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Effect of ethylacetate on the transport of sodium and glucose in the hamster small intestine in vitro

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Fisiologia. — *Effect of ethylacetate on the transport of sodium and glucose in the hamster small intestine* in vitro (*). Nota di GIOVANNI ESPOSITO, ALIDE FAELLI e VITTORIO CAPRARO, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — L'etilacetato, data la sua liposolubilità, permea facilmente la membrana plasmatica dell'enterocita; all'interno della cellula, questa sostanza viene scissa in etanolo ed acetato dalle esterasi aspecifiche intracellulari.

L'etilacetato, messo nel liquido di incubazione mucosale e serosale di un preparato *in vitro* di intestino tenue di criceto, stimola fortemente il trasporto netto transintestinale di sodio e di glucosio senza modificarne le concentrazioni intracellulari.

L'azione stimolante di questa sostanza sembra quella di fornire energia metabolica alla cellula per effettuare un'estrusione attiva di sodio e di glucosio a livello della membrana baso-laterale della cellula assorbente intestinale.

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 [4]); 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 [1, 27].

During the first step, sugar in general accumulates intracellularly; this happens particularly in hamster and bullfrog jejunum [25, 9, 7] and rabbit ileum [32], whereas in rat jejunum such accumulation is not so impressive [14, 16] or it does not take place at all [14]; the different behaviour in the rat occurs under different experimental conditions.

Sugar accumulation has been ascribed to a Na^+ electrochemical potential gradient directed inward from the cell exterior across the luminal membrane [3, 33]; this gradient is in turn mantained by an active Na^+ extrusion (Na^+ pump) located in the baso-lateral membrane of the enterocyte [31, 6].

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 [25, 23]. As mentioned before, Na^+ must be present in the lumen or in the mucosal side in order to interact with a carrier molecule responsable for sugar entry. Such interaction 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 [34].

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(**) Nella seduta dell'8 marzo 1975.

It seems quite probable that the energy supplied to the Na^+ pump derives from an ATP-Na^+, K^+-activated ATPase system located mainly in the baso-lateral membrane of the enterocyte [5, 29, 30, 21, 18]. In this connection, experiments performed by different authors showed that extracellular ATP stimulates the electrical activity and the Na^+ transport in small intestine [24, 19].

Ethylacetate is a substance which strongly stimulates water transport in rat small intestine [8] by supplying energy to the cell. Owing to its liposolubility it would enter the epithelial cell in such form and then it is split by intracellular esterases [17, 26, 20, 35]; thus, free acetate is released which in turn is a source of energy for the cell. Ethanol has no effect on water transport [8]. Acetate and pyruvate as such do not stimulate Na^+ and water transport in rat jejunum [28], perhaps because they are mostly ionized at the pH of the tissue so that they cannot enter the epithelial cell in appreciable amount.

Aim of the present work has been to examine how ethylacetate can affect transport processes and intracellular solute concentrations in the hamster small intestine.

Male golden hamsters (Mesocricetus auratus) weighing 60–80 g, starved for 16–18 hr with free access to water, were used. Under barbituric narcosis the 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 a thin polyethylene tubing and on the other was fixed to a glass cannula through which 2 ml of serosal solution were introduced [12]. The intestine was then incubated at 28 °C in 50 ml of proper solution gassed with 95 % O_2 and 5 % CO_2. Gas mixture was first bubbled in a solution of 28 mM ethylacetate, only in experiments in which this substance was used, in order to prevent its evaporation from the mucosal solution.

Mucosal and serosal fluids were a Krebs-Ringer bicarbonate solution added with 5.55 mM glucose (control) or 5.55 mM glucose+ethylacetate 28 mM.

All other reagents were Analytical Reagent Grade. In order to determine the extracellular space [12] and the net transepithelial fluid transport, ^{14}C polyethyleneglycol (^{14}C-PEG, New England Nuclear Corp.) was introduced in the mucosal and serosal solutions (0.5 μCi/ml).

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 was scraped off at 0 °C and immediately weighed [14, 15]; the cells were broken up by osmotic shock with 2 ml of a 3 mM monoiodoacetic acid in order to prevent glucose breakdown. It has been previously demonstrated [14] that such a procedure minimizes glucose breakdown in the rat. The suspension was then frozen at —30 °C and subsequently thawed. After centrifuging, 0.3 ml of supernatant fluid were analyzed for ^{14}C radioactivity by a liquid scintillation spectrometer and by using 10 ml of a high efficiency

scintillation liquid (Insta Gel, Packard Instr. Co.). One ml of the same supernatant fluid was deprotenized with 1 ml of 0.6 N perchloric acid. After centrifuging, the limpid supernatant fluid was analyzed for glucose by an enzymatic method [22] and for Na^+ and K^+ by flame photometer. The dry weight of scraped mucosa is 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 analyzed for ^{14}C radioactivity; an additional 0.1 ml sample was analyzed for glucose in order to know its actual initial concentration in the serosal fluid. At the end of the experiment 0.1 ml sample of mucosal and serosal solutions were taken to determine ^{14}C radioactivity and 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^+.

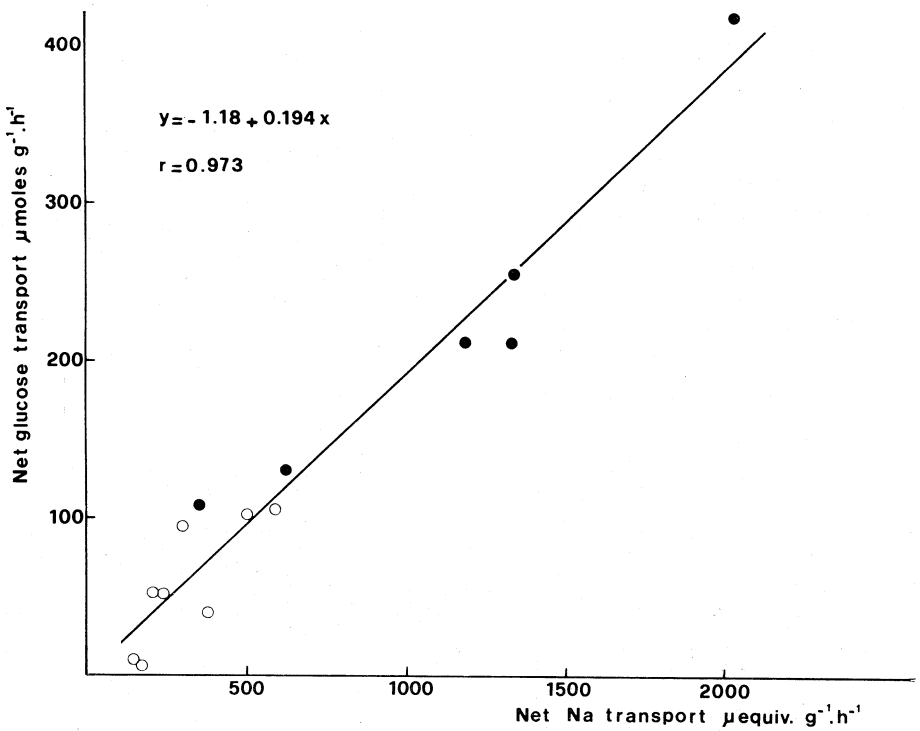

Fig. 1. – Relationship between net transepithelial glucose and Na^+ transport in control (○) and ethylacetate (●) experiments.

Fig. 1 shows a relationship between net glucose and Na^+ transport in control and ethylacetate experiments. Such a relation has been previously demonstrated in rat jejunum, both *in vivo* and *in vitro* [10, 11]. 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,

thus 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 ethylacetate experiments (0.182 ± 0.026 S.D., $n = 6$) does not statistically differ ($P > 0.4$) from that of controls.

Table I shows cell concentration of electrolytes, glucose and water, as well as the ratio of glucose between cell and mucosal solution. As already known in the literature [25, 9, 2], present results show that actively transported sugars are always concentrated within the cell under all experimental conditions as T/M ratio clearly shows. Cell Na^+ is higher and cell K^+ is lower than the corresponding values found *in vivo* both in the rat and hamster jejuna [11, 13].

TABLE I.

The effect of ethylacetate on cell glucose, sodium and potassium concentration as well as on cell volume and serosal glucose concentration in hamster jejunum.

Intracellular solutes concentration is referred to ml of intracellular water; cell water is given in ml/g of dry weight of scraped mucosa and represents the volume of cell compartment. 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 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 (K–R *b*) added with 5.55 mM glucose (control) or added with 5.55 mM glucose + 28 mM ethylacetate. Initial mucosal volume was 50 ml; initial serosal volume was 2 ml. ^{14}C polyethyleneglycol ($^{14}CPEG$) was initially present in both mucosal and serosal fluids(0.5 μC/ml). Values given are the mean ± 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 ($ml \cdot g^{-1}$)
K–R *b* + 5.55 mM glucose (control)	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]
K–R *b* + 5.55 mM glucose + 28 mM ethylacetate	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]

(*) $P < 0.01$.

Table II shows that ethylacetate has a strong stimulatory effect on net Na^+, water and glucose transport. Since the intracellular concentrations of Na^+ and glucose are not affected by this treatment, it seems that such a 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, data of Tables I and II seem to bring

additional arguments in favour of the existence of an active extrusion mechanism of glucose localized in the baso-lateral membrane [11]. For instance comparing the ethylacetate 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 is only 25 % of the transport in ethylacetate experiments. In addition, glucose concentration at cell-intercellular space boundary, calculated from the ratio between glucose and fluid transport (see Table II), approaches a value higher (24.0 ± 4.8 mM, $n = 11$ in control experiments and 30.5 ± 3.0 mM, $n = 6$ in ethylacetate 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 ethylacetate groups respectively).

TABLE II.

The effect of ethylacetate on net glucose, sodium and fluid transport in hamster jejunum.

Net glucose and sodium transport is expressed in μmoles/g dry tissue weight of intestine per hour, while net fluid transport is given in ml/g dry tissue weight of intestine per hour. Tissue weight represents the total dry tissue weight of the jejunum. The initial fluid was Krebs-Ringer bicarbonate solution (K–R *b*) added with 5.55 mM glucose (control) or added with 5.55 mM glucose + 28 mM ethylacetate. Values given are the mean ± S.E.M. with the number of experiments in parentheses.

Incubating fluid	Net glucose transport ($\mu moles \cdot g^{-1} \cdot h^{-1}$)	Net sodium transport ($\mu moles \cdot g^{-1} \cdot h^{-1}$)	Net fluid transport ($ml \cdot g^{-1} \cdot h^{-1}$)
K–R *b* + 5.55 mM glucose (control)	60±11 [11]	317±51 [8]	2.2±0.3 [8]
K–R *b* + 5.55 mM glucose + 28 mM ethylacetate	224±46 [6]	1139±241 [6]	7.8±1.7 [6]
	$P < 0.01$	$P < 0.01$	$P < 0.01$

Obviously the validity of these arguments depend on the accuracy of the cell glucose concentration data, but at present and in our experimental conditions, we have no indications as to the possibility that these data are erroneous.

As a conclusion, ethylacetate 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, whereas the intracellular concentration of these solutes remains unmodified. 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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