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Uranyl Action on Sugar Transport Across Rat Jejunum *

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The effect of uranyl on sugar transport across rat jejunum has been studied *in vitro* and *in vivo*. D-glucose and D-galactose accumulation in jejunum rings at pH 6.0 is inhibited about 40-65 % by 1 mM uranyl nitrate. This inhibition is lower than that produced by 0.5 mM phlorizin. The effect was very similar when the incubation of the rings with the sugar was made in the absence of uranyl, after preincubation with the inhibitor. Washing with 10 mM EDTA reverted uranyl inhibition only slightly. D-fructose entry was weakly inhibited by uranyl. Glucose absorption *in vivo* along perfusion periods of 1 min was not affected by the presence of uranyl (0.001 to 1 mM) in the sugar solution, but the exposure of the mucosa to 0.1 mM uranyl at pH 6.5 for 10 min inhibited sugar absorption at the same pH in the subsequent periods of perfusion. Results suggest that uranyl impairs sugar transport by binding to protein chemical groups at the surface or in deeper sites of enterocyte membranes, a process that requires some minutes to be accomplished.

Key words: Uranyl, Sugar, Transport, Rat, Jejunum.

Uranyl was reported many years ago to inhibit both sugars entry in yeast cells (1, 18) and the *in vivo* absorption of glucose and galactose across rat small intestine (8, 13, 17). The latter effect was ascribed to binding of uranyl to the luminal surface of the epithelium, which could partially be reverted by EDTA (8). Uranyl also impaired sugar transport in sacs of rat everted intestine (9, 10), as well as alanin transport in rabbit ileum (4, 19).

As in some of those works the experimental conditions were not adequate enough for the evaluation of sugar transport, and the shift in the pH of the medium produced by addition of uranyl salts was not sufficiently taken into consideration, the present paper was intended to revise the subjet of the uranyl action on sugar intestinal transport, both *in vitro* and *in vivo*.

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Materials and Methods

White Wistar rats, 100-200 g weight, were used. Rings of jejunum of about 25 mg w.w. were prepared for *in vitro* experiments, after the sugar accumulation technique of CRANE *et al.* (2). The suspension medium was a Krebs-Ringer-Tris solution (KRT) modifield to avoid precipitate production when uranyl had to be added, containing 127 mM NaCl, 10.18 mM KCl, 5.44 mM $CaCl_2$, 15 mM Tris-HCl. Incubations with sugar were for 10 min, at 37°C, under 95 % O_2-5 % CO_2 bubbling. Uranyl was present in the medium during incubation, or during preincubation without sugar.

In vivo experiments were carried out with 24 h fasted animals under urethane anaesthesia, by single pass perfusion of a 150 mM NaCl solution with the added sugar, at a rate of 5.6 ml/min, through the lumen of a jejunum segment about 15-20 cm in length, along several successive absorption periods of 1 min (14).

Labelled sugars were D-(1-^{14}C) glucose, D-(1-^{14}C) galactose and D-(1-^{14}C) fructose from Amersham. The corresponding cold sugars were from Merck (glucose, galactose) and Sigma (fructose). Uranyl nitrate was from Merck. Radioactivity was determined with a LKB Wallac 1215 Rack Beta II liquid scintillation counter. Measure of pH was with a PHM 52 Radiometer, and adjustments of pH were obtained with 2 mM Tris or 2 mM HCl.

Results

Aqueous solution of uranyl nitrate have acidic pH, and on carrying them to neutral or alkaline pH, the UO_2^{2+} changes and some turbidness, even precipitate can be observed. As the pH of the medium influences sugar transport (3, 5, 7, 11, 15, 16), the experiments were usually made at pH 6.0 if 1 mM uranyl had to be added, or at 6.5 with the lower uranyl concentrations, conditions in which turbidness did not appear.

In vitro *experiments.* — The sugar accumulated in the rings of everted jeju-

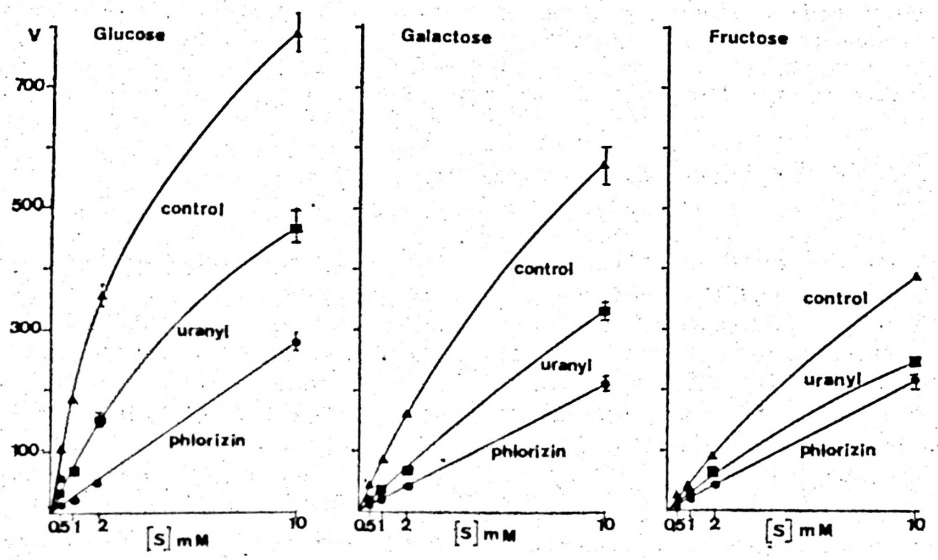

Fig. 1. *Sugar accumulated in rings of rat jejunum after 10 min incubation at pH 6.0.* Control (▲), with 1 mM uranyl (■), with 0.5 mM phlorizin (●). Points are the mean of 9-12 experiments ± SE. V = nmoles of sugar per 100 mg w.w. in 10 min.

num was measured after 10 min incubation, a period of time long enough to reach near maximum sugar levels. Galactose entry into the rings at 1 mM or 10 mM concentrations in the medium under control conditions, as well as under sugar transport inhibition by 0.5 mM phlorizin, was very similar at pH 7.4 and at pH 6.00.

The accumulation of glucose, galactose and fructose for different sugar concentrations in the suspension medium, at pH 6.0 was measured in control experiments, or in the presence of 0.5 mM phlorizin or 1 mM uranyl (fig. 1) With uranyl, the levels of glucose or galactose in the tissue were significatly lower than those found in the control ones, the inhibition amounting to about 40 % for 10 mM sugar in the medium, and to about 65 % for 1 mM concentrations. The inhibitions by 0.5 mM phlorizin were always higher than those obtained with uranyl. Fructose entry into the rings was markedly lower than that of aldohexoses, and it was less affected by uranyl or phlorizin.

If the passive entry of sugar when transport was entirely inhibited by 0.5 mM phlorizin (6), is subtracted from the entry in the absence of phlorizin, the transport mediated entry can be estimated. In this way, assuming that uranyl action is exclusively exerted on transport, the inhibition by 1 mM uranyl would be from 62 % to 80 %.

The effects of uranyl were found to be very alike when the jejunum rings were preincubated for 10 min in a solution with 1 mM uranyl, then thoroughly washed, and finally incubated with the sugar solution. If the washing solution contained 10 mM EDTA, the inhibition was a little lower than or equal to that observed without EDTA treatment. With uranyl in the medium both during preincubation and incubation, a slightly higher inhibition of sugar transport was obtained (figure 2).

Lower uranyl concentrations, between 0.001 and 0.1 mM, did not modify glucose or galactose transport on incubating the tissues for 5 or 10 min, at pH 6.5, in

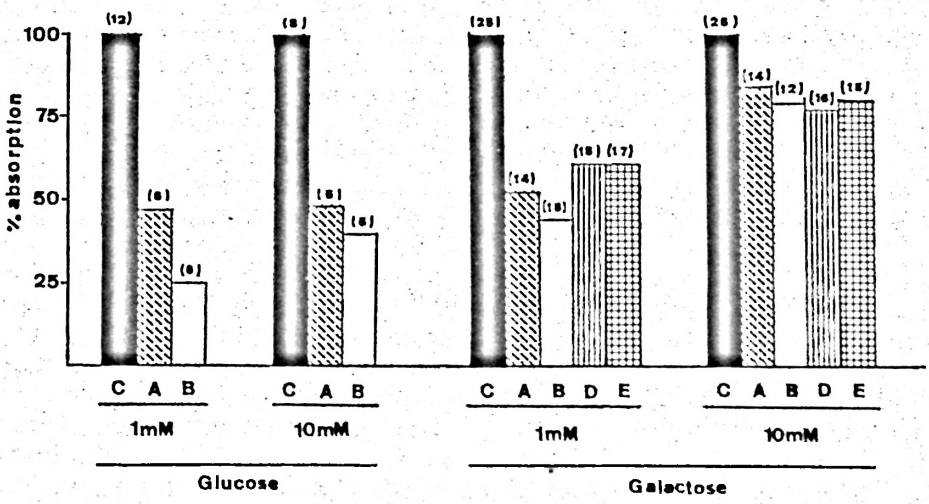

Fig. 2. *Sugar accumulation by rat jejunum rings in percent of control.* The rings were first preincubated (10 min), then thoroughly rinsed, and lastly incubated (10 min) with the sugar. C (control), preincubation and incubation without uranyl. A, 1 mM uranyl during preincubation. B, 1 mM uranyl both in preincubation and incubation. D, and E, like respectively to A and B, but rinsing with 10 mM EDTA before incubation. Number of data in parentheses.

the presence of 1 mM or 10 mM sugar.

In other experiments, jejunum rings were preincubated for 5 min at pH 6.9 without uranyl, or at pH 2.5 in the absence of 0.5 mM uranyl. After washing with KRT, the preparations were incubated for 10 min wit 1 mM galactose at pH 6.9. A strong inhibition (65 %) of sugar transport was produced by the acidic preincubation, but it was not increased by the simultaneous presence of uranyl.

Perfusion experiments in vivo. — The absorption of sugar along a series of consecutive perfusion periods of 1 min, under different experimental conditions was measured. The jejunum segment was thoroughly washed with saline solution before each absorption period. Addition 0.001 to 1 mM uranyl to the perfusion solution did not modify the intestinal absorption of 1 mM glucose. Since a slight desquamation of the epithelium was observed with 0.5 and 1 mM uranyl, those concentrations were henceforth abandoned for *in vivo* experiments.

In a group of animals, after some periods of sugar absorption under control conditions, the lumen was perfused for 10 min with a sugar-free uranyl containing solution, and then sugar absorption in the presence of uranyl was measured. In this way, the absorption of 1 mM glucose became about 24 % inhibited by 0.1 mM uranyl (table I), lower uranyl concentrations (0.001 to 0.05 mM) being ineffective.

Table I. *Glucose absorption by rat jejunum* in vivo. Luminal single pass perfusion (5.6 ml/min) of 1 mM glucose in 150 mM NaCl solution, during consecutive absorption periods of 1 min. Absorption in nmoles · cm^{-1} · Mean values ± SE. Number of data in parentheses.

	Control	Uranyl 0.1 mM
Absorption	30.13 ± 1.23 (29)	22.25 ± 1.06 (6)
Inhibition	—	24.49 %

Lastly, the influence of mucosa exposure for 5 min to a saline solution at pH 2.5 with or without 0.1 mM uranyl on the subsequent absorption of 1 mM glucose at pH 6.5 was tested. Sugar absorption was not significantly different from that of the control before the exposure.

Discussion

Accumulation of glucose or galactose in jejunum tissue at pH 6.5 was not modified by 0.001 to 0.1 mM uranyl. Nevertheless, a clear inhibition was produced by 1 mM uranyl at pH 6.0 which amounts to about 40 or 65 % with 10 mM or 1 mM sugar in the medium, respectively. Fructose entry in the tissue was weakly inhibited by 1 mM uranyl.

NEWEY *et al.* (9, 10) reported inhibition of glucose transport into intestinal sacs by 0.01-0.3 mM uranyl when the sugar was at 27.8 mM concentration. However, with 5.6 mM sugars the inhibition of glucose, galactose and 3,o-methylglucose transfer required uranyl concentrations to reach up to 3 mM. Besides difficulties in explaining the higher sensitivity found with 27.8 mM glucose, it is not clear that changes in pH due to uranyl solutions were sufficiently taken into consideration.

The results with jejunum rings at pH 6.0 show that 1 mM uranyl inhibits sugar accumulation in lower proportion than 0.5 mM phlorizin does. This fact means that uranyl concentration does not entirely block the sugar transport system.

To exert its inhibitory action, uranyl does not have to be added to the incubation solution; it suffices that it be present during preincubation. Thus, uranyl seems to be retained by the tissues thereby inhibiting transport when the rings are subsequently incubated with the sugar. Washing the rings with EDTA after preincubation improves sugar transport

only slightly. This result suggests that most bonds between uranyl and chemical groups of the enterocytes are either not solved by EDTA or not accessible to it.

Galactose accumulation in the tissues after preincubation at pH 2.5 was much lower than that after preincubation at pH 6.9. The presence of 0.5 mM uranyl in the preincubation solution did not add any further decrease in transport.

The *in vivo* absorption of 1 mM glucose across rat jejunum along perfusion periods of 1 min was not significantly affected by 0.001 to 1 mM uranyl. However, when the intestine had been previously exposed to even 0.1 mM uranyl solutions for some minutes, an inhibitory effect was observed. The inhibitory action of uranyl seems to need a certain amount of latency time to exert itself.

These *in vivo* results essentially agree with much older ones, also in rats (8,13, 17) but under very diferent experimental conditions (300 mM sugar concentrations, 30 min absorption periods, and with disregard of the Na^+ and H^+ concentrations in the luminal solutions). Both sets of experiments reveal inhibition of intestinal sugar absorption by 0.1 mM uranyl, if the mucosa is exposed to the inhibitor for enough time.

The impairment of sugar transport, observed both *in vitro* and *in vivo* has to be ascribed to binding of uranyl with chemical groups at the luminal surface or deeply in the enterocytes, which may belong to proteins. A delay of some minutes is required for the inhibition to be manifested, suggesting a hard accessibility of the uranyl ligands involved in transport. Nevertheless, a minor part of the uranyl bound can be released and sequestered by EDTA treatment, slightly diminishing inhibition.

It is not easy to establish which form of the metal may be the active one on inhibiting sugar transport. When solution of uranyl nitrate are carried out from their acidic pH to pH 6.0 or 6.5, the UO_2^{2+} changes into several transformation derivatives (12) although turbidness does not appear.

Resumen

Se estudia *in vitro* e *in vivo* el efecto del uranilo sobre el transporte de azúcares a través del yeyuno de rata. La acumulación de D-glucosa y D-galactosa en anillos de yeyuno a pH 6 se inhibe un 40-65 % por nitrato de uranilo 1 mM. Esta inhibición es inferior a la que produce la florricina 0.5 mM. El efecto es muy parecido cuando la incubación de los anillos con el azúcar se hace en ausencia de uranilo, pero después de haberlos preincubado con el inhibidor. El lavado con EDTA 10 mM reduce sólo ligeramente la inhibición por uranilo. La entrada de D-fructosa se inhibe débilmente por acción del uranilo. La absorción de glucosa *in vivo*, a lo largo de períodos de perfusión de 1 min, no se afecta por la presencia de uranilo (0,001 a 1 mM) en la solución de azúcar, pero la exposición de la mucosa a uranilo 0,1 mM a pH 6,5 durante 10 min produce inhibición de la absorción del azúcar al mismo pH en los siguientes períodos de absorción. Los resultados sugieren que el uranilo perturba el transporte de azúcar por unión a grupos químicos de proteínas en la superficie o en regiones más profundas de la membrana de los enterocitos, proceso que requiere algunos minutos para completarse.

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