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Received Nov. 25, 1968. P.S.E.B.M., 1969, Vol. 131.

Enhancement of Net Sodium Transport in Isolated Bullfrog Intestine by Sugars and Amino Acids (33801)

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Many examples of coupling between the transport of sodium and that of other solutes, including sugars and amino acids, by mammalian small intestine have been reported (1–3). As yet, however, the nature of the mechanisms involved in these coupling phenomena remains largely obscure (3). Recent studies have suggested that the isolated small intestine of the bullfrog is a particularly useful model system for the *in vitro* investigation of coupled transfer processes. Levin (4) has shown that this preparation maintains a remarkably stable transmural potential difference (pd) for several hours *in vitro*, both in chloride and in sulfate media, that the potentials developed under these conditions are increased by actively transported sugars and amino acids, and that the effects of sugars and amino acids on the transmural pd are additive. These results have been confirmed and extended (5). In addition, it was shown that the effects of sugars and amino acids on transmural pd are paralleled by equivalent increases in short circuit current (i.e., these substances do not cause any significant change in tissue resistance). In sulfate media, short circuit current was found to correspond to net sodium transport (5, 6) so that, under these conditions, measurements of short circuit current can be used to estimate the net rate of sodium transfer and the effects on it of added solutes. The present paper reports the results of a preliminary survey of the effects of a number of sugars and amino acids on the short circuit current across the isolated small intestine of the bullfrog, under conditions where this current corresponds to net sodium movement.

Materials and Methods. Adult bullfrogs were used in this investigation. The animals were stunned by a blow on the head, the abdomen was opened, and a segment of intestine either immediately proximal or distal to the hepato-pancreatic duct was removed rapidly and transferred to oxygenated Ringer's solution. The segment was opened lengthwise and mounted between the two halves of a conventional Ussing chamber (7) having a circular aperture with an area of 0.33 cm^2 between them. Both sides of the chamber were filled with equal volumes of identical Ringer's solutions. The chamber contents were maintained at 26 $\pm$ 0.2° and continuous circulation and oxygenation of the solutions in both halves of the chamber during the experiment were effected as described by Ussing and Zerahn (7).

The bathing solutions were phosphate Ringer's of the type described by Adrian (8)

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and contained 106 m*M* Na, 2.5 m*M* K, 1.8 m*M* Ca, and 3 m*M* phosphate. All chloride ions in these solutions were replaced by sulfate ions. The total osmolality was maintained at 230 mOsm by the use of mannitol and the pH was adjusted to 7.2 using small amounts of Tris [tris(hydroxymethyl)amino methane] where necessary.

In initial experiments, transmural pd and short circuit current were measured as described by Schultz and Zalusky (9) except that agar bridges from calomel half cells, rather than Ag/AgCl electrodes, were used to lead in external current for short-circuiting. In later experiments an automatic voltage clamp device was used (10). This device allows for compensation of the potential drop, due to the resistance of the bathing medium, between the potential sensing electrodes. In each experiment, the system was checked for leaks and for electrical offset between the potential sensing electrodes.

The experimental procedure was as follows: The tissue was allowed to come to equilibrium between identical Ringer's solutions without substrate and the steady state open circuit transmural pd and short circuit current were recorded. When the short circuit current had stabilized a small measured aliquot of a stock solution containing the substrate under investigation was added to either or both sides of the chamber and the resulting changes in transmural pd and short circuit current were recorded until a new steady state was established. At this time a further aliquot of stock solution was added and the process was repeated. This was continued until further addition of substrate resulted in no measurable increment in transmural pd or short circuit current. Stock solutions of substrate were prepared in substrate free Ringer's solution containing mannitol. Thus, sequential additions of substrate were made without changing either the sodium concentration or the total osmolality of the bathing medium.

The substrates investigated were D-glucose, D-galactose, 3-O-methyl glucose, L-alanine, L-valine, and L-cysteine. Reagent grade chemicals and on line distilled water which had been further purified by passing it through a mixed bed ion exchanger were used throughout.

Results. Addition of actively transported solutes to the solution bathing the serosal surface of the isolated bullfrog intestine did not, with one exception, alter the potential difference or short circuit current across the tissue. The exception was glucose. Occasionally, addition of glucose to the serosal solution produced a small increase in short circuit current. For example, in one experiment addition of glucose in a final concentration of 4 m*M* to the mucosal solution alone caused an increase, over the value observed in substrate free solution, of 9.1 neq/cm^2/min in the short circuit current[2]. The same concentration of glucose, added to the serosal solution only, caused an increase of 0.6 neq/cm^2/min. These small, probably metabolic, effects of serosal glucose were not further investigated and, in most experiments, substrate was added in equal amounts to both the mucosal and the serosal bathing solutions.

It became apparent early in the investigation that as long as the sodium concentration of the medium was kept constant, the effects of any sugar or amino acid on transmural pd and on short circuit current were similar; in particular, both parameters were saturated at the same substrate concentration. For this reason, only the short circuit current data, which may be taken as representing net sodium transport under the conditions of the present experiments (5, 6) will be further considered.

The evaluation of the kinetic parameters for the effect of added solutes on short circuit current and, by inference, on net sodium transport, is illustrated in Fig. 1 and 2. These figures show the results of a typical experiment with L-alanine. In Fig. 1 the increase in short circuit current over the base line value in substrate-free solution (ΔI_{sc}) is plotted

[2] Since, as already reported (5), short circuit current and net sodium flux are equal in sulfate media, the former has been converted to an equivalent mucosal to serosal flow of monovalent cations (neq/cm^2/min) and is so expressed in the present paper.

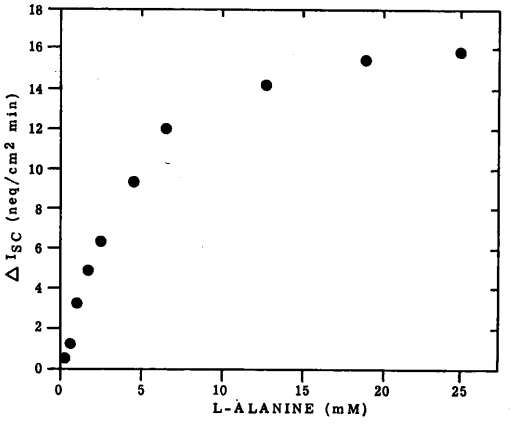

FIG. 1. Saturation of short circuit current response to L-alanine.

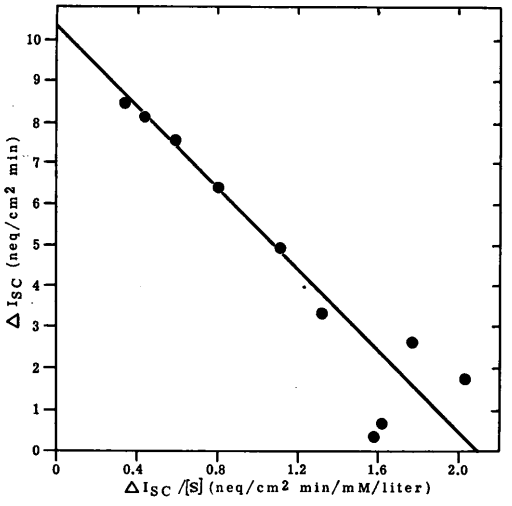

FIG. 2. Augustinsson plot of the data in Fig. 1.

as a function of alanine concentration. The saturable nature of the effect observed is clearly apparent. For this reason, as shown in Fig. 2, the data were further analyzed in terms of simple Michaelis-Menten kinetics.

Figure 2 shows an Augustinsson plot (11, 12) of the data from Fig. 1. This plot was chosen rather than the more usual double reciprocal plot of Lineweaver and Burk (13) because it gave a more even distribution of the experimental points as a function of the reciprocal of substrate concentration, thus avoiding the excessive weighting of statistical analyses of the data by points corresponding to low substrate concentrations which is a frequent consequence of the Lineweaver–Burk treatment (14, 15). The form of the Michaelis-Menten equation appropriate to the plot illustrated in Fig. 2 may be written

$$\Delta I_{sc} = (\Delta I_{sc})_{max} - \frac{\Delta I_{sc} \cdot K_m}{[S]}. \qquad (1)$$

Where ΔI_{sc} is the increase in short circuit current, over the baseline value, observed at a given substrate concentration $[S]$; $(\Delta I_{sc})_{max}$ is the maximum or saturation value of ΔI_{sc}, and K_m is the Michaelis constant. It is apparent from Eq. (1) that a plot of ΔI_{sc} as a function of $\Delta I_{sc}/[S]$ gives a straight line with an intercept on the Y-axis equal to $(\Delta I_{sc})_{max}$ and a slope equal to $-K_m$.

Essentially similar results to those shown in Fig. 1 and 2 were obtained in other experiments with L-alanine and in experiments with other transported solutes. The values of K_m and $(\Delta I_{sc})_{max}$ obtained by linear regression analysis of the data, plotted as illustrated in Fig. 2, are given in Table I which also includes the standard errors of the means for these parameters. The K_m values given in Table I for D-glucose, D-galactose, and L-alanine are in good agreement with those reported by Schultz and Zalusky (16, 17) for the effect of these substances on sodium transport across the isolated rabbit ileum.

If a second actively transported sugar was added to the mucosal bathing solution after maximal stimulation of the short circuit current by another actively transported sugar, no further effect was observed. However, it was found that a typical saturable increase in short circuit current could be obtained by adding glucose in the presence of a relatively high concentration of L-valine and vice versa. The kinetic parameters of the response of the short circuit current to L-valine did not appear to be altered significantly by D-glucose. For example, in an experiment in which serial additions of L-valine were made in the presence of 11 mm of glucose the K_m was 3.0 mM and the $(\Delta I_{sc})_{max}$ was 8.9 neq/cm^2/min. In another experiment carried out in the presence of 20 mM glucose, the values obtained for these parameters were 4.2 mM and 5.3 neq/cm^2/min, respectively.

TABLE I. Kinetic Parameters of Stimulation of Na Transport by Sugars and Amino Acids.

Substrate	K_m (m*M*)	$(\Delta I_{sc})_{max}$ (neq/cm²/min)	No. of expts.
D-Glucose	2.8 ± 0.7	11.7 ± 3.2	6
D-Galactose	16.3 ± 1.7	17.7 ± 2.7	3
3-*O*-methyl glucose	6.6	37	1
L-Alanine	5.3 ± 0.3	19.8 ± 5.7	3
L-Valine	3.5 ± 0.6	6.0 ± 1.3	4
L-Cysteine	1.1	15.3	1

On the other hand, valine appeared to have an effect on the stimulation of the short circuit current by glucose. In an experiment in which the mucosal solution contained 27 m*M* valine throughout, the K_m for glucose was 0.9 m*M* and the $(\Delta I_{sc})_{max}$ was 2.5 neq/cm²/min. In the presence of 46 m*M* valine the values obtained were 0.6 m*M* and 0.9 neq/cm²/min.

Discussion. The results of the present study demonstrate the direct interaction between active sugar and amino acid transport and active sodium transport in isolated bullfrog intestine. Under the conditions of the present experiments net sodium flux and short circuit current are equal and are equally stimulated by glucose as well as by valine in the presence of glucose (6). Thus, valine does not appear to contribute directly to any significant extent to the total transmural current observed under these conditions. This may also be inferred from the known dissociation constants for L-valine (18). From these it can readily be shown that L-valine is virtually completely in the zwitterion form at pH 7.2. Similarly, it can be shown that L-alanine and L-cysteine are effectively without any net charge at this pH. The electrical response of the bullfrog intestine in sulfate media to the addition of glucose and valine occurs solely through changes in transmural potential with no effect on tissue resistance. The results presented herein are, therefore, consistent with a direct interaction between solute transfer and an electrogenic sodium pump rather than an indirect coupling via generalized, solute-induced, permeability changes. Thus, in the absence of active chloride transfer, the coupling of sodium fluxes to the transport of a representative sugar and amino acid in the bullfrog intestine is directly comparable to the coupling of these processes in the isolated rabbit ileum which does not possess a net chloride transport capability under short circuit conditions. On this basis the present results serve to confirm and extend the results of Schultz and Zalusky (16, 17) on sodium transport in the isolated rabbit ileum and to indicate the suitability of the bullfrog preparation, by virtue of its stability *in vitro*, for further studies of the co-transport process.

An interesting aspect of the co-transport of sugars and amino acids with sodium is the question of the similarities and differences of the manner in which the transport of these two types of solute is linked to that of sodium. Evidence from various sources (19–21) suggests that significant differences do indeed exist. This is supported by the preliminary indications of the nonreciprocal nature of the effects of valine and glucose together on short circuit current reported in the present paper. The bullfrog intesting *in vitro* can maintain a low but finite rate of active sodium transport in the absence of exogenous substrate (5) and responds similarly to the mucosal addition of sugars of differing metabolic utility (D-glucose, D-galactose, and 3-*O*-methyl glucose) indicating that sugar stimulation of net sodium flux cannot be primarily the result of metabolic changes. Nevertheless, it is logical to expect that the metabolic fate of the actively transported solute will influence the ultimate nature of the response. Consideration of the metabolic state of the tissue may provide some insight into differences between sodium-dependent transport of

sugars and amino acids. Interpretation of studies on the effect of actively transported amino acids on intestinal sugar transport (22, 23) and on the effect of transported sugars on amino acid transport (24–26) have also required consideration of the metabolic utility of the transported substrates. However, the primary purpose of the present study was to show that the isolated small intestine of the bullfrog should prove to be a valuable model system for future investigations of dynamic interactions between transport processes in the intestinal epithelium.

Summary. When isolated bullfrog small intestine is mounted between identical Ringer solutions in which chloride is replaced by sulfate, short circuit current and net mucosal–serosal Na^+ flux are identical. This identity is preserved in the presence of actively transported sugars and amino acids. In the presence of a constant concentration of Na^+, both sugars and amino acids increase the transmural pd and short circuit current when added to the mucosal bathing fluid. Addition of these substances to the serosal medium only has no effect. The increases in transmural pd and short circuit current show identical saturation characteristics with respect to the concentration of added solute and can be described in terms of simple Michaelis-Menten kinetics. These effects are not dependent on metabolism of the added solute. Addition of a second actively transported sugar to the mucosal solution during maximal stimulation by another sugar had no effect on transmural pd or short circuit current. Addition of an amino acid under these conditions caused an additional saturable increase in these parameters. Similarly, addition of an actively transported sugar during maximal stimulation by an amino acid caused a saturable increase in transmural pd and short circuit current. The kinetics of the response of the short circuit current to L-valine were unaffected by D-glucose. L-Valine decreased both the K_m and the maximal short circuit current obtained with glucose.

This work was supported by grant number 2-67-761 from the American Heart Association, by facilities provided by USPHS Grants GM 10971 and HE 06308, and by a research grant from Eli Lilly and Company, Indianapolis. One of us (J.F.Q.) was supported by USPHS Predoctoral Fellowship F1-GM 29918. The above work forms part of a thesis submitted by him to Indiana University in partial fulfillment of the requirements for the Ph.D. degree. We acknowledge with pleasure the advice and assistance of Dr. P. L. Yu of the Institute of Psychiatric Research, Indiana University Medical Center, Indianapolis, in the statistical aspects of the work. Computations were carried out at the Research Computation Center, Indiana University Medical Center with support from USPHS Grant FR 00162. The technical assistance of Miss Laura F. Gale is gratefully acknowledged.

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Received Nov. 25, 1968. P.S.E.B.M., 1969, Vol. 131.

Lack of Active Transfer of ^{14}C Tetraethylammonium and Para-Aminohippuric Acid by the Term Sheep Placenta* (33802)

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The placenta is generally considered to behave as a lipoid membrane analogous to the blood–brain barrier in being poorly permeable to ionized substances (1). However, placental transfer is not completely passive, since active transport has been demonstrated for a number of materials, *l*-amino acids being one example (2). The possibility that the placenta may actively remove substances from the fetal circulation has seldom been studied. Of substances known to be actively transported by such organs as the liver and kidney, only BSP has been administered to the fetus (3). It was demonstrated that BSP was not actively transported from the fetal compartment. The present study was designed to determine whether the term sheep placenta actively removes a typical organic acid or base from the fetal compartment.

Methods. Mixed breed pregnant ewes, weighing 48–68 kg, 128–140 days from dated breeding (normal duration of pregnancy is approximately 150 days), were fasted for 36 hr, and anesthetized with phencyclidine HCl (Sernylan), 1 mg/kg, intramuscularly and chloralose, 50 mg/kg intravenously. Intermittent supplemental chloralose was administered as needed. Respiration was maintained by a Bird respirator (Mark 8). Surgical preparation included laparotomy and hysterotomy with catherization of maternal (femoral) artery (MA), uterine vein (UV), fetal (cotyledonary) artery (FA), and umbilical (cotyledonary) vein (FV). Additional maternal veins and a fetal femoral artery and vein were cannulated for measurement of pressures and infusion of drugs. A total of 12 experiments were performed, comprising 4 equal groups. Para-aminohippuric acid (PAH) was administered via the maternal circulation in one group and by the fetal circulation in another group. The remaining two groups received ^{14}C tetraethylammonium bromide (^{14}C TEA) sp act 2.47 mCi/mmole, (New England Nuclear Corp.). The labeled compound was administered via the maternal circulation in one group and the fetal circulation in the other. In each experiment, infusion into the fetus of antipyrine (A) at a constant rate was employed to measure effective maternal and fetal placental flows, according to the method developed by Meschia (4) and validated by Rudolph (5). Transplacental gradients of A were also utilized as indices of the passive transfer characteristics of the placenta, providing a basis for evaluation of possible active transfer of PAH or ^{14}C TEA.

The experimental protocols were as follows: A was infused via a fetal vein at 2 $\times$ maintenance rate for 5 min, and at maintenance rate (see section "Calculations" below) for the remainder of the experiment. Twenty min was allowed to elapse for equilibration of A concentrations. The PAH or ^{14}C TEA was then administered as a single bolus injection to either mother or fetus. A maintenance

* Supported by NIH Grant GM 14270.