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EFFECT OF ETHANOL AND OTHER ALCOHOLS ON THE TRANSPORT OF AMINO ACIDS AND GLUCOSE BY EVERTED SACS OF RAT SMALL INTESTINE*

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SUMMARY

1. Using everted sacs of rat small intestine, 3% ethanol was found to inhibit the transport of L-phenylalanine, L-leucine, glycine, L-alanine, L-methionine, and L-valine. The transport of D-glucose was partially inhibited.

2. Similar effects on L-phenylalanine transport were observed with 3% methanol; 2% *n*-propanol and isopropanol; 1% *n*-butanol and isobutanol and with 0.5% cyclohexanol and phenethyl alcohol.

3. The effect of ethanol on L-phenylalanine transport increased with ethanol concentration, and was reversed by washing the section of intestine with buffer solution following exposure to ethanol.

4. Ethanol produced some increase in the permeability of the everted sac in 1 h, as measured by diffusion of D-phenylalanine.

5. The rapid inhibition of amino acid transport, the ease of reversibility of this inhibition, and the relatively small increase in back-diffusion, indicate that ethanol inhibits active transport under the conditions of these experiments.

INTRODUCTION

Most studies with ethanol have been concerned with possible systemic effects at concentrations generally found in the blood and tissues; but little attention has been directed toward the effect of higher concentrations of ethanol on intestinal absorption. Evidence has accumulated that non-lethal concentrations of ethanol will inhibit the active transport of cations by many different types of cells[1–5], possibly being related to the inhibition of (Na^+-K^+-Mg^{2+})-stimulated ATPase which has been associated with transport function[3,4]. Few reports have appeared on the effects of ethanol on amino acid transport. FREINKEL *et al.*[6] found that the uptake of alanine by rat-liver slices was reduced in the presence of 10 mM ethanol. CHANDLER,

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GEORG AND BASS[7] reported significant inhibition of α-aminoisobutyric acid uptake by the isolated perfused rat liver. SPENCER, BRODY AND LUTTERS[8] used everted sacs of hamster small intestine for transport studies, finding significant inhibition of glycine and L-proline transport with 1 M ethanol (4.6%).

We first became interested in the effect of drugs on intestinal transport following reports by COCKBURN *et al.*[9] that low phenylalanine diets produced cytoplasmic vacuoles in the marrow cells of normal infants, morphologically similar to those observed by MCCURDY, PIERCE AND RATH[10] in cases of acute alcoholism. Although this could be the result of a direct toxic effect of ethanol on the marrow cells, as indicated by BEARD AND KNOTT[11], nutritional deficiencies arising from interference with intestinal absorption might also be a contributing factor. Our earlier experiences with the inhibition of intestinal transport with chloramphenicol[12], with *S*-(1,2-dichlorovinyl)-L-cysteine (ref. 13), and with a large number of anorexiant, antihistaminic and antidepressant drugs[14,26], led to further studies with ethanol.

MATERIALS AND METHODS

Everted sac technics

Using the procedure described by WILSON AND WISEMAN[15], everted sacs of rat small intestine were used to examine the transport characteristics of a number of amino acids and glucose in the presence and absence of ethanol or other alcohols. 6–8 sacs were prepared from each animal, alternate sections being used as controls to minimize variations in transport by different sections of the intestine. ^{14}C-Labelled amino acids in 10 mM concentrations were added to KREBS–HENSELEIT bicarbonate buffer[16] containing 0.3% glucose. Glucose transport was studied with solutions containing 11 mM [^{14}C]glucose. The test solutions were placed on the inside (1 ml) and outside (5 ml) of the everted sacs. Diffusion studies with D-phenylalanine were carried out with 10 mM solutions in buffer, added either to the serosal side or to the mucosal side of the sacs. Where ethanol was added to the system, identical concentrations were placed on both sides of the sacs. The sacs were incubated for 1 h at 37° with gentle shaking, under an atmosphere of O_2 *plus* 5% CO_2. At the end of this period, the solutions were assayed for ^{14}C activity by liquid-scintillation counting. The concentration ratio of ^{14}C activity in the serosal/mucosal solutions was used as an index of transport function.

The open sac technic described by CRANE AND WILSON[17] was also used with everted sections of rat small intestine to obtain repeated samples (20 μl) of serosal solution at different time intervals during the experiment. Details of the technic were described earlier[13]. In the reversal studies, the open sacs were exposed to buffer containing 3% ethanol for 20 min, and then were washed with ethanol-free buffer for a 10-min period. Fresh buffer solution containing L-[^{14}C]phenylalanine was then added to both sides of the sac, and measurement of L-phenylalanine transport was resumed.

Tissue uptake studies

The technic described by AGAR, HIRD AND SIDHU[18] was used to study the uptake of L-Phe in the presence and absence of 3% ethanol. Segments of rat small intestine about 1 cm in length were cut open to expose the mucosa, and immersed

in buffer containing 3% ethanol for 10 min. They were then transferred to 2 ml of buffer containing different concentrations of [^{14}C]phenylalanine and 3% ethanol. A control series was run in the absence of ethanol. The segments were incubated for 20 min at 37° under an atmosphere of O_2 *plus* 5% CO_2, rinsed briefly to remove adherent solution, and total ^{14}C activity was determined by oxygen-flask combustion and liquid-scintillation counting[13]. Duplicate tissue segments were employed for each concentration of L-phenylalanine, which ranged from 1 to 8 mM.

Analytical methods

Radioactivity measurements were made by liquid-scintillation counting, employing technics described previously[13]. D-Phenylalanine was assayed fluorimetrically by the micro procedure described by WONG, O'FLYNN AND INOUYE[19].

Materials

L-Phenylalanine, L-valine (Aldrich Chem. Co., Milwaukee, Wisc.); L-leucine, glycine, isobutanol, *sec.*-butanol, cyclohexanol (Eastman Kodak Co., Rochester, N.Y.); L-methionine (Calbiochem., Los Angeles, Calif.); D-phenylalanine (Sigma Chem., St. Louis, Mo.); methanol, propanol, isopropanol, *n*-butanol, *tert.*-butanol (J. T. Baker Chem. Co., Phillipsburgh, N.J.); ethanol (Commercial Solvents Corp., Terre Haute, Ind.); phenethyl alcohol (Matheson Scientific, Inc., Chicago, Ill.); uniformly ^{14}C-labelled L-amino acids and D-glucose (New England Nuclear Corp., Boston, Mass.).

RESULTS

Effect of ethanol on intestinal transport

The transport data obtained with everted sacs of rat small intestine, are shown in Table I. In most cases, 8 sacs were used in each series, representing 4

TABLE I

EFFECT OF ETHANOL ON THE TRANSPORT OF ^{14}C-LABELLED AMINO ACIDS BY EVERTED SEGMENTS OF RAT SMALL INTESTINE

Using everted sacs of rat small intestine, 10 mM concentrations of ^{14}C-labelled L-amino acids (11 mM D-glucose) in bicarbonate buffer were placed inside and outside the sacs, which were incubated for 1 h at 37° under an atmosphere of 95% O_2 *plus* 5% CO_2. Where ethanol was present, identical concentrations were placed inside and outside of the sacs (1% or 3% ethanol). At the end of the incubation period, ^{14}C assays were carried out on the serosal and mucosal solutions by liquid-scintillation counting. Means and standard deviations were calculated for 4–8 sacs per series. Student's *t*-test was used to evaluate the significance of the difference between the ethanol and control series. N.S. stands for not significant (where $P \geqslant 0.05$).

^{14}C-Labelled substrate (10 mM)	*Number of sacs per series*	*Serosa-mucosa concentration ratios (mean ± S.D.)*		
		Controls	*+ 1% ethanol (217 mM)*	*+ 3% ethanol (651 mM)*
L-Phenylalanine	8	2.1 ± 0.4	2.0 ± 0.2 (N.S.)	1.2 ± 0.1 ($P < 0.001$)
L-Leucine	8	1.9 ± 0.2	1.7 ± 0.3 (N.S.)	1.2 ± 0.2 ($P < 0.001$)
Glycine	8	1.7 ± 0.3	1.7 ± 0.3 (N.S.)	1.2 ± 0.1 ($P = 0.001$)
L-Alanine	8	1.7 ± 0.3	1.7 ± 0.3 (N.S.)	1.2 ± 0.1 ($P = 0.001$)
L-Methionine	8	1.6 ± 0.4	1.5 ± 0.1 (N.S.)	1.1 ± 0.1 ($P < 0.01$)
L-Valine	4	2.7 ± 0.4	2.3 ± 0.4 (N.S.)	1.5 ± 0.1 ($P < 0.01$)
D-Glucose	8	4.8 ± 0.3	4.0 ± 0.8 ($P < 0.05$)	2.9 ± 0.6 ($P < 0.001$)

different animals. The means and standard deviations are given in Table I, together with P-values obtained by application of Student's t-test. The mean serosal/mucosal (S/M) ratios ranged from 1.6 to 2.7 for different amino acids. These were not altered significantly in the presence of 1% ethanol (217 mM); but in all cases there was a significant reduction of the S/M ratio with 3% ethanol ($P < 0.001$–0.01). The observations with glucose indicate a mean S/M ratio of 4.8 in the controls, dropping to 2.9 in the presence of 3% ethanol ($P < 0.001$). Water transport did not appear to be a significant factor, with the serosal fluid volumes following the incubation period showing slight changes (0.9–1.2 ml).

Similar observations were made with other alcohols using a smaller number of sacs for each test, and using only L-phenylalanine (10 mM) as the test substrate. Methanol was only partly effective in 3% solution (S/M, 1.3–1.4); *n*-propanol and isopropanol were effective at 2% concn. (S/M, 1.0–1.1); *n*-butanol and isobutanol were effective at 1% (S/M, 1.0–1.1); while *sec.*-butanol (S/M, 1.3) and *tert.*-butanol (S/M, 1.7–1.9) showed only slight inhibition at this level. Cyclohexanol was highly effective in suppressing L-phenylalanine transport, with complete inhibition at 0.5% (50 mM) concn. (S/M, 1.0). Phenethyl alcohol (40 mM) also produced complete suppression of L-phenylalanine transport in this system (S/M, 0.9).

Reversal studies

Using the open-sac technic described by CRANE AND WILSON[17], the transport of L-phenylalanine was followed in the presence of different concentrations of ethanol, with the results shown in Fig. 1. Slight inhibition was seen with 1% ethanol.

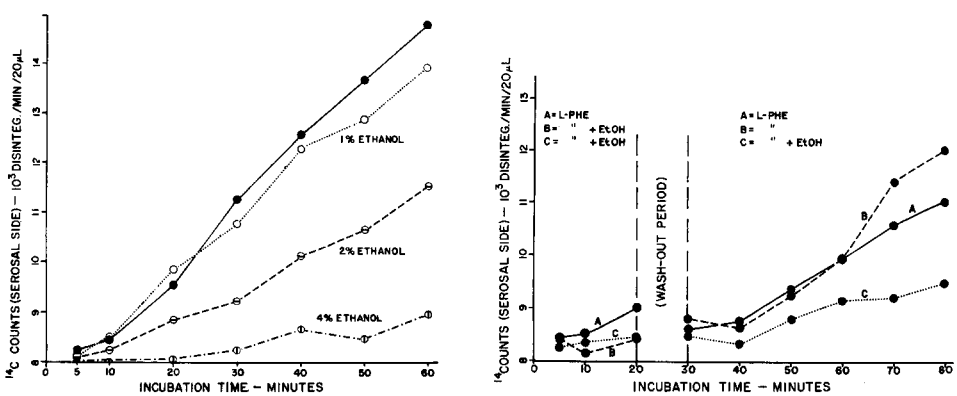

Fig. 1. Effect of ethanol on intestinal transport of L-phenylalanine. 10 mM concentrations of L-[^{14}C]phenylalanine in bicarbonate buffer were placed inside and outside the everted intestinal sacs. The concentration of ethanol ranged from 0 to 4% (v/v). Assays of ^{14}C activity were run on 20-µl samples of the solution inside the sacs by liquid-scintillation counting. Samples were taken at 10-min intervals during the course of the experiment.

Fig. 2. Reversal of ethanol inhibition of L-phenylalanine transport with everted sacs of rat small intestine. 10 mM concentrations of L-[^{14}C]phenylalanine in bicarbonate buffer were placed inside and outside the sacs. Initially, 3% ethanol was added to the buffer solutions inside and outside of Sacs B and C. Samples of 20 µl were taken from inside the sacs (serosal side) for radioactivity counts at 5, 10 and 20 min. All sacs were then washed with fresh buffer for 10 min, and L-phenylalanine was added again to the serosal and mucosal solutions; but ethanol was added only to Sac C in the same concentration used previously. Sampling was continued at 10-min intervals for the duration of the experiment.

There was a marked drop in the L-phenylalanine transport with 2% ethanol present, and essentially complete inhibition with 4% ethanol.

This technic was also used to determine whether the inhibitory effects of ethanol could be reversed by simple washing procedures. Following a 20-min exposure of the open sacs to 3% ethanol in buffer containing 10 mM L-phenylalanine, the sacs were washed with ethanol-free buffer for 10 min to remove the ethanol, and measurement of L-phenylalanine transport was then resumed. Control sections of intestine from the same animal were run simultaneously in the presence of 3% ethanol, and in the absence of pre-exposure to ethanol. The results are summarized in Fig. 2. Line A indicates the rate of transport in the control segment of intestine, with no pre-exposure to ethanol. Line C shows the inhibitory effect of 3% ethanol on L-phenylalanine transport. Line B indicates a resumption of L-phenylalanine transport following the 10-min wash-out period. There is a suggestion of an overshoot in Line B similar to that observed in experiments with other inhibitors such as chloramphenicol, which are readily removed by washing[12]. This did not occur with inhibitors such as dichlorovinylcysteine[13] and imipramine[14,26], which are firmly bound to the tissues. From a physiological standpoint, the 20-min period of exposure to ethanol did not appear to damage the intestine irreversibly, and transport functions returned to normal following removal of the ethanol.

Effect of ethanol on permeability

Possible changes in the permeability of the rat small intestine were measured by the passage of D-phenylalanine in either direction through the walls of everted sacs. Preliminary trials with equal concentrations of D-phenylalanine on both sides of the sacs showed that this isomer did not accumulate on the serosal side (S/M = 1), under conditions where L-phenylalanine was readily transported against a concentration gradient. When D-phenylalanine (10 mM) was placed only on the serosal side or only on the mucosal side of the everted sac, the amount passing to the opposite side in 1 h was measured in the presence and absence of 3% ethanol. No attempt was made to correct for tissue uptake or retention of D-phenylalanine. The results are shown in Table II, representing the mean values for 3 sacs in each series.

TABLE II

DIFFUSION OF D-PHENYLALANINE THROUGH EVERTED SACS OF RAT SMALL INTESTINE

Buffered solutions of D-phenylalanine (10 mM) were placed either on the mucosal side or on the serosal side of the intestinal sacs. These were incubated at 37° for 1 h, and fluorimetric assays were run for D-phenylalanine in the mucosal and serosal solutions. Values in parentheses represent concentrations on D-phenylalanine remaining at the location where originally introduced. (Mean of 3 sacs per series.)

Direction of diffusion	*Conditions*	*Mean wet weight of sacs (mg)*		*Terminal volume of fluid (ml)*		*Mean D-phenylalanine concn. (μg/ml)*		*Mean amount transported (μg)*
		Before	*After*	*Serosal*	*Mucosal*	*Serosal*	*Mucosal*	
Mucosa to serosa	Control	427	387	0.9–1.0	4.6–4.7	330	(1620)	320
	+3% ethanol	470	340	0.9–1.1	4.6–4.7	424	(1500)	427
Serosa to mucosa	Control	413	323	1.0–1.2	4.5–4.7	(819)	102	469
	+3% ethanol	423	310	0.9–1.2	4.6–4.7	(790)	117	542

In all cases, the ethanol-treated sacs showed slightly greater net movement of phenylalanine in either direction than the controls, indicating some increase in the permeability of the sacs under the conditions of the experiment. Tissue damage undoubtedly occurred, with some shedding of mucosal cells and with a greater weight loss occurring in the sacs exposed to ethanol. However, the 'back-leakage' of phenylalanine due to increased permeability was not great enough to account for the sharp reduction in L-phenylalanine transport which occurred in the presence of ethanol. In similar experiments with everted sacs of rat small intestine using only a 30-min incubation period, no significant differences in D-phenylalanine transport were noted in the presence or absence of 3% ethanol.

Effect of ethanol on tissue uptake of L-*phenylalanine*

Using the technic described by AGAR, HIRD AND SIDHU[18], the uptake of L-phenylalanine by segments of rat small intestine was studied with solutions containing 1, 2, 4 and 8 mM concentrations of L-phenylalanine, in the presence and absence of 3% ethanol. Results of the individual assays are shown in Fig. 3. The reciprocals of the substrate concentration and rate of uptake are plotted in a form

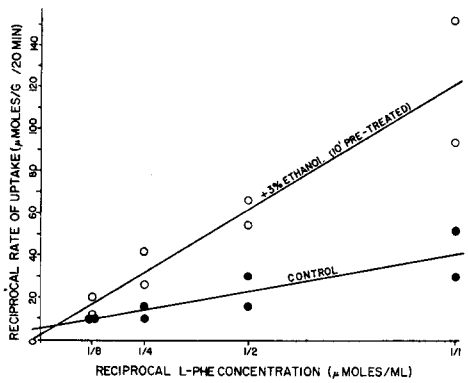

Fig. 3. Lineweaver–Burke plot of L-phenylalanine uptake by segments of rat small intestine in the presence and absence of 3% ethanol. The initial concentrations of L-[^{14}C]phenylalanine in the incubation medium ranged from 1 to 8 mM. Segments of rat small intestine approx. 1 cm in length were cut open and dropped into these solutions, using duplicate sample for each time period; segments used in the ethanol series were pre-exposed to 3% ethanol in buffer for 10 min prior to the uptake study. These were incubated for 20 min at 37° under an atmosphere of 95% O_2 *plus* 5% CO_2, following which they were rinsed briefly and assayed for ^{14}C activity by oxygen-flask combustion and liquid-scintillation counting. The best straight lines for each set of data were calculated by the method of least squares. Lower line represents the control series; upper line represents the series with 3% ethanol present.

suitable for kinetic analysis[20]. The lines of regression for each set of data were obtained by the method of least squares. In this and in a number of related experiments the slopes of the double reciprocal plots were always greater in the presence of ethanol than in its absence; but the intercepts with the *y*-axis were lower than the controls, with a tendency to pass through the origin unless the tissue was pre-exposed to ethanol before the uptake study. These observations do not indicate that a true competitive type of inhibition is involved. They are quite different

from the observations of ISRAEL, KALANT AND LAUFER[3], who demonstrated a competitive type of antagonism between ethanol and K^+ in their effect on (Na^+-K^+-Mg^{2+})-stimulated ATPase.

DISCUSSION

The effect of ethanol on the everted intestinal sacs at first sight appears to be one of denaturation and tissue damage, with some sloughing of cells. These changes were not evident with more potent inhibitors used in lower concentrations[14]. However, following a 20-min exposure to ethanol followed by a 10-min wash in ethanol-free buffer, the inhibitory effect on L-phenylalanine transport was eliminated, and normal transport characteristics were restored. The diffusion of D-phenylalanine through the walls of the sac showed a slight increase in the 1-h test period employed; but the changes were not significantly different from the controls in 30 min. Consequently, under the conditions of these experiments, there is little doubt that active transport processes are inhibited by ethanol, and that this can be reversed by washing out the excess ethanol. It seems likely that both the duration of exposure and concentration of alcohol may influence the extent of damage to the cellular membranes.

The effects of higher alcohols on the transport of L-phenylalanine is of considerable interest. The greater activity of long-chain aliphatic alcohols has been noted by THORE AND BALTSCHEFFSKY[21,22] in their work on the inhibition of electron transport and phosphorylation mechanisms in plants. Similar observations have been reported by ISRAEL, KALANT AND LEBLANC[4] on the inhibition of K^+ uptake by potassium-depleted rat cerebral cortex. Reports of the apparent inhibition of protein, RNA and DNA synthesis in *Escherichia coli* by derivatives of phenethyl alcohol[23,24] may in fact be due to the inhibition of transport mechanisms for essential amino acids and purines by intact bacterial cells, rather than to interference with synthesis at the ribosome level.

Although the kinetic study of L-phenylalanine uptake by intestinal tissue does not clearly define the nature of the inhibition by ethanol, interference with the attachment of amino acids to a hypothetical protein carrier in the cell membrane cannot be ruled out. Similarly, ethanol could interfere with the formation or utilization of energy-rich intermediates required for active transport. A non-specific depression of cellular respiration would produce this effect, and appears to be more likely than a specific effect on the synthesis or utilization of ATP. These questions have been considered at some length by ISRAEL and co-workers[1–4], who have suggested that the inhibition of ion transport by ethanol resulted from the inhibition of membrane ATPase[3,4]. Of greatest interest to us, perhaps, are the relevant observations that chlorpromazine, promethazine and diphenhydramine which were found to be effective inhibitors of amino acid transport with everted sacs of rat small intestine[14,26], also suppressed electrolyte transport in other cellular systems and inhibited (Na^+-K^+-Mg^{2+})-stimulated ATPase in liver microsomes[25].

Relatively high concentrations of ethanol were required to inhibit intestinal transport of amino acids in our experiments, but concentrations of this magnitude would certainly be found in the intestinal tract following large doses of ethanol. The observations *in vitro* suggest that ethanol may have an effect on the active transport

mechanisms in the intestinal tract of living animals, although general pharmacological effects such as alterations in gastric emptying time, intestinal motility and blood flow would undoubtedly be superimposed. Further studies are under way in this area.

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