has no effect on the I_{sc} whereas the addition of these sugars to the mucosal side alone results in the rapid rise in the I_{sc} which we have described.

Previous investigations, cited above, have indicated that active sugar transport by the intestine is stimulated by the presence of Na^+ in the bathing medium. The observation that ouabain inhibits active sugar transport suggests that active Na^+ transport and active sugar transport are somehow linked; however, the possibility that ouabain independently inhibits active sugar transport has not been excluded. The present observations that the short-circuit current is markedly stimulated by the addition of an actively transported, non-metabolized sugar to the solution bathing the mucosal surface clearly establishes an interaction between the mechanisms responsible for the active transport of Na^+ and sugar. These results further indicate that in addition to the role of glucose as a source of metabolic energy for active-transport processes, the active transport of glucose *per se* stimulates active Na^+ transport in the isolated rabbit ileum.

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PN 1243

Further investigations on the carbohydrate moiety of egg albumin

It has been suggested that the bond linking carbohydrate to protein in egg albumin is that of an *N*-(β-aspartyl)glycosylamine[1,2] of *N*-acetylglucosamine[3–5]. Partial acid hydrolysis of a purified glycopeptide from the protein gave rise to small amounts of a substance, containing glucosamine and aspartic acid but no mannose, which behaved electrophoretically and chromatographically like 2-acetamido-1-β-(L-β-aspartamido)-1,2-dideoxy-D-glucose[6]. Further high-tension paper-electrophoretic studies (Whatman 3 MM, 40 V/cm, 60 min, pH 1.87) of partial acid hydrolysates (2 N HCl, 12 min, 100°) of the latter compound and of the glycopeptide from egg albumin have revealed a great similarity in the "fingerprint" obtained by staining the paper strip with the ninhydrin reagent described previously[7] (Fig. 1). In this Figure, Spot 2 in each case