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EFFECT OF ETHYL ACETATE ON THE TRANSPORT OF SODIUM AND GLUCOSE IN THE HAMSTER SMALL INTESTINE IN VITRO

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SUMMARY

The effect of ethyl acetate on Na^+, water and glucose transport, as well as on glucose and electrolyte intracellular concentrations in everted and cannulated sacs of hamster jejunum, have been studied.

Ethyl acetate, a substance that easily penetrates and delivers energy to the cell, strongly stimulates net glucose and Na^+ transport.

The explanation of the experimental results takes into account the possibility of the existence of an active extrusion of glucose at the level of the basolateral membrane of the enterocyte.

INTRODUCTION

Transintestinal transport of sugars and other actively transported solutes takes place in two steps: first, the sugar enters the cell by means of a carrier-mediated mechanism which requires Na^+ in the lumen or in the mucosal side (it seems that the concentration of Na^+ in the serosal side is not critical [1]); second, the sugar leaves the cell at the opposite pole by means of another carrier-mediated mechanism which does not require Na^+ and is phlorhizin insensitive [2, 3].

During the first step, sugar in general accumulates intracellularly; this happens particularly in hamster and bullfrog jejunum [4–6] and rabbit ileum [7], whereas in rat jejunum such accumulation is not so impressive [8, 9] or it does not take place at all [8]; the different behaviour in the rat occurs under different experimental conditions.

Sugar accumulation has been ascribed to an Na^+ electrochemical potential gradient directed inward from the cell exterior across the luminal membrane [10, 11]; this gradient is in turn maintained by an active Na^+ extrusion (Na^+ pump) located in the basolateral membrane of the enterocyte [12, 13]. This gradient provides the energy for sugar accumulation in the epithelial cell; thus a specific active sugar entry at the luminal plasma membrane is not necessarily involved, as earlier suggested [4,14]. As mentioned before, Na^+ must be present in the lumen or in the mucosal side in order to interact with a carrier molecule responsible for sugar entry. Such inter-

action could result either in an increased affinity of the sugar for the carrier or to an increased mobility of the ternary complex, namely the carrier-glucose-Na^+ compound [15].

It seems quite probable that the energy supplied to the Na^+ pump derives from an ($Na^+ + K^+$)-activated ATPase system located mainly in the basolateral membrane of the enterocyte [16–20]. In this connection, experiments performed by different authors showed that extracellular ATP stimulates electrical activity and Na^+ transport in the small intestine [21, 22].

Ethyl acetate is a substance which strongly stimulates water transport in rat small intestine [23] by supplying energy to the cell. Owing to its liposolubility, it would enter the epithelial cell in such form and then be split by intracellular esterases [24–27]; thus, free acetate is released which is in turn a source of energy for the cell. As a matter of fact, the presence of an esterase inhibitor (tetraethyl pyrophosphate) completely prevents the stimulating effect of ethyl acetate. Ethanol has no effect on water transport [23] or on amino acid and glucose transport when used at low concentration [28]. Acetate and pyruvate as such do not stimulate Na^+ and water transport in rat jejunum [29], perhaps because they are mostly ionized at the pH of the tissue, so that they cannot enter the epithelial cell in any appreciable amount.

The aim of the present work has been to examine how ethyl acetate can affect transport processes and intracellular solute concentrations in the hamster small intestine.

METHODS

Preparation of the animal

Male golden hamsters (*Mesocricetus auratus*) weighing 60–80 g, starved for 16–18 h with free access to water, were used. Under barbituric narcosis, a tract of small intestine (jejunum) was removed, placed in a gassed Krebs-Ringer-bicarbonate solution kept at 28 °C and everted. The intestine was then secured on one end to thin polyethylene tubing and on the other to a glass cannula, through which 2 ml of serosal solution were introduced [30]. The intestine was then placed in a water-jacketed glass vessel containing 50 ml of incubating solution gassed with 95 % O_2 and 5 % CO_2. The gas mixture was first bubbled in a solution of 28 mM ethyl acetate, in experiments in which this substance was used, in order to prevent evaporation of the ethyl acetate from the mucosal solution. The temperature was kept constant at 28 °C by recirculating water at this temperature through the jacket. The content of the serosal side was readily mixed by raising and lowering at intervals the polyethylene tubing attached to the lower end of the intestine.

Perfusing solutions

Mucosal and serosal fluids were Krebs-Ringer-bicarbonate solutions with 5.55 mM glucose (control) or 5.55 mM glucose + 28 mM ethyl acetate added. In order to see if the stimulating effect of ethyl acetate is still present when Na^+ and water transport are abolished (elimination of a possible solvent drag effect) a set of experiments was performed in which the mucosal fluid was made hypertonic by adding 100 mM mannitol to the Krebs-Ringer-bicarbonate solution containing 5.55 mM glucose and 28 mM ethyl acetate.

All reagents were Analytical Reagent Grade. In order to determine the extracellular space [30] and the net transepithelial fluid transport poly[^{14}C]ethyleneglycol (New England Nuclear Corp.) was introduced into the mucosal and serosal solutions (0.5 μCi/ml).

Experimental procedure

The experiment lasted for 30 min after a 5 min of preincubation. At the end of the experiment the intestine was removed, cut open along its mesenteric border, blotted on Whatman No. 1 filter paper on both sides and the mucosal layer scraped off at 0 °C and immediately weighed [8, 31]; the cells were broken up by osmotic shock with 2 ml of a 3 mM monoiodoacetic acid in order to prevent glucose breakdown [32]. It has been previously demonstrated that a similar procedure minimizes glucose breakdown in the rat: histological control of the mucosal scraping has shown [8] that the tissue consists almost completely of intestinal epithelial cells. The suspension was then frozen at −30 °C and subsequently thawed. After centrifuging, 0.3 ml of supernatant fluid (in duplicate) was analyzed for ^{14}C radioactivity by a liquid scintillation spectrometer (Tri-Carb, Packard Inst. Co. mod. 3315), using 10 ml of a high efficiency scintillation liquid (Insta Gel, Packard Instr. Co.). 1 ml of the same supernatant fluid was deproteinized with 1 ml of 0.6 M perchloric acid. After centrifuging the limpid supernatant fluid was analyzed for glucose by an enzymatic method [33] and for Na^+ and K^+ by flame photometry. The sediment was washed several times with distilled water in order to remove perchloric acid. The test tube with the sediment was dried overnight at 100 °C and then weighed to calculate the small amount of tissue debris lost during sampling. This small fraction of the dry weight must be added to the dry weight of scraped mucosa obtained by drying the tissue sediment and its remaining supernatant fluid overnight at 100 °C. After the 5 min of preincubation, 0.1 ml sample of the serosal fluid was taken in duplicate to measure ^{14}C radioactivity; an additional 0.1 ml sample was analyzed for glucose to determine its actual initial concentration in the serosal fluid. At the end of the experiment a 0.1 ml sample of mucosal and serosal solutions (in duplicate) was taken to determine ^{14}C radioactivity and a 0.1 ml sample of the serosal fluid was analyzed for glucose in order to determine its final serosal concentration. Two additional 0.1 ml samples of the final serosal fluid were analyzed for Na^+ and K^+.

Further control of the determination of tissue glucose concentration

It has been recently found by Leese [34] that in in vivo rat intestine glucose accumulates within the intestinal wall. These results are entirely different from those previously reported by us [35] in rat intestine in vivo. However, our results refer to enterocyte glucose or 3-*O*-methylglucose (a non-metabolized sugar) concentration and not to a total tissue glucose concentration. Since an accurate determination of cell glucose concentration is critical to the interpretation of glucose transport mechanism across the intestinal wall, a set of experiments was carried out using exactly the same technique as employed by Leese, to avoid any criticism about the chemical determination of glucose and loss of sugar from the mucosa during the sampling time.

Leese's method briefly consists of perfusing the rat jejunum, under ether anesthesia, with Krebs-Ringer-bicarbonate solution for 5 min and then for a subsequent 10 min with the same solution with 5.55 mM glucose. At the end of the experi-

TABLE I

COMPARISON BETWEEN TWO DIFFERENT TECHNIQUES USED TO DETERMINE GLUCOSE CONCENTRATION IN SMALL INTESTINE

Glucose concentration in cell compartment and in total tissue wall, as well as in the lumen and serum, are reported. Cell concentration refers to ml of intracellular water; total tissue concentration refers to total tissue water calculated according to Leese [34]. For in vitro experiments the everted sac technique has been used; for in vivo experiments a perfusion technique has been used. The incubation fluid was a Krebs-Ringer-bicarbonate solution with 5.55 mM glucose, on both sides. Rat and hamster jejunum have been employed. Values are means ± S.E.M. with number of experiments in parentheses.

Technique	Final glucose concentration (mM)			
	Mucosal scraping (Cell concn.)	Total tissue (Wall concn.)	Lumen*	Serum
Used by us (mucosal scraping, freezing and thawing, see ref. 35)				
Glucose oxidase (11) (rat, in vivo)	2.9 ± 1.0	--	4.1 ± 0.1	8.5 ± 0.6
Glucose oxidase (3) (rat, in vitro)	2.6 ± 0.2	—	--	-
Hexokinase (3) (rat, in vitro)	2.7 ± 0.5	—	—	—
Glucose oxidase (4) (hamster, in vitro)	37.2 ± 4.1	—	—	-
Hexokinase (4) (hamster, in vitro)	37.3 ± 3.7	--	--	-
Used by Leese (shorter incubation, liquid N_2)				
Glucose oxidase (3) (rat, in vivo)	...	5.3 ± 1.4	5.0 ± 0.1	12.6 ± 1.0
Hexokinase (6) (rat, in vivo)	...	5.4 ± 0.8 (blotted) 5.0 ± 0.5 (non blotted)	4.8 ± 0.1	8.6 ± 0.7

* Average value between initial and final concentration.

ment the tissue was immediately frozen in liquid N_2. All details are reported in Leese's paper [34]. Table I shows our obtained results. By freezing the whole intestine immediately in liquid N_2 at the end of the experimental period (15 min), tissue glucose concentration determined with the hexokinase method [36] (as used by Leese) is always lower than that found by Leese (5.0 ± 0.5, $n = 6$ vs. 15.3 ± 2.3, $n = 8$) [34]. It seems that blotting the intestine before dipping it into liquid N_2 does not alter the result. Anyway, tissue glucose concentration is always lower than serum concentration. At present we have no explanation for the difference between our results and those of Leese unless we ascribe it to a different way of keeping and housing the animals. Moreover, the table shows that, by using our technique, enterocyte glucose concentration in rat jejunum in vitro is similar by both the glucose oxidase and the hexokinase method. The same is true for hamster jejunum in vitro, but here the concentration is much higher because of the known peculiarity of this animal to accumulate glucose [5, 37–39].

Transport rate of glucose, sodium and water

Net amount of glucose and Na^+ transport from the mucosal to the serosal side is expressed in μmol/g dry tissue weight of intestine per h, while the net water transport is given in ml/g dry tissue weight of intestine per h. It has to be pointed out that the dry tissue weight of the intestine is the sum of the dry weight of the scraped mucosa and that of the remaining submucosal layer. T/M ratio represents the ratio between cell glucose concentration and the concentration of the sugar in the mucosal side (5.55 mM); the latter does not vary appreciably throughout the experiment because of the large volume (50 ml) of the mucosal compartment. Cell water is given in ml/g dry weight of scraped mucosa and represents the volume of cell compartment. Na^+ and K^+ cell concentrations are expressed in mM (μmol/ml of intracellular water). All values are means $\pm$S.E.M.

RESULTS AND DISCUSSION

Fig. 1 shows a relationship between net glucose and Na^+ transport in control and ethyl acetate experiments under isosmotic incubation. Such a relation has been previously demonstrated in rat jejunum, both in vivo and in vitro [35, 40]. The linear correlation indicates that the more the Na^+ is transported, the more the glucose is transported as well. The straight line starts from the origin, indicating that in the absence of transport of Na^+ the glucose transported is nil. The ratio between glucose and Na^+ transport is 0.194 ± 0.013 S.D., $n = 14$; this value is not statistically different ($P > 0.4$) from that of controls (0.206 ± 0.06 S.D., $n = 8$); also the ratio found in ethyl acetate experiments (0.182 ± 0.026 S.D., $n = 6$) does not statistically differ ($P > 0.4$) from that of controls.

Table II shows cell concentration of electrolytes, glucose and water, as well as the ratio of glucose between the cell and mucosal solution. The present results confirm previous findings [4, 5, 37–39] that actively transported sugars are concentrated within the cell under all experimental conditions as clearly shown by the T/M ratio, (Table II). The value of this ratio is very consistent with that found by others [5].

Table III shows that ethyl acetate has a strong stimulatory effect on net Na^+, water and glucose transport. Since the intracellular concentrations of Na^+ and glucose

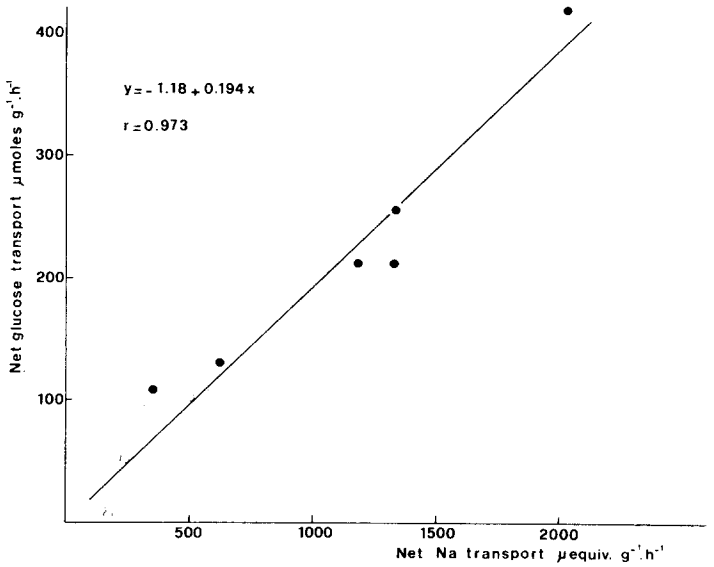

Fig. 1. Relationship between net transepithelial glucose and Na^+ transport in control (○) and ethyl acetate (●) experiments.

are not affected by this treatment, it seems that such stimulation goes with a facilitated entry of glucose and Na^+ into the cell and a concomitant stimulation of glucose and Na^+ extrusion across the basolateral membrane of the enterocyte; as a consequence, more glucose and Na^+ are transported. Furthermore, the data of Tables II and III bring additional arguments in favour of the existence of an active extrusion mechanism of glucose, localized in the basolateral membrane [35]. For instance, comparing the ethyl acetate experiments with control experiments, it is clear that, in spite of a more favourable cell to serosal concentration gradient of glucose in control group, net glucose transport in the control group is only 25 % of the transport in ethyl acetate experiments. In addition, the apparent glucose concentration at the cell-intercellular channel boundary calculated from the ratio between glucose and fluid transport (see Table III), approaches a value higher (24.0 ± 4.8 mM, $n = 11$ in control experiments and 30.5 ± 3.0 mM, $n = 6$ in ethyl acetate experiments) than the value found in the intracellular compartment (18.4 ± 1.4 mM, $n = 9$ and 18.1 ± 1.3 mM, $n = 6$, in control and ethyl acetate groups, respectively). In the rat, the calculated concentration at the same boundary approaches a value much higher (36.5 ± 5.5 mM, $n = 5$) than cell concentration (7.2 ± 0.9 mM, $n = 5$) and serosal concentration (8.7 ± 0.6 mM, $n = 5$), (data from this laboratory).

One objection concerning the action of ethyl acetate on net glucose transport is that this effect could be due to an indirect consequence of the drag effect of the volumetric flow. To avoid this objection a set of experiments has been carried out to counteract the mucosa to serosa volumetric flow by means of an osmotic gradient opposing this flow; this has been achieved by raising the osmolarity of the mucosal fluid with 100 mM mannitol. The results reported in Table III show that glucose transport is not significantly affected by the mucosal hypertonicity; on the contrary, net water and Na^+ transport from mucosa to serosa is almost abolished. This can

TABLE II

EFFECT OF ETHYL ACETATE ON CELL GLUCOSE, SODIUM AND POTASSIUM CONCENTRATION AS WELL AS ON CELL VOLUME AND SEROSAL GLUCOSE CONCENTRATION IN HAMSTER JEJUNUM

Intracellular solute concentration is referred to in ml of intracellular water; cell water is given in ml/g dry weight of scraped mucosa and represents the volume of cell compartment. T/M ratio is the ratio between cell glucose concentration and the concentration of sugar in the mucosal side (5.55 mM); the latter concentration does not vary appreciably throughout the experiment because of the large volume (50 ml) of the mucosal compartment. The initial fluid was Krebs-Ringer-bicarbonate solution (A) with 5.55 mM glucose (control), (B) with 5.55 mM glucose + 28 mM ethyl acetate or (C) with 5.55 mM glucose + 28 mM ethyl acetate + 100 mM mannitol (the latter compound only in the mucosal side). Initial mucosal volume was 50 ml; initial serosal volume was 2 ml. Poly[^{14}C]ethyleneglycol was initially present in both mucosal and serosal fluids (0.5 μCi/ml). Values given are the mean $\pm$S.E.M. with the number of experiments in parentheses.

Incubating fluid	Cell glucose (mM)	Serosal glucose (mM)	T/M	Cell sodium (mM)	Cell potassium (mM)	Cell water (ml · g^{-1})
A	18.4±1.4 (9)	7.8±0.4 (12)	3.3±0.3 (9)	64±6 (9)	99±6 (9)	5.5±0.3 (9)
B	18.1±1.3 (6)	10.7±0.8 (6)	3.3±0.2 (6)	63±2 (6)	92±6 (6)	5.9±0.1 (6)
C	42.9±3.9 (4)	12.0±0.6 (4)	7.7±0.7 (4)	65±3 (4)	83±4 (4)	5.0±0.3 (4)

TABLE III

EFFECT OF ETHYL ACETATE ON NET GLUCOSE, SODIUM AND FLUID TRANSPORT IN HAMSTER JEJUNUM

Net glucose and sodium transport is expressed in μmol/g dry tissue weight of intestine per h, while net fluid transport is given in ml/g dry tissue weight of intestine per h. Tissue weight represents the total dry tissue weight of the jejunum. The initial fluid was Krebs-Ringer-bicarbonate solution with (A) 5.55 mM glucose (control), (B) with 5.55 mM glucose + 28 mM ethyl acetate or (C) with 5.55 mM glucose + 28 mM ethyl acetate + 100 mM mannitol (the latter compound only in the mucosal side). Values given are the means ± S.E.M. with the number of experiments in parentheses.

Incubating fluid	Net glucose transport (μmol · g^{-1} · h^{-1})	Net sodium transport (μmol · g^{-1} · h^{-1})	Net fluid transport (ml · g^{-1} · h^{-1})
A	60 ± 11 (11)	317 ± 51 (8)	2.2 ± 0.3 (8)
B	224 ± 46 (6)	1139 ± 241 (6)	7.8 ± 1.7 (6)
C	258 ± 16 (4)	82 ± 45 (4)	0.6 ± 0.3 (4)

probably be explained by a back movement of Na^+ from the intercellular channels to the mucosal side through the tight junctions; these junctions, on the other hand, prevent the glucose back movement.

As a conclusion, ethyl acetate is a substance which can easily penetrate into the cell and supply energy to it, so that the net transepithelial transport of Na^+ and glucose is greatly enhanced. Finally, our data are consistent with the hypothesis of the presence of an active extrusion mechanism located in the serosal pole of the enterocyte.

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