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Fisiologia. — *Cyclic* AMP *and passive permeability of the intestinal brush border* (*). Nota di GIOVANNI ESPOSITO, ALIDE FAELLI e VITTORIO CAPRARO, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — L'adenosin-3',5'-monofosfato (AMP ciclico) messo nel lato serosale di un preparato di intestino tenue (digiuno) di ratto fa aumentare la permeabilità transintestinale all'acetamide, una sostanza non carica, idrosolubile che diffonde passivamente attraverso la parete intestinale.

Sembra che l'AMP ciclico influenzi relativamente di più la permeabilità all'acetamide dell'orletto a spazzola che non quella del polo serosale della cellula assorbente intestinale, nonostante questo composto ciclico sia presente inizialmente soltanto nel compartimento serosale.

The ionic composition of the incubation fluid is of the utmost importance as far as transport activities of the intestine are concerned [1–3]. This holds both for active transport of electrolytes [4, 5] and non-electrolytes [2, 6] and for the passage of passively diffusing substances [7, 8].

As to the latter mechanism, it has been demonstrated that the decrease of sodium concentration in the incubating solution strongly reduces the permeability of acetamide [7, 8].

It has been shown that adenosine-3',5'-monophosphate (cyclic AMP) is also involved in the permeability of some epithelia [9, 10]; it has been recently demonstrated that this compound increases the net glucose transport as well as the passive permeability of an uncharged and hydrosoluble substance, such as acetamide, through the whole intestinal wall [11].

Since a molecule crossing the intestine has to move through two plasma membranes in series (the apical and basal pole of the epithelial cell), aim of the present work is to ascertain which of these membranes is more affected by the presence of cyclic AMP as far as the passive permeability of acetamide is concerned.

The jejunum of semistarved male albino rats [12] (Sprague-Dawley, 250 g body weight) was everted and perfused at 28° C on both sides with a Krebs-Henseleit bicarbonate solution containing glucose 1.4 mM and acetamide 10 mM (control) or glucose 1.4 mM, acetamide 10 mM and dibutyryl cyclic AMP 4 mM (experimental). The latter compound was present on the serosal side only. The solutions were gassed with O_2 95% and CO_2 5%.

In order to determine the serosa to mucosa unidirectional flux in the absence of a concentration gradient, ^{14}C-acetamide was initially added to the serosal space only. The reason why only the serosa to mucosa flux was

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measured is that the mucosal solution constitutes a very large compartment (50 ml) if compared to the serosal one (5 ml). When the volume of both compartments was equal [13], the results obtained in the case of a serosa to mucosa unidirectional flux were the same as those obtained in the case of an opposite unidirectional flux.

As a marker to determine the extracellular space, ^{3}H–inulin was added both to the mucosal and serosal compartments [14]. The intracellular concentration of labelled acetamide was calculated as follows: the amount of this substance in the mucosal extracellular space and the higher amount present in the serosal extracellular space were subtracted from the total quantity of acetamide found in the scraped mucosa. This calculation is based on previous findings [14, 15] which indicate that total extracellular space of the everted gut is 50% serosal and 50% mucosal.

By knowing the flux and the mean mucosal and serosal concentrations of labelled acetamide in the second 30 min period of perfusion, transepithelial mobility coefficient (ω) was calculated [8, 14, 16]. ΔC_{cs} and ΔC_{cm} (see table) were calculated on the data obtained at the end of the experiment.

We also assume that the ratio $\Delta C_{cm}/\Delta C_{cs}$ is equal to the ratio ω_{cs}/ω_{cm}.

The results obtained (see Table) confirm previous data demonstrating that the basal pole of the absorbing cell is less permeable to acetamide than the apical pole (brush border) and that cyclic AMP increases the mobility of acetamide through the whole intestinal barrier [11, 14].

TABLE

Labelled acetamide concentration difference between serosal and cell compartment (ΔC_{cs}) and between cell and mucosal compartment (ΔC_{cm}) and mobility of acetamide (ω) across rat jejunum, are reported.

The labelled acetamide concentration of the above fluids was calculated by dividing the c.p.m./ml value by the specific radioactivity of the initial incubating serosal solution. The term ω_{cs}/ω_{cm} represents the ratio between acetamide mobility across the serosal pole (ω_{cs}) and the brush border (ω_{cm}) of the cell. Mean values ± S.E.M., referred to 1 g dry tissue weight and 1 h, are reported. Number of experiments in parentheses.

INCUBATING FLUID	MOBILITY COEFFICIENT (ω) (μmoles g^{-1} h^{-1} atm^{-1})	ΔC_{cs} (mM)	ΔC_{cm} (mM)	ω_{cs}/ω_{cm}
Krebs-Henseleit bicarbonate+glucose 1.4 mM (6)	519±78	5.92±0.30	1.27±0.09	0.221±0.026
Krebs-Henseleit bicarbonate+glucose 1.4 mM + cyclic AMP 4 mM (6)	758+47	6.15±0.40	0.56±0.06	0.096±0.016
P	<0.05	>0.6	≪0.01	<0.01

Furthermore, it seems that the permeability increasing effect of cyclic AMP for acetamide is relatively greater at the brush border than it is at the basal pole of the cell. The ratio between acetamide mobility across the serosal pole of the cell (ω_{cs}) and the mobility across the brush border (ω_{cm}) is reduced to a half, which means that the relative permeability of the brush border, doubles.

It is interesting to note here that the substitution of NaCl of the medium with Tris–Cl also affects the permeability of the brush border relatively more than that of the serosal pole of the cell [14].

An objection might be raised: cyclic AMP modifies the thickness of the intestinal wall by influencing the smooth muscle tone and consequently the acetamide and inulin movements. However, at this point, it is reasonable to suppose that the resistance to acetamide and to inulin movements offered by the subepithelial layers is not appreciably affected; the epithelium is always the actual limiting factor so that a smooth muscle tone change would not affect the movement of acetamide.

As a conclusion, cyclic AMP appears to act on the brush border, in spite of the fact that initially it is present in the serosal compartment only. This suggests that such a compound can easily enter the cell through the basal pole and then affect the permeability of the brush border.

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