

Acute and chronic exposure to ethanol and the electrophysiology of the brush border membrane of rat small intestine

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SUMMARY In this study we have investigated the effects of (a) chronic ethanol intake on glucose and galactose absorption across the rat jejunum *in vivo* and on the potential difference across the isolated brush border membrane (V_m) and (b) acute exposure to ethanol (4% or 8%) and acetaldehyde (0.25%) on changes in V_m associated with Na^+ -dependent galactose absorption across the jejunum and ileum. Chronic ethanol intake was associated with hyperpolarisation of V_m and an enhanced galactose but not glucose transport. Acute ethanol and acetaldehyde were without effect on V_m whether or not galactose was present. We conclude that while a greater electrochemical gradient across the brush border membrane is a likely explanation for the stimulation of galactose absorption induced by ethanol feeding, factors other than changes in V_m are responsible for the inhibitory effects of acute ethanol.

It is well known that acute exposure of the intestinal mucosa to ethanol reduces the transport of those nutrients which use the Na^+ -gradient for movement across the brush border membrane (BBM),¹⁻³ one of which is the hexose galactose.^{4,5} In marked contrast, we have noted an enhanced galactose absorption in rats maintained for four weeks on a liquid diet containing 5% ethanol,⁶ a finding which is a likely consequence of an early maturation of enterocytes.⁷

The potential difference across the brush border membrane (V_m) is an important driving force for Na^+ -dependent sugar movement into enterocytes.⁸ In order to examine further the mechanisms involved in the functional response to acute and chronic exposure to ethanol, we have compared the effects of ethanol feeding on the active absorption of glucose and galactose *in vivo* and report those changes in V_m , measured *in vitro*, associated with active galactose uptake after acute or chronic exposure to ethanol. Finally, because acetaldehyde, an intestinal metabolite of ethanol, inhibits Na^+ -dependent sugar movement across the brush border membrane,² we have assessed the effect of acetaldehyde on V_m .

Methods

DIETS AND ANAESTHESIA

Sprague-Dawley rats (RFHSM bred, initial weight 140–150 g) were individually caged and pair-fed for 26–32 days on a liquid control diet containing Complan (Farley Health Products, Nottinghamshire, UK), casein, glucose, and corn oil to provide 34% of the total calories as carbohydrate, 18% as proteins and the remainder as fat.⁹ For the alcohol diet, ethanol (5% w/v) was substituted for a proportion of the fat content so as to provide 36% of the total calories. Additional vitamins (Orovite 7, Beecham, Brentford, UK) 5 g/l were added to each diet to satisfy the requirements for growth.¹⁰ The alcohol diet was replaced by the control diet 18 hours before experimentation. Chow fed animals (200–250 g) were maintained on Diet 41B (Grain Harvesters Ltd, Kent, UK) up to the time of use.

Pentobarbitone sodium (90 mg/kg ip, Sagatal, May and Baker, Essex, UK), was used for *in vivo* absorption studies and to enable tissue to be removed for *in vitro* experiments.

MEASUREMENTS OF V_m

Full details of the *in vitro* preparation used, the techniques for the preparation and use of micro-electrodes and the criteria adopted for accepting

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impalements have been previously described.¹¹ Jejunal tissue was taken approximately 10 cm from the ligament of Treitz and ileal tissue approximately 10 cm from the ilealcaecal junction.

SUGAR ABSORPTION *IN VIVO*

The techniques used to measure glucose and galactose absorption *in vivo* are similar to those described previously.¹² In brief, uptake was determined by circulating the sugar (64 mM) dissolved in gassed (95% O₂:5% CO₂) bicarbonate saline through a 25 cm length of upper jejunum. After 30 minutes, the perfusate in the intestine and circulation system was washed out, deproteinised and estimated for either glucose or galactose.¹³ The segment of intestine was removed and its length measured. Sugar uptake was calculated as luminal loss and expressed as nmol/cm/min. Uptake data were corrected for non-saturable absorption¹² in order to obtain data for active, Na⁺-dependent transport.

STATISTICAL ANALYSIS

Data are given as mean (SE). Difference between means were evaluated by Student's *t* test for unpaired samples. Differences between the magnitude of the depolarisation obtained upon addition of galactose to the mucosal fluid were compared using an Interaction test.¹⁴ For both tests, differences were considered not significant at $p > 0.05$.

CHEMICALS

D-galactose (glucose free) and phlorhizin were obtained from Sigma UK. Absolute ethanol was purchased from James Burrough, Essex, UK. All other chemicals were Analar Grade from BDH, Dorset, UK.

Results

The data for ethanol intake and body weight for animals on the two liquid diets over the 26–31 day feeding period are shown in Figure 1. The gain in body weight per day was very similar in control (4.5 (0.23) g/day) ($n=8$) and ethanol fed animals (4.76 (0.27) g/day) ($n=9$) over the second half of the feeding period during which time ethanol consumption expressed per kg body weight was remarkably constant. The initial weight loss in the ethanol group may be because of the diuretic effect of ethanol. No evidence of diarrhoea was apparent in rats fed either diet.

V_m AFTER CHRONIC ETHANOL INGESTION

Approximately 15% of impalements resulted in electrical stability for a minimum period of 10 seconds. Examples, showing the profiles of three

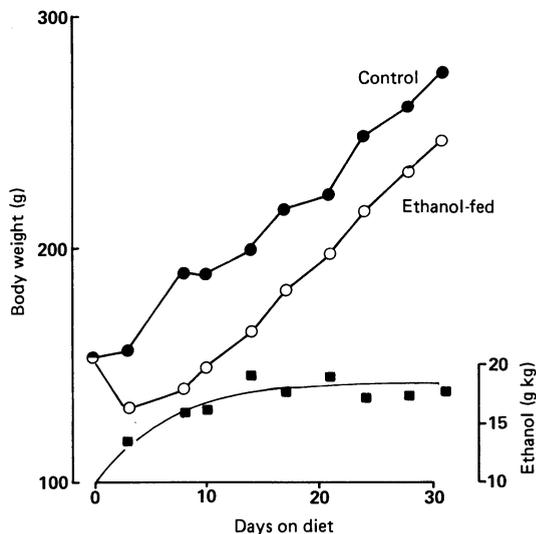


Fig. 1 Body weight in ethanol fed and pair fed control rats. Also shown is the amount of ethanol consumed expressed as g/kg body weight at various times over the 32 day feeding period.

successful impalements whilst exposing jejunum from chow-fed rats to oxygenated bicarbonate buffer are shown in Figure 2. In all cases, an immediate deflection occurred upon microelectrode penetration of the cell and the potential difference returned to its original baseline following withdrawal of the electrode giving a mean potential difference of -43.7 (2.1) mV ($n=36$). This value is similar to previously reported measurements of V_m in rat small intestine.^{15,16} The time between removal of tissue from the animal and exposure to oxygenated buffer never exceeded one minute as further delays affect

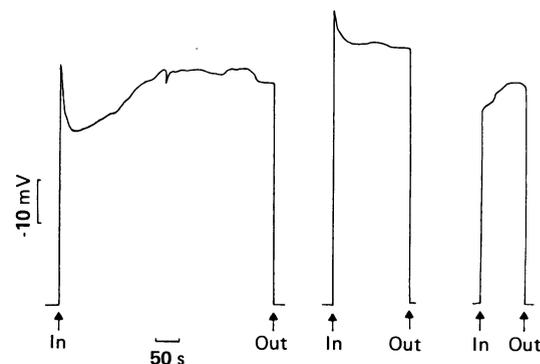


Fig. 2 Three examples of changes in potential difference observed during microelectrode impalement of enterocytes. Arrows refer to impalement and withdrawal of the microelectrode. In each case, electrical stability was maintained for a minimum of 10 seconds.

Table 1 Potential difference across the brush border membrane (V_m) in control and ethanol fed rats

	V_m (mV)		
	- Galactose	+ Galactose (10 mM)	<i>p</i>
Control diet	-42.9 (1.5) [81]	-34.4 (1.2) [61]	<0.001
Ethanol diet	-51.2 (1.5) [79]	-36.1 (1.4) [60]	<0.001
<i>p</i>	<0.001	>0.05	

Values are given as mean (SEM) with number of impalements in square brackets. At least three animals were used for each group of experiments.

tissue viability.¹⁵ For non-gassed tissue, V_m was found to be -7.6 (1.3) mV ($n=7$).

Ethanol feeding resulted in a significant hyperpolarisation of the brush border membrane of some 8.3 mV (Table 1); the distribution of individual impalements in intestine from control and ethanol fed rats is shown in Figure 3. The addition of D-galactose (10 mM) to the mucosal fluid caused a depolarisation of 8.5 mV and 15.1 mV, respectively, in intestine from control and ethanol fed rats ($p<0.05$) (Table 1).

The effects of Na^+ concentration on V_m was studied by using mucosal solutions of varying Na^+ concentration, mannitol being used to maintain isotonicity. A comparison of the response using intestine from control and ethanol fed animals is shown in Figure 4. Under control conditions, Na^+ concentration had a profound effect on the magnitude of V_m and analysis of the region between

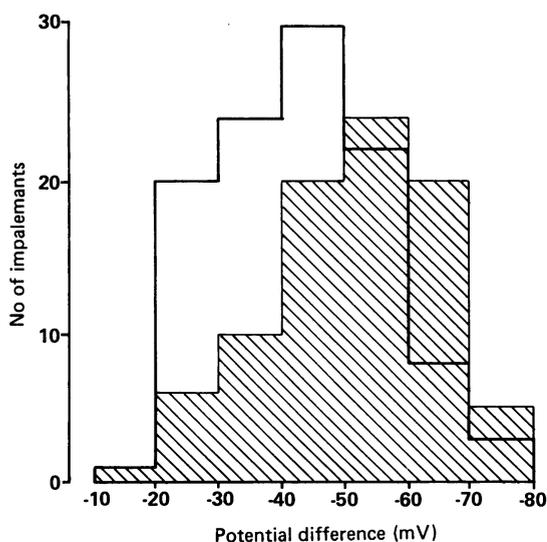


Fig. 3 Histogram showing the distribution of measured values of V_m in intestine from control (clear area) and ethanol fed (shaded area) rats.

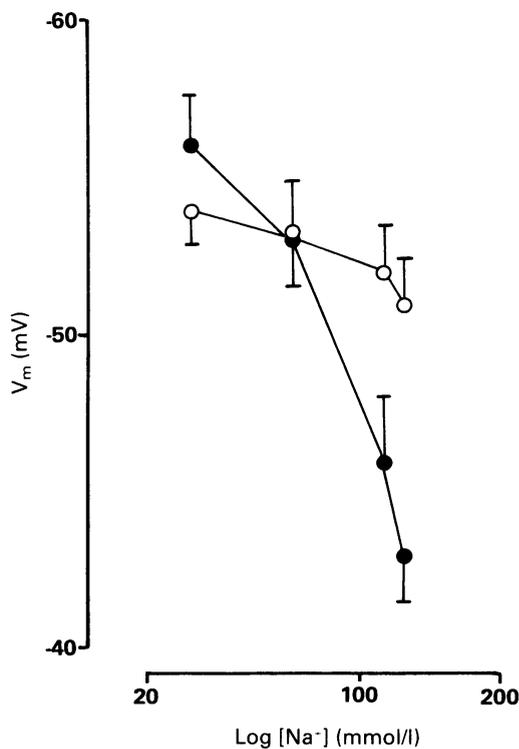


Fig. 4 Effect of chronic ethanol ingestion on the relationship between V_m and mucosal sodium concentration. Animals were maintained on either ethanol (○) or control liquid diet (●). Means (SEM) are given with 15–81 impalements for each mean value.

61 and 143 mM Na^+ revealed that the slope of the response for a 10-fold change in Na^+ was 25.5 mV. After ethanol, however, a decrease in Na^+ from 143 to 25 mM was without significant effect on V_m ($p>0.05$).

ACUTE EXPOSURE TO ETHANOL AND ACETALDEHYDE

Jejunal and ileal tissue from chow fed rats was used to study the effect of acute exposure to ethanol (4% or 8% w/v) and acetaldehyde (0.25% w/v) on V_m . At these concentrations, these agents have been shown to inhibit sugar transport across the BBM.³ In their absence, V_m was very similar to that obtained from tissue exposed to the control liquid diet ($p>0.5$, Tables 1, 2). In contrast with the hyperpolarisation seen after chronic ethanol feeding, however, the presence of ethanol or acetaldehyde in the mucosal fluid were without effect on V_m and, furthermore, these agents did not influence the depolarisation of V_m induced by the presence of D-galactose in the mucosal fluid (jejunum: 4% ethanol $p>0.5$, 8%

Table 2 Acute effect of ethanol and acetaldehyde on V_m of jejunum and ileum

	Jejunum		Ileum	
	No galactose	+ Galactose (10 mM)	No galactose	+ Galactose (10 mM)
Bicarbonate buffer	-43.7 (2.1) [36]	-38.4 (1.5) [42]†	-40.5 (1.1) [65]	-36.6 (1.1) [43]†
+ Ethanol (4%)	-43.8 (1.9) [62]*	-36.4 (1.3) [49]**	-42.7 (1.5) [50]*	-36.4 (1.1) [49]**
+ Ethanol (8%)	-43.1 (1.2) [80]*	-33.6 (1.4) [41]**	-39.2 (1.1) [44]*	-36.8 (1.5) [38]*
+ Acetaldehyde (0.25%)	-41.6 (1.1) [82]*	-35.2 (1.3) [42]**	-41.8 (1.3) [38]*	-35.8 (1.6) [32]**

Tissue was obtained from chow-fed rats and values (mV) are given as mean (SEM) with number of impalements in square brackets. At least three animals were used for each group of experiments. * $p > 0.05$ compared with V_m in the presence of bicarbonate buffer alone; † $p < 0.05$ compared with V_m in the absence of galactose.

ethanol $p > 0.2$, acetaldehyde $p > 0.7$; ileum: 4% ethanol $p > 0.05$, 8% ethanol $p > 0.9$, acetaldehyde $p > 0.1$.

SUGAR ABSORPTION IN VIVO

Chronic ethanol feeding resulted in a 31.3% stimulation of active galactose absorption ($p < 0.05$, Fig. 5), but was without significant effect on glucose absorption ($p > 0.4$).

Values for glucose and galactose absorption in rats maintained on the liquid control diet were very similar to jejunal uptakes using chow fed animals (glucose: 398.1 (31.0) nmol/cm/min ($n=6$); galactose: 317.4 (26.5) nmol/cm/min ($n=6$)).

Discussion

Previous studies in the rat on the functional effects of acute ethanol exposure on intact intestinal mucosa or isolated brush border membrane (BBM) have reported a diminished active sugar uptake.¹⁻³ In contrast, there is conflicting information on the response to chronic ethanol ingestion on hexose absorption.¹⁷ The discrepancies between the various studies are likely to be caused by such variables as the time scale of ethanol ingestion and the alcohol and carbohydrate composition of the control and ethanol diets. A crucial factor of the dietary regime adopted in this study was that the control diet used fat as a calorie substitute for ethanol. Carbohydrate substitution was avoided as variations in the dietary level of this nutrient have been shown to influence sugar uptake.¹⁸ Animals on control and experimental diets gained weight at a similar rate and this, together with our previous observations of an unchanged villus height and enterocyte number after ethanol,⁶ suggests that both groups were of a comparable nutritional state. It must also be emphasised that rats were not subject to overnight starvation before experimentation as this procedure is associated with profound changes in V_m and sugar absorption.^{11,19} The ethanol concentrations used for both chronic and acute studies were within the range found in the

upper intestine of man after moderate alcohol ingestion.²⁰

For *in vivo* absorption studies, a sugar concentration of 64 mM was chosen as in control animals, uptake at this concentration gives a good approxima-

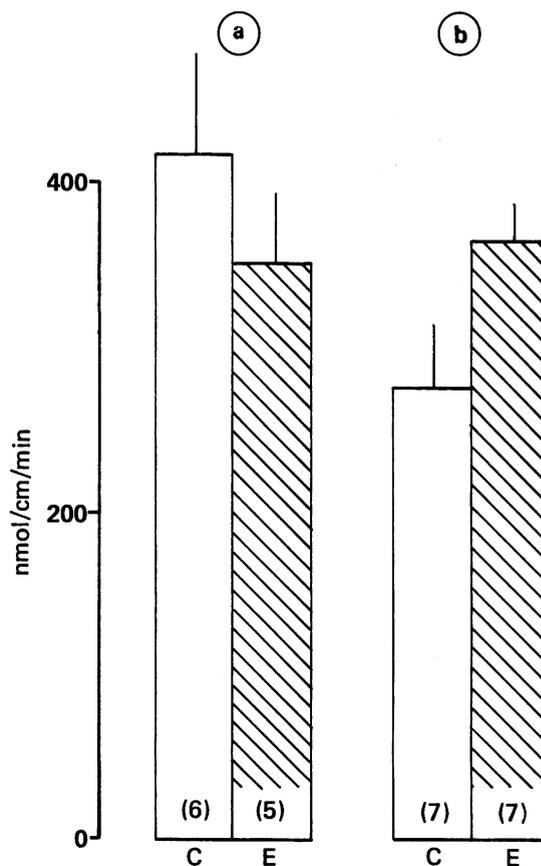


Fig. 5 Glucose (a) and galactose (b) absorption *in vivo* in rats fed control (C) or ethanol (E) liquid diets. Data have been corrected for the linear uptake process (see Methods) and are given as means (SEM) with number of observations in parentheses.

tion of V_{\max} .¹² Hypertonicity of the circulating sugar containing buffer is unavoidable because even modest reductions in Na^+ concentration to compensate for this osmolality change cause a significant inhibition of absorption.²¹ Our results confirm and extend a previous report of an enhanced capacity for active galactose absorption in rats maintained on ethanol.⁶

The differential effect of ethanol feeding on galactose and glucose absorption may reflect separate carrier systems for the two sugars.²² This observation may also be a consequence of the ability of enterocytes to metabolise glucose²³ and it is of interest that a previous study has reported a greater inhibition, by ethanol, of glucose compared with galactose absorption.⁴ The use of galactose, a non-metabolisable sugar²³ to assess changes in intestinal uptake avoids problems of data interpretation imposed by intracellular metabolism of absorbed sugar and alterations in galactose uptake after ethanol give a better indication of changes in membrane transport *per se*.

The stability and magnitude of V_m measurements in our study were a consequence of rapid mounting and oxygenation of the isolated tissue. It has been argued elsewhere¹⁵ that tissue anoxia is a likely cause of the low V_m values obtained previously. As the magnitude of the electrical gradient across the BBM is an important driving force for active sugar entry into the enterocyte,⁸ our finding of an enhanced V_m following ethanol feeding provides an obvious explanation for the stimulation of galactose absorption *in vivo*.

The process of enterocyte migration along the jejunal villi is associated with hyperpolarisation of V_m ,²⁴ and we have previously suggested that the adaptation to chronic ethanol ingestion may be related to a reduced enterocyte transit resulting in a higher proportion of mature cells on the villus.⁶ In this context, it is of interest that prolonged ethanol feeding is associated with an increased lactase activity⁶ and a stimulation of iron absorption²⁵ but no change in villus height or enterocyte number.⁶

Data obtained in Na^+ replacement experiments in this present study imply that a reduction in Na^+ permeability of the brush border membrane is responsible, at least in part, for the hyperpolarisation seen in ethanol fed rats. The electrical response is unlikely to be the result of alterations in Cl^- concentration of the mucosal fluid during these experiments, as Cl^- diffusion makes no contribution to V_m .¹⁵ Interestingly, membrane hyperpolarisation together with an increased Na^+ -dependent nutrient uptake occur in two other experimental conditions, fasting and hyperglucagonaemia,^{26,27} a reduced enterocyte transit being a recognised feature of both conditions.

Acute exposure to ethanol at concentrations similar to, or below, those used in this present work have been shown to inhibit Na^+ -dependent galactose transport.^{4,5} As we were unable to detect changes in V_m after acute exposure to ethanol, previous explanations of the inhibition of active nutrient uptake by ethanol or its intestinal metabolite acetaldehyde² based on an increased sodium conductance of the BBM^{1,2} can be eliminated as depolarisation of V_m would be expected in the presence of these agents. The precise mechanism of the inhibitory effect of acute ethanol on brush border membrane sugar transport must await further studies, but the osmotic effects of ethanol can be excluded.² Other possibilities include direct conformational effects on the hexose transporter and increased fluidity of the BBM.^{2,28}

In conclusion, we have shown that the jejunal epithelium of ethanol fed rats displays an enhanced ability for galactose transport, the likely explanation being a higher electrical driving force for Na^+ -coupled movement across the BBM. The significance of our findings to absorption studies in alcoholics is unclear because an associated poor nutritional state will complicate the interpretation of uptake data. There is, however, some evidence for an enhanced absorption of glucose and xylose in well nourished alcoholics.²⁹ The chronic effects of ethanol resulting from a slower enterocyte transit may compensate, at least in part, for its acute inhibitory action on sugar uptake.

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