Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

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Relationship between passive permeability and active transport of the isolated rat intestine. Possible involved mechanism

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **52** (1972), n.4, p. 558–562.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1972_8_52_4_558_0>

Fisiologia. — *Relationship between passive permeability and active transport of the isolated rat intestine. Possible involved mechanisms* (*). Nota di ALIDE FAELLI, GIOVANNI ESPOSITO, GIANNI GAROTTA, ROBERTO PAROTELLI e VITTORIO CAPRARO, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — Nell'intestino di ratto perfuso *in vitro* si manifesta una evidente correlazione tra trasporto attivo di glucosio e mobilità dell'acetamide. Anche la conduttanza elettrica trasversale dell'intestino sembra aumentare con l'aumento del trasporto di glucosio. L'AMP ciclico aggiunto al liquido di perfusione ha lo stesso effetto dell'elevato trasporto di glucosio nell'incrementare la mobilità dell'acetamide e potrebbe costituire il meccanismo di insorgenza del predetto effetto.

INTRODUCTION

In previous papers [1–3] we have pointed out that the mobility coefficient of acetamide and thiourea of the rat jejunum perfused *in vitro* with a basic perfusion fluid (Krebs–Henseleit bicarbonate solution added with D–glucose 13.9 mM), decreases when the active transport of this intestinal tract was inhibited by replacing NaCl with Tris–Cl in the basic incubation fluid.

The decrease of permeability seems not to be due to a shrinkage of epithelial cell taking place in the above reported modification of the perfusing fluid [3].

Furthermore, the decrease of acetamide permeability in Tris–Cl perfusing fluid seems not to be related only to the decrease of intestinal active transport. If we compare a group of intestinal tracts perfused with a low glucose content to a group of intestinal tracts perfused with the above Tris–Cl solution, we observe the same fluid and glucose transepithelial transport, nevertheless the mobility of acetamide is higher in the intestines perfused with a low glucose containing solution (Table I).

Therefore, the lowering of Na concentration could affect directly the physico-chemical properties of cell membrane due, for instance, to the varied Na : Ca ratio of the perfusing fluid. However, the influence of the active transport on passive permeability of acetamide cannot be disregarded, as appears from the present work.

(*) Lavoro eseguito nell'Istituto di Fisiologia Generale dell'Università di Milano, con un finanziamento del Consiglio Nazionale delle Ricerche, Roma.

(**) Nella seduta dell'8 aprile 1972.

TABLE I.

Glucose and fluid transepithelial transport, mobility of acetamide across the intestinal wall.

Number of experiments in parenthesis.

INCUBATION FLUID	Glucose transepithelial (*) transport μmoles $g^{-1} h^{-1}$		Fluid transepithelial (*) transport ml $g^{-1} h^{-1}$		ω (*) μmoles $g^{-1} h^{-1}$ Atm^{-1}	
Krebs–Henseleit bicarbonate+glucose 1.39–13.9 mM	35 ± 9	(10)	2.59 ± 0.25	(10)	439 ± 24	(10)
Krebs–Henseleit bicarbonate+glucose 13.9 mM–NaCl substituted isosmotically with Tris Cl	46 ± 14	(6)	2.57 ± 0.74	(6)	315 ± 30	(6)

(*) Mean values (± S.E.M.) obtained when the range of glucose transport is between 0 and 100 μmoles $g^{-1} h^{-1}$. All data are referred to one gram dry tissue weight and one hour.

METHODS

The experiments were performed by recirculating the perfusion fluid through an open and everted intestinal tract [2]. Sprague–Dawley albino male rats, initially weighing about 250 g, semistarved over a 15 day period (final percent weight decrease: 15–25 %), were used. Under barbituric narcosis, a tract of small intestine 10–15 cm long was removed from the animal at about 10 cm from pylorus. Each tract was everted and perfused at 28° C. The basic perfusion fluid, as above reported, was gassed with a mixture of 95 % O_2 and 5 % CO_2 and was added with acetamide 10 mM; labelled ^{14}C–acetamide was present in the serosal space only. In a second set of experiments glucose was present at a concentration of 1.39 mM only. At the end of each individual experiment the dry weight of the intestine was determined. Transepithelial mobility coefficient, disregarding the drag effect of the volumetric flow, and net glucose transport throughout a 1 hour perfusion period, were calculated [2].

In another set of experiments the electrical conductance of the intestinal barrier was investigated by perfusing the intestine with different solutions. The transepithelial electrical potential and the current crossing the barrier at different electrical fields were determined and the corresponding resistance (or conductance) calculated [4].

RESULTS AND DISCUSSION

From the mobility experiments, it seems that there is a linear correlation between the mobility of acetamide and the net active fluid and glucose transport through the intestinal wall (fig. 1), in normal perfused intestines. In

this case there is a good correlation between glucose transport and fluid transport [5]. On the contrary in phlorhizin poisoned intestines the absence of glucose transport does not indicate the absence of any transport activity because the fluid transport is still high.

The relationship between the mobility of acetamide and the net glucose transport has been confirmed by experiments in which the same intestinal tract was initially perfused for 20 min with a low glucose perfusion fluid

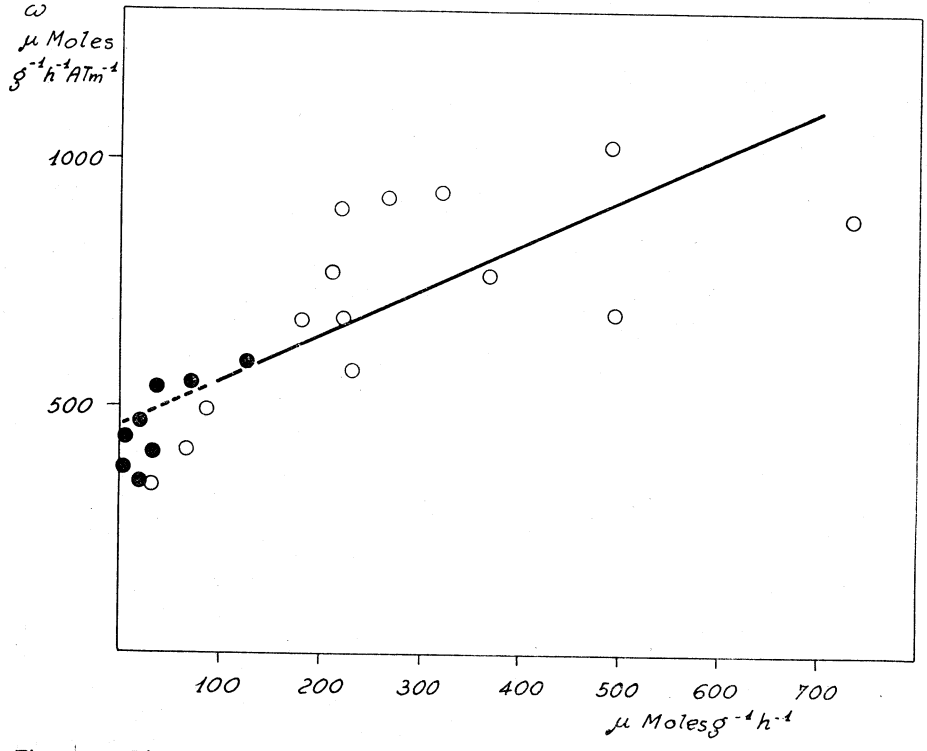

Fig. 1. – Linear correlation between net glucose transport (abscissa) and mobility coefficient of acetamide (ordinate). Solid (●) and open (○) circles represent experiments in which glucose concentration of the incubating fluid was 1.39 and 13.9 mM respectively.

(1.39 mM), and then for an equal period with a high glucose perfusion fluid (13.9 mM). In the second period of experiment, net glucose transport increases together with the mobility coefficient of acetamide (16 ± 3 %; number of experiments = 10). In control experiments (low glucose content) and during the same experimental periods there is no change of acetamide permeability.

The results from the resistance experiments are reported in Table II which shows that the presence of glucose increases the conductance of the intestinal barrier. Such an increase can also be obtained in the same intestinal tract by adding glucose 13.9 mM to the Krebs–Henseleit bicarbonate solution (percent increase = 120 ± 36 %; number of experiments = 10). As to the mechanism of the relationship between the glucose transport and the mobility of the acetamide, the hypothesis may be put forward that the intracellular

TABLE II.

Conductance ($\Omega^{-1} g^{-1}$) of the intestinal wall in different experimental conditions.

Mean values ± S.E.M. referred to one gram dry tissue weight, are reported.

INCUBATION FLUID	NUMBER OF EXPERIMENTS	CONDUCTANCE $\Omega^{-1} g^{-1}$
Krebs–Henseleit bicarbonate .	8	4.62 ± 0.61
Krebs–Henseleit bicarbonate + glucose 13.9 mM	9	7.17 ± 0.60
Krebs–Henseleit bicarbonate + glucose 13.9 mM–NaCl substituted isosmotically with Tris Cl	5	1.42 ± 0.17

level of cyclic AMP is here involved. It is known that cyclic AMP increases the permeability of the mucosal border of some epithelial cells [6]. In fact when cyclic AMP 4 mM (dibutyryl derivative) is added to the serosal perfusion fluid containing a low glucose concentration (1.39 mM) the mobility coefficient of acetamide becomes as high as that obtained by perfusing the intestine with a high glucose concentration (13.9 mM). Presumably also the increase of glucose transepithelial transport could be explained by an increase of permeability of the brush border. Control experiments in which Na–butyrate 4 mM was added to the serosal side, show no effect (Table III).

TABLE III.

Glucose transepithelial transport and mobility of acetamide across the intestinal wall.

Mean values ± S.E.M. referred to one gram dry tissue weight and one hour, are reported.

INCUBATION FLUID	Number of experiments	Glucose transepithelial transport μmoles $g^{-1} h^{-1}$	ω μmoles $g^{-1} h^{-1}$ Atm^{-1}
Krebs–Henseleit bicarbonate + glucose 1.39 mM	8	37 ± 14	465 ± 31
Krebs–Henseleit bicarbonate + glucose 13.9 mM	14	279 ± 51	721 ± 55
Krebs–Henseleit bicarbonate + glucose 1.39 mM + cyclic AMP 4 mM	8	141 ± 21	813 ± 24
Krebs–Henseleit bicarbonate + glucose 1.39 mM + Na–Butyrate 4 mM	7	86 ± 14	554 ± 49

Besides the above explanation and if we consider the whole absorbing intestinal barrier, the widening of the intercellular spaces as a consequence of the increased glucose and water transport could be taken into account. As a matter of fact this condition increases the total serosal surface available for the diffusion of acetamide. The opening of the intercellular spaces has been observed in bullfrog intestine [7] when there is an osmotic flow from the mucosal to the serosal side of the intestine due to an osmotic gradient.

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