

EFFECTO DEL ETANOL SOBRE LA ABSORCION DE SODIO, CLORO, AGUA, GLUCOSA Y TRIPTOFANO EN INTESTINO DELGADO DE RATA *IN SITU*.

In situ effect of ethanol on sodium, chloride, water, glucose and triptofan absorption in the rat small intestine.

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RESUMEN

Noradrenalina (2.5×10^{-5} M), etanol (652 mM), glucosa (5 mM) y triptofano (0.2 mM) aumentan la absorción de sodio, cloro y agua en intestino delgado de rata. Se sugiere que los dos últimos actúan mediante la estimulación de un sistema transportador glucosa-sodio y triptofano-sodio respectivamente.

El transporte de glucosa y triptofano y la diferencia de potencial transepitelial disminuyen en presencia de etanol colocado en el lumen intestinal.

La dibencilina, no así el propranolol, inhibe la acción de la noradrenalina y del etanol. Lo anterior indica que el efecto está mediado por un receptor alfa adrenérgico y no por un receptor beta adrenérgico.

Se sugiere que el etanol libera noradrenalina desde las terminaciones nerviosas simpáticas de la mucosa intestinal.

ABSTRACT

Norepinephrine (2.5×10^{-5}), ethanol (652 mM), glucose (5 mM) and tryptophan (0.2 mM) increase the absorption of sodium, chloride and water in the small intestine of the rat. The suggestion is made that glucose and tryptophan act by stimulating a glucose-sodium and a tryptophan-sodium carrier systems respectively.

Glucose and tryptophan transport as well as the transepithelial potential difference are decreased when ethanol is present in the intestinal lumen.

Dibenzylamine, but not propranolol, inhibited the action of norepinephrine and ethanol, indicating that the effect is mediated by an alfa and not a beta adrenergic receptor.

We suggest that ethanol causes the release of norepinephrine from sympathetic nerve endings of the intestinal mucosa.

Keywords: Rat gut. Ethanol action. Gut absorption. Physiology.

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INTRODUCTION

Numerous studies on the absorption of ions, sugars and amino acids by the small intestine have established certain characteristics of the physiological processes involved.

Using optimal concentrations of electrolytes and non-electrolytes it has been shown that the luminal and serosal sides of the intestine carry a negative and positive charge respectively (Clarkson, et al. 1961; Hubel, 1969).

The small intestine absorbs water against hydrostatic and osmotic pressure gradients (Visscher, et al. 1945). Water absorption takes place against a water activity gradient and it is now generally believed that water transport is not in itself an active process, but, that it is linked to solute transfer (Albers, 1969).

On the other hand, ethanol is absorbed by simple diffusion (Simposio Internacional sobre alcohol y alcoholismo, 1969). Sodium (Whitlam and Wheeler, 1970; Levinson and Schedl, 1966), chloride (Hubel, 1969; Simposio Internacional sobre alcohol y alcoholismo, 1969), sugars (Levinson and Schedl, 1966; Quay and Armstrong, 1969) and amino acids (Quay and Armstrong, 1969; Csáky, 1965; Munck and Rasmussen, 1975) are actively transported by the small intestine, the latter two being coupled to sodium transport.

The purpose of this work was to study the intestinal absorption of sodium, chloride, water, glucose and tryptophan, in the presence and absence of ethanol in the lumen. The effects of norepinephrine, in the presence and absence of dibenzylamine and propranolol, are compared to those of ethanol.

MATERIAL AND METHODS

Albino rats (250 to 300 g), fasted for 48 hours prior to the experiments, were anesthetized with one ml of a 20% solution of urethane per 150 g of body weight.

The concentration of ethanol chosen for this work (652 mM) was based on the observation that, during a moderate to high intake of alcohol, the intestinal (intraluminal) concentration is of the same order.

The experimental set-up consisted (Fig. 1) of an "in situ" 30 cm segment of jejunum, to the proximal end of which was tied a small plastic funnel containing a perfusion cannulae and an agar-Ringer bridge connected to a mucosal calomel electrode. Saline, at 37°C, was perfused at a constant flow of 0.5 ml/min by means of a polystaltic pump. The intraluminal pressure was measured by means of a manometer placed between the pump and the proximal end of the segment. The potential difference (P. D.) was measured by means of two calomel electrodes placed on the mucosal and serosal side of the preparation, these in turn were connected to a Grass model 5 Polygraph. The test solutions were perfused through the proximal cannulae and collected in a glass cylinder at the distal portion of the intestinal segment. Volume measurements were made every 15 minutes.

In each experiment the same rat served as both test and control in order to eliminate possible individual differences that might arise due to the administration of ethanol.

FIG. 1

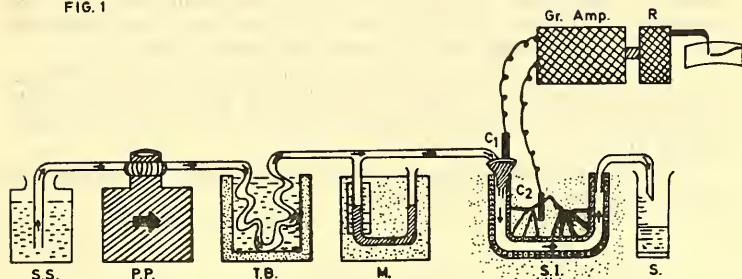


Fig. 1.- Schematic diagram of the preparation of small intestine of the rat. S.S. = saline; P.P. = polistaltic pump; T.B. = bath at a normal temperature; M = manometer; S.I. = small intestine; GR.AMP. = press amplifier; R = recorder; S = samples.

Water absorption was estimated by measuring the volumen differences obtained between the test and control solutions.

Sodium and chloride ions were determined electrometrically by the use of ion-specific electrodes (Thomas, model 4923-L 10 sodium and Orion model 92-17 chloride electrodes). Glucose was estimated by means of the o-toluidine procedure (Hyvarinen and Esko, 1962) and tryptophan was determined by the procedure of Laties (Laties, 1949).

When the beta-receptor blocker propranolol was used, a final concentration of 1×10^{-6} M was added to the perfusate after collecting a control sample.

In those experiments in which the effects of norepinephrine were studied, one ml of a 1×10^{-5} M dibenzylamine solution in saline was parenterally injected two hours before the infusion of saline containing a final concentration of 2.5×10^{-5} M norepinephrine or 652 mM ethanol.

D-Glucose (5mM) and L-Tryptophan (0.2 mM) were prepared in saline and were perfused in the presence and absence of ethanol.

RESULTS

Fig. 2 shows that 2.5×10^{-5} M Norepinephrine added to the luminal side elicits a statistically significant ($p < 0.001$) increase in the absorption of water, sodium and chloride, with significant changes in P. D. The same results were observed when Norepinephrine was substituted for Ethanol (652 mM) (see Fig. 3).

Dibenzylamine, completely inhibited the effects of norepinephrine and ethanol (Figs. 4 y 5). Propranolol, on the other hand, did not inhibit the stimulating effect of norepinephrine or ethanol (Fig. 6).

When the intestine was perfused, in the presence and absence of ethanol, with saline containing 5 mM glucose, ethanol inhibited the absorption of sodium and glucose, but did not significantly affect the movement of chloride. The P. D. was also decreased. Water transport was increased by ethanol in the presence of glucose (Table 1).

A significant decrease in the absorption of sodium, chloride and tryptophan was observed when the intestine was perfused with 0.2 mM tryptophan in the presence of ethanol. The P. D. was also decreased. (Table 2).

The intraluminal pressure was not significantly altered by any of the treatment previously described.

TABLE Nº 1.

Effects of glucose and ethanol on the absorption of sodium, chloride and water by the rat intestine.

SUBSTANCE	SALINE (n=7)	SALINE + GLUCOSE (n=9)	SALINE + GLUCOSE + ETHANOL (n=9)
WATER uL/cm ² /h	469 ± 10	469 ± 8	788 ± 13.5 p < 0.001
SODIUM uEq/cm ² /h	24 ± 3	49 ± 8	42 ± 8 p < 0.05
CHLORIDE uEq/cm ² /h	11 ± 1.2	17 ± 1.5	18.4 ± 2.4 p < 0.5
P. D. mVots	1.2 ± 0.2	3.5 ± 0.4	2.0 ± 0.4 p < 0.01
GLUCOSE uM/cm ² /h	—	1.8 ± 0.2	1.0 ± 0.2 p < 0.02

TABLE Nº 2.-

Effects of tryptophan and ethanol on the absorption of sodium, chloride and water by the rat intestine.

SUBSTANCE	SALINE (n=7)	SALINE + TRYPTOPHAN (n=9)	SALINE + TRYPTOPHAN + ETHANOL (n=9)
WATER uL/cm ² /h	469±10	472±10	359±6.6 p=0.001
SODIUM uEq/cm ² /h	23.6±3.4	42±6	27±2.3 p=0.001
CHLORIDE uEq/cm ² /h	11±1.2	17±1.6	12.6±1.0 p<0.01
P. D. mVots	1.2±0.2	4.6±0.3	2.9±0.5 p < 0.001
TRYPTOPHAN uM/cm ² /h	—	1.1±0.007	0.07±0.09 p < 0.01

These results are quite different from those observed when glucose was added to the perfusate. Water transport was not increased by ethanol in the presence of tryptophan and the increase in sodium and chloride uptake, due to tryptophan, was completely inhibited by the presence of ethanol. The P D. was affected in a manner similar to the effect of glucose. Tryptophan and glucose transport were inhibited by ethanol.

DISCUSSION

Since the studies of Ussing and Windhager (Schultz, et al. 1974) it has become increasingly clear that solute transport across epithelia may take one of two routes, i. e. a transcellular route through the cellular membrane, or an extracellular route that circumvents the membranes surrounding the epithelial cells. The latter is referred to as the shunt pathway and it comprises the tight junctions and the underlying lateral intercellular spaces. The tight junctions are permeable to some permeable small ions and small non-electrolytes (Kimmich, 1973).

In the absence of transepithelial ionic gradients, the shunt pathway markedly attenuates the difference between the electromotive forces across the mucosal and basolateral membranes, so that these tissues will be, in general, characterized by small transepithelial potential differences. The presence of a transepithelial potential difference reflects diffusion potential differences resulting from the permselectivity of the shunt (Schultz, et al. 1974).

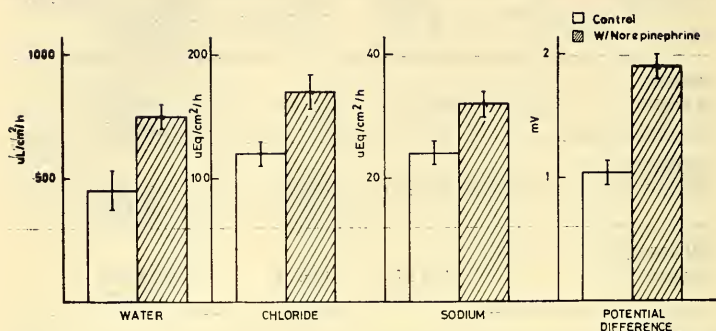


Fig. 2.- Effect of 2.5×10^{-5} M Norepinephrine, on the absorption of sodium, chloride and water in the small intestine of the rat, and on potential difference. $n = 7$.

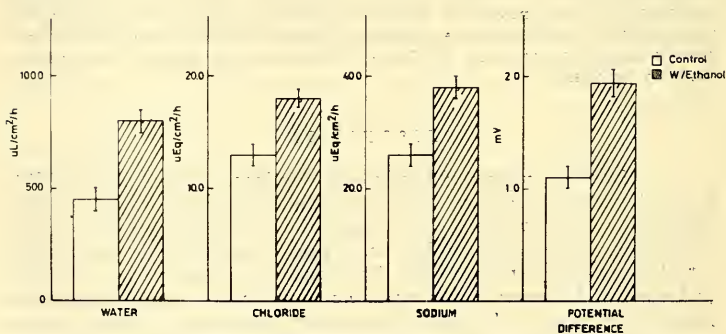


Fig. 3.- Effect of 652 mM Ethanol on the absorption of sodium, chloride and water in the small intestine of the rat, and on potential difference. $n = 7$.

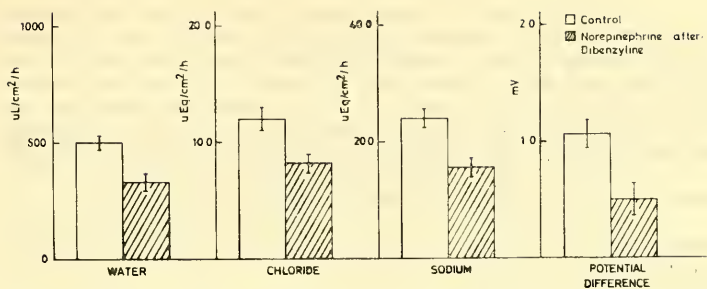


Fig. 4.- Effect of 2.5×10^{-5} M Norepinephrine after 10^{-5} M Dibenzylamine on the absorption of sodium, chloride and water in the small intestine of the rat, and on potential difference. $n = 5$.

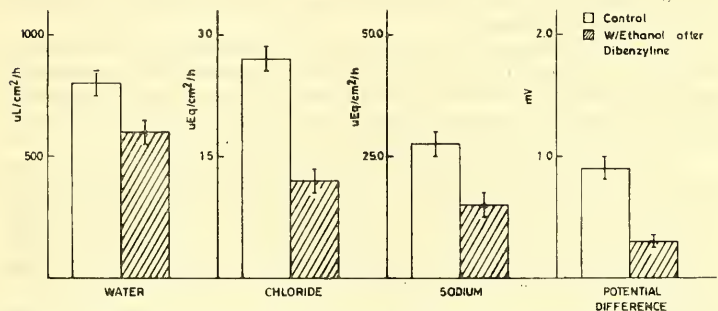


Fig. 5. Effect of 652 mM Ethanol after 10^{-5} M Dibenzylamine, on the absorption of sodium, chloride and water in the small intestine of the rat, and on potential difference. $n = 5$.

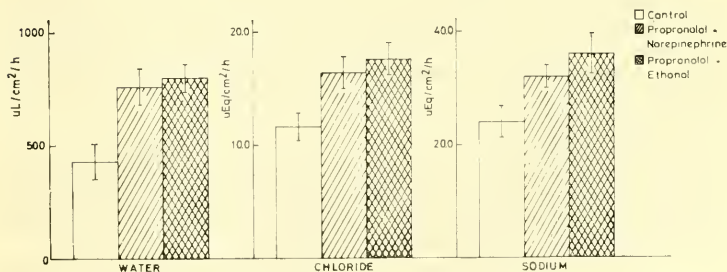


Fig. 6.- Effect of 1×10^{-6} M Propranolol on the stimulating effect of Norepinephrine and Ethanol, on the absorption of sodium, chloride and water in the small intestine of the rat. $n = 5$.

The influx into the shunt pathway from the mucosal solution closely agrees with the unidirectional serosal to mucosal flux of sodium in rabbit ileum and rat jejunum. The above suggest that most, if not all, of the back flux of sodium from the serosal to the mucosal solution takes place through the shunt, and that the basolateral membranes of the epithelial cells are essentially impermeable to sodium, suggesting the presence of an active transport system for this ion (Crane, 1965).

Fordtran, et al. 1968 reported sodium and urea movements across human jejunum are markedly affected by the bulk flow.

Norepinephrine, at a final concentration of 2.5×10^{-5} M (Fig. 2) increased the absorption of sodium, chloride and water. These effects could be due to an increase in both the active transport of sodium and the passage through the shunt pathway.

Ethanol also increased the absorption of sodium, chloride and water, (Fig. 3) suggesting that it causes the release of norepinephrine from sympathetic nerve-endings in the mucosa. Fluorescent histochemical techniques (Douglas, et al. 1971) have shown the existence of compact nervous endings in the submucosa of the small intestine, specially in the region of the cryptae.

The adrenergic action of ethanol was inhibited by the alfablocking agent, dibenzylamine (Fig. 5). This drug decreased the transport of sodium, chloride and water suggesting that the effect might be mediated by alpha adrenergic receptors. Propranolol, a beta adrenergic receptor agonist, had no effect on the transport of these ions.

From the experiments in which glucose was perfused in the presence and absence of ethanol, it can be seen that glucose and sodium transports are coupled. Ethanol inhibited sodium transport with a concomitant decrease in glucose transport (Ghirardi, et al. 1971). These results agree with those of the literature which clearly indicate that this is a sodium-glucose carrier mediated transport system, which can be inhibited by phlorizin (Kimmich, 1973). Chloride transport was not affected by ethanol.

Alvarado, 1966 has shown that glucose, (a non-electrolyte) has an electrogenic action on sodium and chloride transport, but that it has no effect on water absorption. These same results are shown on Table 1; water uptake was not affected by glucose, but was greatly increased by ethanol. On the other hand, glucose increased sodium and chloride transport. Sodium and glucose uptake, but not chloride was partly inhibited by ethanol. The transepithelial P. D. was decreased by ethanol. The presence of glucose in the lumen presumably inhibits the adrenergic action of ethanol.

Amino acid uptake is also a sodium mediated transport system, which can be inhibited by ouabain (Chang, et al. 1972). Our results support these findings. Table 2 shows that the presence of tryptophan in the perfusate increased sodium and chloride uptake. The addition of ethanol completely inhibited these uptakes.

Israel, et al. 1968 have shown that the active transport of amino acids in the intestine depends on the presence of an operative active transport of sodium or of a sodium-potassium-activated ATPase or both.

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