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PARACELLULAR TRANSPORT OF INORGANIC AND ORGANIC IONS ACROSS THE RAT ILEUM

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Abstract—The properties of the absorption route via the tight junctions and the intercellular spaces (the paracellular pathway) in rat ileum were investigated with inorganic and organic ions. Isolated ileal epithelial sheets were used, mounted between two chambers. Biionic and diffusion potentials of the ions were measured with Ag/AgCl electrodes. From the Goldman–Hodgkin–Katz equation relative permeabilities were calculated and selectivity isotherms constructed. The paracellular pathway behaved as an aqueous pore with cation selectivity. The permeability order is $K^+ \simeq NH_4^+ > Na^+ > Cl^- \simeq Li^+ >$ tetramethylammonium$^+$ > tetraethylammonium$^+ \simeq$ choline$^+ >$ carbachol$^+$. TAP$^+$ (2,4,6-triaminopyrimidinium) appeared, in contrast to the situation in other tissues, not to be an inhibitor of this pathway. The permeability for organic cations is high: compounds with a mol. wt of about 150 have a permeability relative to sodium of 0.45. Together with the results of previous studies, in which the transcellular aspects of organic cation transport were investigated, a model for organic cation absorption is developed.

Many efforts have been undertaken to clarify the mechanism of transport of organic cations across artificial and biological membranes [1, 2]. Up to now attention has been payed mainly to the transcellular aspect of this problem. Only one study investigated the paracellular aspects of organic cation transport [3]. That study revealed that in rabbit and frog gall-bladders protonated aliphatic amines up to a mol. wt of 100 could use this pathway for transport.

Like the rabbit gall-bladder the small intestine belongs to the so-called 'leaky' epithelia, all of which are characterized by low transepithelial electrical resistances, low spontaneous transepithelial potential differences and high passive permeabilities to small ions and water. In recent years it has become clear that the characteristic transport properties of these epithelia cannot be attributed to unusually leaky cell membranes or, as in the case of the small intestine, to areas of cell denudation. Instead these properties are consequences of the presence of low-resistance, transepithelial paracellular pathways. The anatomic counterpart of these paracellular shunts is the route traversing the lateral intercellular spaces and the zonulae occludentes, the 'tight junctions', which bind the epithelial cells together at their apical borders. In leaky epithelia these junctional complexes are readily permeable to small inorganic and organic compounds and provide an easy route for transepithelial diffusion. The properties of this paracellular shunt have been reviewed recently by Schultz [4] and Erlij and Martinez-Palomo [5]. The characteristics of inorganic ion transport via this route have been investigated in detail. It was found that paracellular pathways show cation selectivity: the permeability to cations is much higher than to anions. The selectivity sequence in the ion permeablity differs clearly from the permeability sequence in free solution, the free mobility (u)*. This is especially the case for Cl^-.

In this study the contribution of the paracellular pathway to the absorption of quaternary ammonium compounds was investigated. The results show that the permeability of the paracellular shunt for organic cations is high.

MATERIALS AND METHODS

Experimental techniques. Experimental material consisted of terminal ilea from male Wistar rats weighing 250–300 g. Methods for measuring relative *P*s of ions across the rat ileum *in vitro* were in general similar to those described by Moreno and Diamond [3, 6]. The terminal ileum (2–4 cm) was removed from the animal and washed free of intestinal contents with a standard solution (see later for composition). The mesenterium was removed as far as possible and the ileum was cut open along the mesenteric border. A piece of tissue was mounted as a flat sheet in a circular window of 10 mm^2 area between two perspex chambers with the aid of a nylon O-ring to prevent edge damage of the tissue [7]. One of the chambers contained 10 ml of the mucosal solution, the other 10 ml of the serosal solution. The solutions were stirred with an O_2-stream and kept at a temperature of 20 ± 1°.

Electrodes. Ag/AgCl electrodes were prepared by coating a pice of silver wire (about 6 cm) totally with an epoxy resin (Araldit 221:Hardener 837 = 100:30). After drying the resin was removed from the end of the wire (5 mm) and the silver thoroughly cleaned with acetone and distilled water. The silver was then chlorated for 10 min in 0.1 M HCl with a direct current of 0.3 mA. Finally the electrodes were cleaned with distilled water. Electrodes were rejected if the asymmetry potential measured in the same standard solution exceeded 0.2 mV.

* Abbreviations: TMA$^+$, tetramethylammonium; TEA$^+$, tetraethylammonium; TAP$^+$, 2,4,6-triaminopyrimidinium; P, permeability coefficient; u, free mobility.

Electrical measurements. Transepithelial potential differences were recorded on a PHM 62 standard pH meter (Radiometer, Copenhagen, Denmark) with a high input impedance, connected to the serosal and mucosal solutions directly with the Ag/AgCl electrodes. No salt bridges were used. The signal was recorded continuously. All transepithelial potential differences measured with asymmetrical bathing solutions were corrected for the difference in electrode potential (E) [$=(RT/F)\ln(a'_{Cl}/a''_{Cl})$], where a'_{Cl}/a''_{Cl} is the chloride activity ratio of the two solutions as estimated from the activity coefficients [8].

Solutions. The composition of the standard solution used was (in millimolal units): 150 mM Na^+, 151 mM Cl^-, 0.25 mM Mg^{2+}, 0.25 mM Ca^{2+}, 4 mM K^+, 1.8 mM HPO_4^{2-} and 0.4 mM $H_2PO_4^-$ (pH = 7.3) Biionic potentials were measured with mucosal solutions in which NaCl was completely replaced by the Cl^- salt of another cation. NaCl dilution potentials were measured by replacing half the NaCl in the mucosal solution iso-osmotically with mannitol. Serosal solutions always contained the standard solution. All solutions had an osmolality of 285 ± 5 mOsm. This was checked on an osmometer. Experiments with TAP^+ were performed at pH 6.1 according to Ref. 9 by adjusting the $HPO_4^{2-}/H_2PO_4^-$ ratio. Changing the pH from 7.3 to 6.1 had no influence on the relative permeabilities.

Determination of relative permeabilities. Permeability ratios were calculated from measured potential differences as described by Moreno and Diamond [3] by means of the Goldman–Hodgkin–Katz equation:

$$V^m - V^s = (RT/F)\ln\frac{\sum P_M\,\gamma_M^s\,[M]^s + P_{Cl}\,\gamma_{Cl}^m\,[Cl]^m}{\sum P_M\,\gamma_M^m\,[M]^m + P_{Cl}\,\gamma_{Cl}^s\,[Cl]^s} \quad (1)$$

where P_i is the relative permeability coefficient of ion i, γ_i^s and γ_i^m are the ion activity coefficients in the serosal and mucosal bathing solutions, quantities between brackets are concns, and M stands for any cation. The summation is taken over all cations present (Na^+, K^+ and the test cation X^+). $V^m - V^s$ is the voltage difference between the mucosal and the serosal solution (mV) and R, T and F have their usual meanings. Since the γ of chloride salts of most cations tested is unknown at the experimental temp and concns, we assumed γ_{XCl} equal to γ_{NaCl} at the same temp and concn as tabulated by Robinson and Stokes [8] (see Ref. 3 for a justification of this procedure). We made the Guggenheim assumption that $\gamma_X = \gamma_{Cl}\cdot P_{Na}/P_{Cl}$, and P_X/P_{Cl} were calculated from the value of the NaCl dilution potential and the X^+/Na^+ biionic potential. Permeabilities were not corrected for a leakage pathway. To correct for inter-individual differences between the ilea from different animals, selectivity isotherms were constructed as described earlier [6].

Materials. Tetramethylammonium chloride, choline chloride and carbachol chloride were obtained from Merck. Tetraethylammonium chloride and 2,4,6-triaminopyrimidine were supplied by Aldrich. All reagents used were the highest purity available.

RESULTS AND DISCUSSION

Evidence for a paracellular route

The possible involvement of transcellular transport in the translocation of ions was investigated by changing the temp of the mucosal and serosal solutions. At 4° essentially the same relative permeabilities were measured as at 20°. At 37° the P_{Na} was somewhat increased relative to the permeability of the other ions. This is probably because the transcellular Na^+ pathways (for instance Na^+-cotransport systems) are blocked up to 20°, while they are functioning normally at 37°. This indicates that an incubation temp of 20° is low enough to exclude a significant involvement of transcellular transport systems. Also the permeability sequence found (Table 1) is incompatible with transcellular transport, in which, for instance, $P_{Na} >> P_K$. In addition transmembranal permeabilities for ions differ by factors much larger than those of Table 1 [4]. Moreover measurements of organic cation transport across isolated plasma membranes of intestinal epithelial cells revealed that only very lipophilic cations with octanol–water partition coefficients of at least 0.1 can pass these membranes passively [10, 11]. TMA^+, TEA^+, choline and carbachol certainly do not belong to that class of lipophilic cations and are much to hydrophilic to exhibit passive transcellular transport. Other evidence could be supplied by the effects of specific blockers on the paracellular route. Only one such blocker is known, TAP^+ [9]. It works in most membrane preparations, but not in all. In our preparation, the rat ileum, TAP^+ (25 mM) had only a small inhibitor effect on the cation permeability, which was statistically not significant. The same is the case in the frog choroid plexus [9]. Although TAP^+ had no effect in our preparation, from the other evidence we conclude that a significant contribution of a transcellular pathway to the permeabilities of Table 1 is very unlikely.

The outcome of the experiments might be influenced by the presence of free-solution shunts. These are artificial channels in the epithelium for instance caused by the mounting of the epithelial sheets in the experimental apparatus. It is possible to correct for such channels by means of a leakage correction as has been done for instance by Moreno and Diamond [3]. Several findings argue against the necessity of such a correction in our experiments. In the first place there was only a small increase of P_{Cl}/P_{Na} with time. Such an increase is generally considered to be due to the formation of free-solution channels during the experiments and is a criterion for the condition of the tissue [3]. Secondly there was no change in P_X/P_{Na} with time. If there are free-solution shunts, they grow with time and consequently P_X/P_{Na} changes also with time, because then P_X/P_{Na} becomes a changing mixture of the true paracellular (P_X/P_{Na}) and the free-mobility ratio (u_X/u_{Na}). Finally, if a leakage correction would be required, the correction would only have a small effect on the values of Table 1 (maximally 0.1 in the relative permeabilities). Therefore we conclude that leakage if present will not influence our interpretations.

Table 1. Paracellular permeabilities in several tissues

Tissue	Reference	P_X/P_{Na}† ± S.D. NH_4^+	K^+	Li^+	TMA^+	TEA^+	$Choline^+$	$Carbachol^+$	Cl^-‡
Rat ileum	This study	1.47 ± 0.03	1.49 ± 0.06	0.78 ± 0.03	0.68 ± 0.06	0.52 ± 0.05	0.51 ± 0.06	0.48 ± 0.04	0.83 ± 0.15
		(12)§	(29)	(15)	(9)	(14)	(16)	(11)	(36)
Rabbit ileum	7	—	1.14	0.57	—	0.02	—	—	0.55
Rabbit gall-bladder	3, 4, 6	1.42	1.80	0.90	<0.02	0.07	0.09	—	0.33
Frog gall-bladder	3, 6	1.70	1.43	0.59	<0.02	0.06	0.07	—	0.28
Free mobility (u_X/u_{Na})*	3, 6	1.55	1.54	0.73	0.73	0.43	0.39	—	1.61

* u_X/u_{Na} is the free mobility (in aqueous solution) of compound X relative to Na^+.
† P_X/P_{Na} is the permeability of compound X relative to Na^+.
‡ Not via the paracellular shunt.

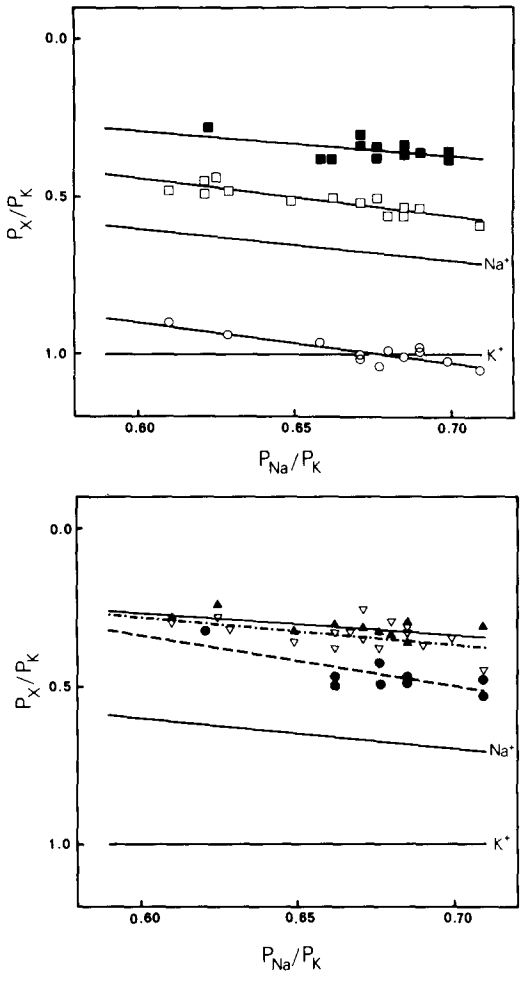

Fig. 1. Selectivity isotherms of the inorganic and organic ions tested. The abscissa gives P_{Na}/P_K values measured in the ileum of each of a number of rats, while the ordinate gives P_X/P_K values in the same ileum, where X stands for the cation tested. Thus, sets of points located along the same imaginary vertical line represent relative permeability coefficients for the ions in a single animal. The K^+ points automatically fall along the horizontal line $P_X/P_{Na} = 1$, and the Na^+ points automatically fall along the line of identity. Ions tested: NH_4^+ (○); Li^+ (□); TEA^+ (■); TMA^+ (●, – – – line); $choline^+$ (▽, – · – · – line); $carbachol^+$ (▲, full line)

Selectivity sequence of the paracellular permeabilities of inorganic and organic ions

Fig. 1 shows the selectivity isotherms in rat ileum for the inorganic and organic ions tested. The isotherms are empirical correlations between variations in P_{Na}/P_K and variations in P_X/P_K. These variations are especially caused by inter-individual differences between the test animals. These empirical correlations can be used for making predictions about permeability ratios in an individual animal, once P_X/P_K has been measured for one cation in this animal. For example if P_{Li}/P_K is measured in a certain rat and found to have the value of 0.50, then P_{TEA}/P_K is predicted to have the value of 0.33 in that particular rat. The prediction is made by locating

P_{Li}/P_K on the isotherm for Li^+, drawing a vertical line through this point intersecting the TEA^+ isotherm, and reading off the value for P_X/P_K from the ordinate. Construction of selectivity isotherms in different biological and non-biological systems is of interest, since this constitutes a means of comparing these systems and gives an indication of the physico-chemical properties of the paracellular shunt in the particular system.

Table 1 gives the relative permeabilities of the different ions and shows that the permeability order is $K^+ \simeq NH_4^+ > Na^+ > Cl^- \simeq Li^+ > TMA^+ > TEA^+ \simeq$ choline$^+$ > carbachol$^+$. Table 1 also summarizes the findings of others in the rabbit ileum [7], rabbit gall-bladder [3, 4, 6], frog gall-bladder [3, 6] and free solution [3, 6]. For all tissues there is a clear difference from the values found in free solution. This is especially the case for the Cl^- permeability ($P_{Cl}/P_{Na} << u_{Cl}/u_{Na}$). For the cations the difference between $P_{X \text{ and } X}$ is smaller. This means that paracellular pathways behave like free-solution channels, with one major exception: they are cation-selective. Rat ileum stands apart from the other tissues in that the permeability to organic cations is very high. Carbachol, a compound with a mol. wt of about 150, still had a permeability relative to sodium of 0.45. It appears therefore that the paracellular pathway can contribute significantly to the absorption of medium sized quaternary ammonium compounds in rat ileum. In addition in rat ileum the Cl^- permeability is much higher than in the other tissues. The only tissue which can be compared to the rat ileum in this respect is the frog choroid plexus [9]. It is of interest that, although TAP^+ is a specific blocker of the paracellular route in most tissues, it is not in the frog choroid plexus [9] and it also appeared not to be in the rat ileum. Taking these facts together it seems that the paracellular pathway in the rat ileum is cation-selective, but consists of aqueous pores larger than in the other tissues of Table 1. This probably results in a cation selectivity which is smaller than in the other tissues. It causes probably also the high permeability for medium sized organic cations and the ineffectiveness of TAP^+.

Model for organic cation absorption

A model for organic cation absorption in the small intestine is developed (Fig. 2), which is based on the results of previous studies [10, 11] and on the paracellular aspects of organic cation transport presented in this study. It was shown that transcellular transport is only possible for lipophilic organic cations with a large hydrophobic tail and a masked charge. These requirements are only met by a very limited number of quaternary ammonium compounds in use today as drugs. Transport across the brush border membrane was passive and stimulated by a transmembrane electrical potential difference, but not by an excess of anions like I^- or taurocholate. Transport across the baso-lateral membrane had the same characteristics, except for the fact that transport was not influenced by a transmembrane electrical potential difference. These findings lead to the following concepts for organic cation absorption *in vivo*.

Transport of lipophilic cations that can pass epithelial cell membrane will be facilitated by the presence of the electrical potential difference across the brush border membrane (*ca.* 60 mV, cell interior negative). The electrical potential difference across the baso-lateral membrane (*ca.* 65 mV, serosa positive) will not influence organic cation transport, since passage across that side of the cell membrane is electroneutral. The bulk of organic cation transport will be via the paracellular route. Although there is a net electrical potential difference between the lumen of the intestinal tract and the serosal side of the paracellular channels (*ca.* 5 mV, serosa positive), this difference is only small and consequently will only influence to a minor extent the absorption process.

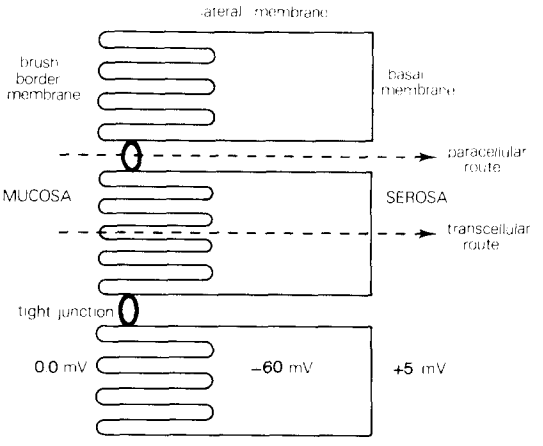

Fig. 2. Model of the intestinal epithelium. Membrane potentials are given relative to the lumen of the intestinal tract. The two absorption routes, transcellular and paracellular, are indicated by arrows.

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