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Effect of Ethanol on Glucose and Tyrosine Transport in the Rat Small Intestine *

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The effect of ethanol on the intestinal absorption of glucose and tyrosine in the rat small intestine has been studied. Ethanol inhibits the active transport of these substrates both in incubation and preincubation experiments. Ethanol, besides, increases diffusion of arabinose, which may indicate an unspecific alteration of intestinal permeability.

Previous studies have demonstrated that ethanol inhibits the intestinal absorption of glucose, water and some aminoacids, in rat and hamster jejunum, both *in vivo* and *in vitro* (5, 13). Ethanol has been shown to depress glucose transport, transmural potential difference and both unidirectional fluxes of sodium in hamster intestine (6). However, there was no correlation between the inhibition of the glucose and sodium transport. Furthermore, it was shown that the depressing effect of ethanol cannot be ascribed to the inhibition of the Na^+, K^+-sensitive ATPase-dependent sodium pump located at the basolateral membrane (7). On the other hand, it appears that the inhibition of water absorption induced by ethanol is related to the morphological alterations in the hamster jejunal mucose (8).

The purpose of the present study is to determine whether ethanol specifically inhibits the active transport of sugars and aminoacids or whether its effect is related to an unspecific alteration of the diffusive characteristics of the intestinal wall.

Materials and Methods

Male Sprague-Dawley rats weighing approximately 220-270 g were used. These animals were starved for 24 hr before the experiment. In each animal, 2 everted intestinal sacs, about 6 cm long, were obtained from the middle portion of the

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small intestine, according to the WILSON and WISEMAN technique (17). Alternate sacs were used as controls to minimize variations in transport by different sections of the intestine (2).

The sacs were incubated in Krebs-Henseleit bicarbonate buffer (15), in a thermostatic bath at 37° C, and were bubbled with carbogen (95 % O_2 and 5 % CO_2) during the whole incubation period. The test solutions were situated on the inside (serosal solution, 1.5 ml) and outside (mucosal solution, 20 ml) of the everted sacs. When ethanol was added to the system, identical concentrations were placed on both sides of the sac or only the mucosal medium. In both cases there were no differences.

The glucose was determined by the HULTMAN method (12) and the arabinose by the SOMOGYI method (16). Tyrosine was determined by the CERIOTTI and SPANDRIO method (4).

Results

Effect of ethanol on the glucose and tyrosine transport. The results obtained when 1 % v/v ethanol was present in the medium, showed that there was no significant variation in the active transport of 2 mM glucose and 1 mM tyrosine (table I). However, when 5 % v/v ethanol was present in the saline, a significant inhibition in the transport was found, in both glucose (86 %) and tyrosine (89 %). The S_F/M_F relation in the control experiments was 2.08 for glucose and 3.07 for tyrosine; with 5 % v/v ethanol it decreased to 1.02 and 1.16 respectively (table I).

Ethanol preincubation influence. Other experiments were made in order to determine whether the preincubation for 5 minutes in a solution with 5 % v/v ethanol at 37° C had some effect on the subsequent active transport of glucose and tyrosine. The control sacs were preincubated for that time in a medium without ethanol. After preincubation the sacs were washed three times in ethanol-free medium.

The results are expressed in figure 1, where it can be observed that the preincubation with ethanol inhibits the active transport of both glucose (55 %) and tyrosine (52 %). This indicates that after 5 minutes ethanol inhibits the influx of these substrates inside the epithelial cells. Moreover, this effect upon active transport persists despite successive washings in order to eliminate the ethanol bound to the intestinal mucosa.

Effect of ethanol on the arabinose diffusion. The ethanol inhibition of active

Table I. *Effect of ethanol on the active transport of glucose and tyrosine in everted sacs of rat small intestine.*

The intestinal sacs were incubated for 60 min, in 20 ml medium containing the appropriate substrate. When ethanol was present, identical concentrations were placed inside and outside the sacs or only in the mucosal. The results are expressed as μmole substrate/hour/100 mg tissue and as final concentrations relation S_F/M_F. Number of data in brackets.

Substrate		μ mol/hr/100 mg	S_F/M_F
Glucose (2 mM)	control	0.96 ± 0.14 (16)	2.08
	ethanol 1 % v/v	0.89 ± 0.12 (8)	2.04
	ethanol 5 % v/v	0.11 ± 0.02 (8)	1.02
Tyrosine (1 Mm)	control	1.07 ± 0.11 (8)	3.07
	ethanol 1 % v/v	1.10 ± 0.10 (4)	3.08
	ethanol 5 % v/v	0.10 ± 0.02 (4)	1.16

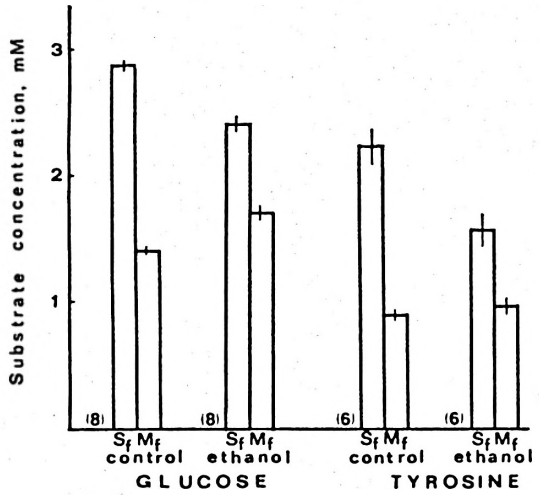

Fig. 1. *Effect of preincubation with ethanol on the active transport of glucose and tyrosine by rat intestine.*
The preincubation was made at 37° C for 5 min, in 5 % ethanol medium. Incubations for 60 min were made, in a medium containing 2 mM glucose or 1 mM tyrosine. The results are expressed in serosal and mucosal final concentrations (S_F, M_F) and are accompanied ± SEM. Number of experiments is given in parentheses.

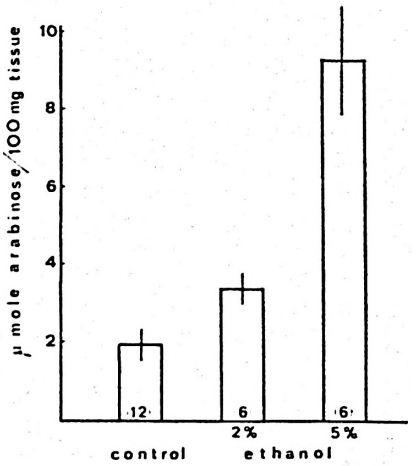

Fig. 2. *Effect of ethanol on diffusion of arabinose by everted intestinal sacs of rats.*
Everted sacs were incubated for 60 min, in 20 ml of saline containing L-arabinose 50 mM in the mucosal medium. The results are expressed in µmole arabinose diffused/100 mg tissue and are accompanied ± SEM. Number of data in parentheses.

transport may be produced by an unspecific alteration of the intestinal permeability. If this is so, the passive diffusion of a sugar non-actively transported as arabinose may be increased when ethanol is present in the medium. In order to study this question, a series of experiments were carried out with arabinose. This sugar, at 50 mM concentration, was placed only in the mucosal side, and after 60 minutes incubation, the sugar in the serosal side was determined.

The results are shown in figure 2, where it can be observed that the presence of ethanol both 5 % and 2 % v/v increases the diffusion of arabinose to the serosal side. Serosal final concentrations of 7.94 mM (control), 13.45 mM (ethanol 2 %) and 15.60 mM (ethanol 5 %) were obtained.

Discussion

The present study was undertaken to elucidate the mechanism through which ethanol inhibits active transport of glucose and tyrosine. Concentrations of 1 to 5 % v/v ethanol were selected because such levels are present in the jejunum of man after moderate drinking (11, 13). The results obtained agree with those of CHANG *et al.* (5) in that 3 % ethanol inhibits active transport of glucose and phenylalanine.

Various hypotheses have been proposed to explain the effect of ethanol on active transport of non-electrolytes: *a*) inhibition of Na^+, K^+ -stimulated ATPase activity, located in the basolateral membrane of the epithelial cell (5, 6); *b*) direct interference with the carrier-mediated entrance of glucose through the brush border membrane (6); *c*) morphological changes of intestinal epithelium provoked by ethanol (1, 3, 10).

In the first case, since ethanol is an easily diffusive molecule, its effects on active transport may be higher when it is in the serosal medium than when it is in the mucosal, such as occurs with the glycoside ouabaine, a classical inhibitor of ATPase Na, K-depending. However, in the results presented here, the inhibition depends on the presence of ethanol in the mucosal medium, independently of the presence or absence of this substance in the serosal. This supports the results obtained by DINDA *et al.* (7). Furthermore, recently, KRASNER *et al.* observed that ethanol does not have a depressing effect on the ATPase activity of guinea pig jejunum (14).

In the second case, for a direct interference between sugar and ethanol in the brush border membrane to occur, the two molecules must be simultaneously in the mucosal. The results obtained in preincubation experiments clearly indicate that ethanol disturbs the intestinal epithelial cells so that in the subsequent incubation without ethanol, the active transport of glucose and tyrosine is strongly inhibited.

In the third case, structural alterations in the intestinal epithelium provoked by ethanol have been studied both in rat (1, 3) and hamster (8, 9, 10) after chronic or acute exposition. In both cases there are alterations; presence of multiple subepithelial blebs mainly at the tip of the villi, the underlying lamina propria is compact and the lacteal are closed (9). With higher magnification alcohol-exposed rats showed separation of intercellular junctions at the apex (18). These alterations increase the permeability through the paracellular pathway to molecules as great as the horseradish peroxidase (18). All this supports the results of the arabinose diffusion, which increases when ethanol 2 % or 5 % v/v is present in the medium (fig. 2). The permeability alteration could explain the inhibition of glucose and tyrosine active transport, since the establishment and sustenance of concentration gradients is made difficult. However, the possibility that ethanol has some effect on the brush border membrane which interferes with the specific system of glucose and tyrosine transport cannot be discarded. Further experiments regarding this question are necessary.

Resumen

Se estudia el efecto del etanol sobre el transporte activo de glucosa y tirosina por sacos evertidos de intestino delgado de rata. El etanol inhibe el paso de estos sustratos al compartimento serosal, tanto en experimentos de incubación como de preincubación. Por otra parte, al estudiar la difusión de arabinosa a favor de gradiente, se observa que el etanol aumenta la velocidad de difusión, lo cual indica que se produce una alteración inespecífica de la permeabilidad intestinal.

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