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Sugar-Induced Potential Difference and Short Circuit Current in Bullfrog Small Intestine: Effect of UO_2^{2+} (39316)

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UO_2 ions inhibit active sugar transport in several cell species, including yeast (1, 2), the epithelial cells of renal tubules (3), and the small intestine (4–7). These inhibitory effects are believed to arise from interaction between UO_2^{2+} ions and membrane ligands which are implicated in sugar transfer (4–8). Since the active step in sugar translocation by the small intestine is generally believed (9) to occur across the luminal border of the mucosal cells, the effect of UO_2^{2+} on mucosal transfer of D-glucose, D-galactose, 3-*O*-methyl glucose, and luminal fluid by isolated rat small intestine was investigated by Newey, Sanford, and Smyth (6, 7). These authors found that mucosal UO_2^{2+}, depending on its concentration, had two effects. At 3×10^{-4} *M*, UO_2^{2+} inhibited mucosal glucose transfer and glucose-dependent fluid transfer. Mucosal transfer of galactose and 3-*O*-methyl glucose were unaffected. UO_2 3×10^{-3} *M* inhibited mucosal transfer, under aerobic conditions, of all three sugars. These results were interpreted (7) as indicating that there are two pathways for the mucosal entry of glucose into the epithelial cells of rat intestine, a relatively UO_2^{2+}-sensitive pathway which is not shared by galactose, and a less sensitive pathway which involves a common carrier for glucose and other actively transported sugars.

Coupling between active sugar transport and net m → s Na^+ transfer in the small intestine is well known and has been widely documented (9–12). This coupling is reflected in the occurrence of sugar-induced increases in transmural potential difference (P.D.) and short circuit current (I_{sc}) (13, 14). These electrical changes appear to involve a transfer mechanism which is shared by glucose and other actively transported hexoses (14). Hence, one may predict that, under suitable conditions, UO_2^{2+} should inhibit sugar-induced increases in P.D. and I_{sc}. The present paper reports the results of a study designed to test this prediction.

Materials and Methods. These experiments were performed on isolated segments of bullfrog (*Rana catesbeiana*) small intestine mounted as flat sheets between the two halves of an Ussing chamber. Both halves of the chamber were filled with an oxygenated sodium sulfate medium. Details of the preparation and mounting of the intestinal tissue, the Ussing chamber, the measurement of P.D. and I_{sc}, and the method used to circulate and oxygenate the fluid in each half of the chamber have been given elsewhere (15, 16). The bathing medium used was a modification of the sodium sulfate Ringer solution previously employed in this laboratory (15). A major modification was the omission of phosphate. Initially, the basic medium had the following composition (in mEq/liter): Na^+ 102.4, K^+ 4, Ca^{2+} 1.8, SO_4^{2-} 53.2, gluconate$^-$ 1.8. In addition, 67.5 mmole/liter mannitol was included to make the medium approximately isosmotic with frog plasma. Since UO_2^{2+} displays a complex set of interactions with OH^- at neutral pH (8), all experiments were run at pH 5.5. The pH of the medium was adjusted to this value with dilute H_2SO_4. Earlier investigations in this laboratory (Gerencser and Armstrong, unpublished observations) had indicated that the response of P.D. and I_{sc} in isolated bullfrog small intestine to actively transported sugars was essentially unchanged between pH 5.5 and 7.0. A preliminary set of experiments in the present study confirmed this finding. UO_2^{2+} was incorporated in the medium as $UO_2(NO_3)_2$. When this was done, an osmotically equivalent amount of mannitol was omitted. Early in this study we noticed that when $UO_2(NO_3)_2$ was present in the fluid bathing one side only of the tissue, artifactual P.D.s was induced. That these were

probably streaming potentials (17) arising from different transmural mobilities of NO_3^- and SO_4^{2-} is suggested by the fact that their magnitude appeared to depend on the transmural $UO_2(NO_3)_2$ concentration gradient and their orientation depended on the side of the chamber to which $UO_2(NO_3)_2$ was added. Also, these potentials could be suppressed by incorporating an equivalent amount of NO_3^- (added as $NaNO_3$) in the medium which did not contain UO_2^{2+}. The following procedure was therefore adopted in later experiments. The tissue was first equilibrated in a medium which contained an amount of $NaNO_3$ equal to twice the molar concentration of $UO_2(NO_3)_2$ used later in the experiment (the latter was usually 1.3 or 2.6 m*M*). An amount of Na_2SO_4 equivalent to the $NaNO_3$ added was omitted from this medium and its mannitol concentration was adjusted to keep the total osmolality constant. When P.D. and I_{sc} had reached a steady state, the fluid in one or both halves of the chamber was removed and replaced by an equal vol (4.7 ml) of a medium containing $UO_2(NO_3)_2$. After 2–3 min this initial aliquot of UO_2^{2+} medium was removed and replaced. This procedure was then repeated. Following this, electrical recordings were resumed. During electrical recording, the tissue was maintained in the "open circuit" condition except for occasional interruptions to measure I_{sc}.

All chemicals used in this study were "Analytical Reagent" grade and all solutions were made in distilled water which had been further purified by two passes through a mixed bed ion exchanger. It has been found (18) that, under conditions approximating those employed in the present experiments, the enhancing effect of actively transported sugars and amino acids on P.D. and I_{sc} are solely due to the solute in the mucosal medium. Therefore, to avoid the imposition of osmotic gradients across the tissue, 50-μl samples of concentrated solutions of D-glucose, 3-*O*-methyl glucose, and L-valine were added directly to both sides of the Ussing chamber to give final concentrations of 11, 26, and 15 m*M*, respectively. These correspond approx to a relative concentration (19) of 4 in each case (18). All experiments were performed at 25 ± 0.5°.

Results and Discussion. Effect of UO_2^{2+} on sugar-induced P.D. and I_{sc}. Preliminary experiments showed that UO_2^{2+} concentrations below 1 mEq/liter had no significant effect on glucose-induced P.D. and I_{sc}. When the medium bathing both sides of the tissue contained 1.3 mEq/liter of UO_2^{2+}, inhibition of these parameters was consistently found. Further experiments showed that the inhibitory effect of UO_2^{2+} was observed only when this agent was present in the mucosal medium. Serosal UO_2^{2+} alone had no effect on glucose-induced P.D. or I_{sc}. Figures 1 and 2 show representative results from these experiments. Two experiments are illustrated in Fig. 1. In one of these glucose was added to the mucosal and serosal media following 50-min incubation of the tissue in a glucose-free medium, and P.D. and I_{sc} were allowed to reach maximal values. At this point the mucosal medium was removed and replaced by a medium containing 1.3 mEq/liter of UO_2^{2+} and 11 m*M* glucose. It is apparent that, following

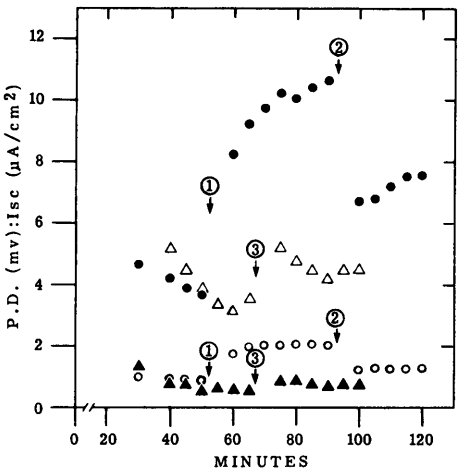

FIG. 1. Effect of mucosal UO_2^{2+} on glucose-induced P.D. and I_{sc}. Two experiments are shown. In one, glucose was added to both the mucosal and the serosal media at the time indicated by arrow (1). The responses of P.D. (○) and I_{sc} (●) to glucose were allowed to develop fully. Then, at the time indicated by arrow (2), the mucosal aspect of the tissue was exposed to 1.3 m*M* UO_2^{2+}. In the second experiment (P.D. – ▲; I_{sc} – △) the mucosal side of the tissue was exposed simultaneously at the time indicated by arrow (3) to glucose and UO_2^{2+}.

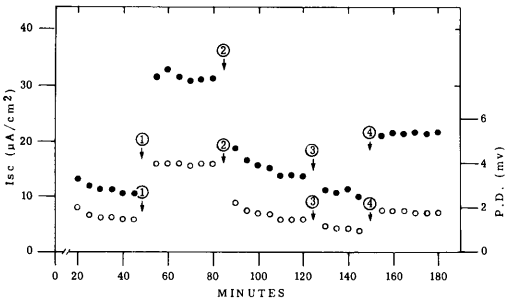

FIG. 2. Effect of mucosal UO_2^{2+} on glucose-induced P.D. (○) and I_{sc} (●). Arrow (1): glucose added to both mucosal and serosal media; (2): both media replaced with glucose-free media; (3): 1.3 mEq/liter of UO_2^{2+} added to mucosal medium; (4): glucose added to both media.

addition of UO_2^{2+} to the mucosal medium, there was a substantial decrease in both P.D. and I_{sc}. Although the degree of inhibition of P.D. and I_{sc} was the same in any single experiment, the inhibitory effect of UO_2^{2+} in different experiments was somewhat variable. In five experiments of the type under discussion the percentage inhibition of glucose-induced I_{sc} ranged from 21 to 66 (mean 42 ± 7 SEM). The average inhibition of P.D. in these experiments was 44 ± 6 percent.

The effect of mucosal UO_2^{2+} concentration on the degree of inhibition of glucose-induced P.D. and I_{sc} was not systematically studied. Three experiments in which the UO_2^{2+} concentration was 2.6 mEq/liter gave results within the range quoted above for 1.3 mEq/liter of UO_2^{2+}. Virtually complete inhibition of glucose-induced increases in P.D. and I_{sc} was consistently observed with 13 mEq/liter of UO_2^{2+}, but in most of these experiments, steady state P.D. and I_{sc} following addition of UO_2^{2+} were significantly less than their initial baseline values. It was shown previously (15) that, under similar conditions to those used in the present study, I_{sc} in the absence of transported solutes reflects net m → s Na^+ transfer in bullfrog small intestine. It may therefore be concluded that, in concentrations of the order of 13 mEq/liter, UO_2^{2+} inhibits the basic mechanism of net Na^+ transport in this tissue.

The inhibitory effect of UO_2^{2+} on glucose-induced P.D. and I_{sc} was quite rapid. This is illustrated in the second experiment shown in Fig. 1. In this experiment glucose and UO_2^{2+} (1.3 mEq/liter) were added simultaneously to the mucosal medium. It is apparent that, under these conditions, the increase in P.D. and I_{sc} was very slight and rapidly reached a steady state. This is consistent with the suggestion (4) that, in small intestine, as in other tissues (8, 20), the inhibitory effects of UO_2^{2+} involve an interaction between this ion and anionic ligands (specifically, phosphoryl and/or carboxyl groups) in the epithelial cell membrane. The fact that glucose induced increases in P.D. and I_{sc} are only observed when this sugar is added to the medium bathing the mucosal surface of the epithelial cells (9, 12, 19) suggests that these ligands are located in the brush border membrane.

In the experiment represented by Fig. 1, P.D. and I_{sc} following addition of glucose and of UO_2^{2+} always reached a higher steady state level than their initial values under control conditions. Hence one could argue that these experiments do not provide unequivocal evidence that UO_2^{2+} specifically inhibits the glucose induced moiety of P.D. and I_{sc}. Similar results to those shown in Fig. 1 could be predicted on the assumption that UO_2^{2+} inhibits P.D. and I_{sc} in the absence of glucose or partially inhibits both the glucose-dependent and glucose-independent P.D. and I_{sc}. Fig. 2 shows one of a series of experiments designed to test this possibility. In this experiment glucose was added to both the mucosal and the serosal medium (arrow 1) and the expected increases in P.D. and I_{sc} were allowed to reach stable values. At this time (arrow 2) both media were replaced by a glucose-free Ringer solution, and P.D. and I_{sc} were permitted to decline to values close to those observed initially. Following this, the mucosal medium was replaced by a medium containing 1.3 mEq/liter of UO_2^{2+} (arrow 3 of Fig. 2). Finally (arrow 4), glucose was again added to both the mucosal and the serosal medium. It is apparent from Fig. 2 that whereas mucosal UO_2^{2+} had virtually no effect on P.D. and I_{sc} in the absence of glucose, the response of these parameters to

glucose was markedly reduced when UO_2^{2+} was present in the mucosal medium. Control experiments showed that, following an initial stimulation of P.D. and I_{sc} by glucose and reexposure of the tissue to a glucose-free medium, a second addition of glucose elicted an undiminished response to these parameters. Fig. 2 clearly indicates that the inhibitory effect of UO_2^{2+} at a concentration of 1.3 mEq/liter is restricted to the glucose-evoked increases in P.D. and I_{sc}.

Figure 3 indicates that, as expected, the inhibitory effect of mucosal UO_2^{2+} on glucose-induced changes in P.D. and I_{sc} extends to other actively transported sugars and sugar analogs which share a common transport pathway with glucose (14). It is apparent from this figure that the increases in P.D. and I_{sc} elicited by 3-*O*-methyl glucose are rapidly and strongly inhibited when 1.3 m*M* UO_2^{2+} is added to the mucosal medium. As was found with glucose, serosal UO_2^{2+} at this concentration did not inhibit the increases in P.D. and I_{sc} elicited by 3-*O*-methyl glucose.

Frizzell and Schultz (21) have presented evidence that anionic groups in the mucosal cell membrane (most probably, in these authors' opinion, carboxyl groups, although they do not rule out phosphoryl groups as a possibility) are involved in the influx of L-alanine across the brush border of isolated rabbit ileum and have shown that, at pH 5, alanine influx in this tissue is markedly reduced by 1.0 m*M* mucosal UO_2^{2+}. Further, Foulkes (20) had shown that the transport of amino acids by rabbit kidney *in vivo* is inhibited by UO_2^{2+} poisoning, though, in this instance, the inhibitory effect appears to be located in the peritubular rather than the luminal cell membrane. Because of these findings, we examined the effect of UO_2^{2+} on valine-evoked increases in P.D. and I_{sc} (18). In these experiments mucosal UO_2^{2+} (1.3 mEq/liter) had no effect on the P.D. and I_{sc} induced by 20 m*M* valine (Fig. 4). Similarly, serosal UO_2^{2+} at the same concentration did not affect these parameters.

In view of the well-known coupling between the transport of sodium and that of amino acids in the brush border of the small intestine (9) and since mucosal UO_2^{2+} inhibits alanine transport in this tissue (21), its

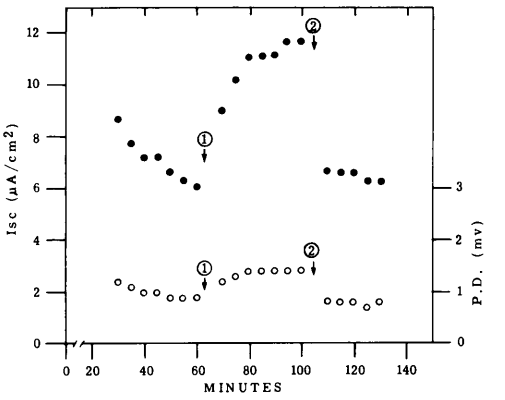

FIG. 3. Inhibition of P.D. (○) and I_{sc} (●) elicited by 35 m*M* 3-*O*-methyl glucose (added at arrow 1) by mucosal UO_2^{2+} (1.3 mEq/liter, added at arrow 2).

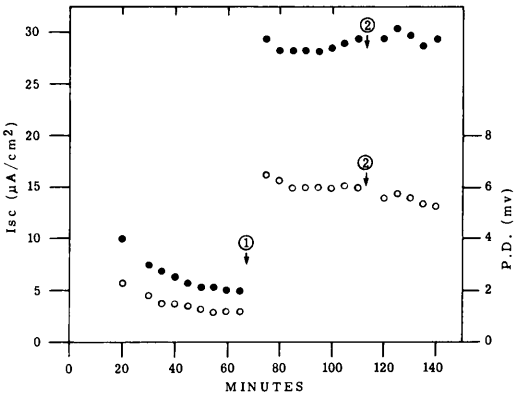

FIG. 4. Failure of mucosal UO_2^{2+} (1.3 mEq/liter, added at arrow 2) to inhibit P.D. (○) and I_{sc} (●) evoked by 20 m*M* valine (added at arrow 1).

failure to inhibit valine-induced P.D. and I_{sc} in our experiments is somewhat unexpected. The reason for this apparent discrepancy is not entirely clear at present. It may be that phosphoryl groups, rather than carboxyl groups, are implicated in intestinal sugar uptake. The former have been shown to possess a higher binding affinity for UO_2^{2+} than the latter both *in vitro* and *in vivo* (8). Hence sugar-evoked increases in P.D. and I_{sc} might be more sensitive to UO_2^{2+} inhibition than the changes induced in these parameters by amino acids. In the light of the results reported by Frizzell and Schultz (21) this view would seem to require a lower sensitivity of amino acid transport to UO_2^{2+} inhibition in bullfrog small intestine than in rabbit ileum. Alternatively, significant inhi-

bition of amino acid influx without any observable decrease in amino acid-induced P.D. and I_{sc} could occur if the coupling ratio for the cotransport of Na^+ and valine across the brush border were altered by mucosal UO_2^{2+}. It is known that the stoichiometry of Na^+/alanine cotransport is sensitive to a variety of factors including the pH of the bathing medium (21) and luminal Na^+ concentration (22). Whatever the origin of this discrepancy, it seems clear that, under the conditions of our experiments, UO_2^{2+} selectively inhibits sugar-induced P.D. and I_{sc} in isolated bullfrog small intestine and, by implication (6, 7, 9), also selectively inhibits mucosal uptake of sugars by this tissue under these conditions.

Summary. UO_2^{2+} 1.3 m*M* added as $UO_2(NO_3)_2$ to the mucosal solution consistently inhibited the P.D. and I_{sc} evoked by 11 m*M* glucose and 35 m*M* 3-*O*-methyl glucose across isolated strips of bullfrog small intestine bathed by symmetrical Ringer solutions in which SO_4^{2-} was the major anion. The average degree of inhibition in the presence of glucose was 42 ± 7 (SEM) percent. P.D. and I_{sc} in the absence of transported solutes were not significantly altered by mucosal UO_2^{2+} at this concentration. Increasing the mucosal UO_2^{2+} concentration to 2.6 m*M* did not significantly increase its inhibitory action on glucose-evoked P.D. and I_{sc}. Further increasing the UO_2^{2+} concentration to 13 m*M* completely inhibited glucose-induced P.D. and I_{sc} but also markedly reduced these parameters in the absence of glucose. Serosal UO_2^{2+} (1.3 m*M*) had no effect on the P.D. and I_{sc} evoked by glucose and 3-*O*-methyl glucose. It is suggested that the inhibitory action of UO_2^{2+} involves binding of this ion to anionic sites located in the apical membrane of the absorptive cells. Mucosal or serosal UO_2^{2+} (1.3 m*M*) had no effect on the P.D. and I_{sc} elicited by 20 m*M* valine, indicating that under the conditions of these experiments UO_2^{2+} selectively inhibits sugar-induced P.D. and I_{sc} and, by implication, mucosal sugar uptake.

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