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Permeability properties of apical and basolateral membranes of the guinea pig caecal and colonic epithelia for short-chain fatty acids

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Abstract

Unidirectional fluxes of short-chain fatty acids (SCFA) indicated marked segmental differences in the permeability of apical and basolateral membranes. The aim of our study was to prove these differences in membrane permeability for a lipid-soluble substance and to understand the factors affecting these differences. Apical and basolateral membrane fractions from guinea pig caecal and colonic epithelia were isolated. Membrane compositions were determined and the permeability of membrane vesicles for the protonated SCFA was measured in a stopped-flow device. Native vesicles from apical membranes of the caecum and proximal colon have a much lower permeability than the corresponding vesicles from the basolateral membranes. For the distal colon, membrane permeabilities of native apical and basolateral vesicles are similar. In vesicles prepared from lipid extracts, the permeabilities for the protonated SCFA are negatively correlated to cholesterol content, whereas no such correlation was observed in native vesicles. Our findings confirm that the apical membrane in the caecum and proximal colon of guinea pig is an effective barrier against a rapid diffusion of small lipid-soluble substances such as SCFAH. Besides cholesterol and membrane proteins, there are further factors that contribute to this barrier property.

Keywords: Apical membrane; Basolateral membrane; Caecum; Distal colon; Proximal colon; Permeability; SCFA

1. Introduction

Short-chain fatty acids (SCFA) such as acetic, propionic and butyric acid are the major end products from anaerobic breakdown of polysaccharides by intestinal microorganisms in the hindgut. These SCFA occur in two forms: the non-ionized (SCFAH) and the ionized ($SCFA^-$). Both forms are absorbed by enterocytes [1,2]. Whereas SCFAH are mostly able to permeate epithelial membranes rapidly by passive diffusion due to their lipid solubility [3–5], the transport of $SCFA^-$ across the colonic epithelia is mediated by a postulated $SCFA^- – HCO_3^-$ exchanger [6–9]. Obviously, there are major regional and species-specific differences in the transport of the ionized and of the protonated forms of SCFA [1,2,10,11].

Furthermore, marked differences in the unidirectional fluxes of SCFA have been seen between the caecum, the proximal and the distal colon of the guinea pig, indicating that the distal colon has a much higher permeability for the protonated form of SCFA than the proximal colon or the caecum when SCFA were added to the mucosal side [1,12]. These findings have been interpreted as differences in the permeability of the apical and the basolateral membranes. Based on these flux measurements it has been speculated that, in the caecum and in the proximal colon of the guinea pig (less so in the distal colon), the permeability of the apical membranes are much lower than that of the basolateral membranes. These assumptions are supported by findings on the regulation of the intracellular pH (pH_i) [13]. When added to the basolateral side, SCFA has had a pronounced effect on pH_i; whereas the addition to the luminal side has elicited only minor changes in pH_i [4–6,13]. The apical membrane thus seems to be a significant barrier to SCFAH diffusion. In this context, the question arises as to whether such a phenomenon could be explained

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by structural features, especially complex lipid and fatty acid patterns, cholesterol content, and the pattern of epithelial membrane proteins.

Our findings confirm that the apical membranes in the caecum and in the proximal colon of the guinea pig are an efficient barrier against the rapid diffusion of small lipid-soluble substances such as the non-ionic form of short-chain fatty acids. In the distal colon of the guinea pig, the apical membranes, however, do not represent such a barrier. The analysis of membrane composition and studies on the permeability of protein-free vesicles from lipid extracts of the membranes indicate that, in addition to cholesterol and protein, other factors have an impact upon membrane permeability.

2. Materials and methods

2.1. Animals

The guinea pigs used in this study were males (body weight 400–550 g and 2–3 months of age) fed on a pelleted standard diet (altromin 3122; Altromin, Lage, Germany) with water and food available ad libitum. The guinea pigs were maintained on a 12-h dark photoperiod with two animals per cage.

2.2. Preparation of epithelial membranes

The guinea pigs were killed by decapitation. The proximal and distal colon as well as the caecum were excised and used as for membrane preparation. Apical and basolateral membranes of these different epithelial segments were isolated in a parallel procedure as described previously [14]. After homogenization of mucosal scrapings and various centrifugation steps, the purity of membrane preparations were controlled by marker enzymes, basolateral membranes Na^+–K^+–ATPase, and apical membranes of the distal colon K^+–H^+–ATPase. The purification of the membranes was 10–12-fold for these marker enzymes. Because of the lack of a suitable enzyme marker, the purification of apical membrane preparations of the guinea pig caecum and proximal colon were qualitatively proofed by staining apical membrane components prior to isolation with Texas Red-X (Molecular Probes, Leiden, Netherlands) [14].

2.3. Membrane composition

The total lipids were extracted from the membranes and quantified by the method of Bligh and Dyer [15]. Phospholipids were separated from these extracts by aminopropyl solid-phase extraction [16] and fatty acids were transformed into their methyl esters [17] for gas chromatographic detection (Varian 3400, double capillary column device; stationary phase Supelcowax 10, 30 m, 0.32 mm ID, 0.5 μm *df*; detection system, flame ionization FID) to give the fatty acid pattern. Diheptadecanoyl phosphatidylcholine was used as an internal standard [18].

The cholesterol content of membrane preparations was estimated by a commercial test kit (Chol MPR 1, Boehringer, Mannheim, Germany). The basic principle of this assay is the enzymatic oxidation of cholesterol to cholestenone by the release of hydrogen peroxide, which causes a peroxidase-catalyzed color reaction detectable spectrophotometrically at a wavelength of 546 nm. To minimize unspecific reactions of membrane proteins which can possibly interfere with test reagents delivered with the cholesterol test system, total lipid extracts were used as sample material instead of whole membrane fractions. Under these experimental conditions, we found a cholesterol level—especially in the apical membrane fractions of both the distal and proximal colon—which differs from that reported earlier [14].

The protein content of the isolated membrane fractions was determined by the method described by Lowry et al. [19] with bovine serum albumin as the standard.

2.4. Vesicle preparation

Two types of vesicles were used in this study. The first type, designated "native vesicles," was prepared from the highly purified apical and basolateral membrane fractions of distal and proximal colon and caecum. Starting material was diluted at least 1:4 with buffer A consisting of 100 mM NaCl, 50 mM gluconic acid (sodium salt) and 10 mM HEPES at pH 7.5, and centrifuged at $26{,}700 \times g$ for 10 min at 15 °C. The resulting pellet was washed three times with buffer A and resuspended in a final volume of 5 ml buffer A containing 0.1 mM carboxyfluorescein (CF; Molecular Probes) by a Dounce-type homogenizer (Wheaton-33, 15 ml, glass–glass, tight pestle). This material was extruded by 17 passages through a polycarbonate membrane (pore diameter 5 μm; Avistin, Ottawa, ON, Canada) mounted in an extruder (Avistin) fitted with two 1.0-ml gas-tight Hamilton syringes (Hamilton, Reno, NV, USA) [20]. The extruding procedure was repeated after replacement of the polycarbonate membrane with another (pore diameter 1 μm). These native large unilamellar vesicles with a diameter of approximately 1 μm containing entrapped fluorophore CF were separated from free CF by Sephadex G-25 chromatography (PD-10 column; Amersham Pharmacia Biotech, Freiburg, Germany) and used for stopped-flow studies.

For the second type, designated "lipid vesicles," lipid extracts of the isolated apical and basolateral membranes were prepared from distal and proximal colon and caecum [15], evaporated to dryness and redissolved in 2 ml of chloroform. After removal of chloroform under a stream of nitrogen, the remaining film was suspended in a final volume of 5 ml buffer A containing 0.1 mM CF. To prepare vesicles of the same diameter, further treatments of the

sample were carried out as described for native vesicles. Lipid vesicles and native vesicles represent both crypt and surface epithelial cells.

Vesicles from phosphatidylcholin (egg yolk; Sigma-Aldrich, Deisenhofen, Germany) were used as a control and prepared as described for native and lipid vesicles. Since we prepared large vesicles, their size could be determined by confocal laser scanning fluorescence microscopy (Leica TCS SP2, Heidelberg, Germany). The size distribution was found to be uniform; the mean diameter of the vesicles was 1.06 ± 0.20 μm.

2.5. Non-ionic SCFA uptake into native vesicles or lipid vesicles

Permeabilities of native vesicles and lipid vesicles for acetic acid, propionic acid and butyric acid in their protonated forms were measured by monitoring the pH-sensitive quenching of the entrapped CF in the vesicle at 25 °C [21]. Vesicles were abruptly exposed to a SCFA gradient by rapid mixing of equal volumes of vesicle preparation and SCFA (25 mM; sodium salt) dissolved in buffer B in a microvolume stopped-flow spectrometer (SX.18MV, Applied Photophysics, Leatherhead, UK). Buffer B contained 100 mM NaCl, 25 mM gluconic acid (sodium salt) and 10 mM HEPES, pH 7.5. The excitation wavelength was set at 492 nm. The emission wavelength was filtered with a 515-nm cut-off filter. The measuring interval depended on type of vesicle and type of SCFA used (for acetic acid, 5.0–12.5 ms; for propionic acid, 2.5–5.0 ms; and for butyric acid, 0.5–1.25 ms). Fluorescence data from the stopped-flow device from three individual determinations were averaged and fitted to a single exponential floating end-point curve using software supplied by Applied Photophysics. According to Lande et al. [21] the initial slope (dpH/dt) can be used for determination of permeability coefficient (P_{SCFAH}) by the equation: P_{SCFAH}=(dpH/dt) (V_v/A_v) ($\beta/\Delta c$), where V_v is the volume and A_v the surface area of the vesicle, β the buffer capacity of the solution estimated by titration and Δc the initial difference in concentration of the protonated SCFA between the inside and outside of the vesicle. Fig. 1 shows examples of original stopped-flow tracings with vesicles prepared from apical and basolateral membranes of the proximal colon and with vesicles prepared from lipid extracts.

2.6. Statistical analysis of data

Unless stated otherwise, statistical data was evaluated by one-way analysis of variance (ANOVA) followed by application of the Student–Newman–Keuls method. Using both vesicles from caecal and colonic lipid extracts and native vesicles prepared from the caecal and colonic membranes, apical and basolateral fractions were compared according to the Kruskal–Wallis ANOVA on ranks, followed by application of Dunn's method. Results were presented as means ± S.D. Differences were considered significant when $P<0.05$, and n is the number of vesicle preparations analyzed. Between four and six membrane preparations from a corresponding number of animals were pooled for each vesicle preparation.

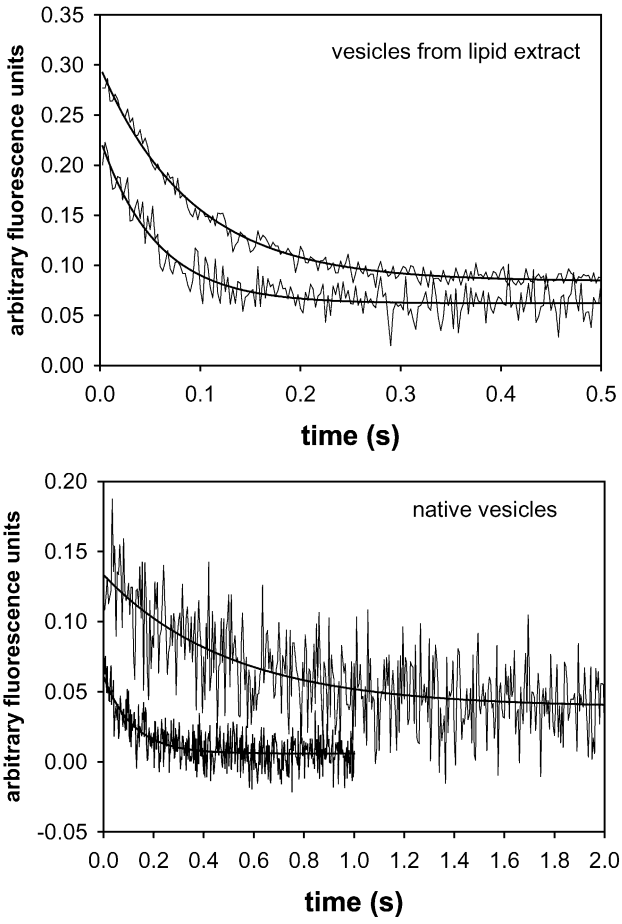

Fig. 1. Stopped-flow measurements showing the fluorescence signal of carboxyfluorescein entrapped within vesicles prepared from apical and basolateral membranes of guinea pig proximal colon (lower chart) and of lipid extracts from these membranes (upper chart) during propionic acid influx. The upper tracing in each diagram corresponds to the apical membrane, the lower tracing to the basolateral membrane of the enterocytes. The fitted single exponential curves are superimposed on the data record. The time scales differ in the two charts. Results are representative of similar determinations performed on four to six different preparations.

3. Results

3.1. Permeability properties of native vesicles

In all segments of the large intestine of the guinea pig, the permeability of native vesicles increased significantly with the chain length of the SCFAH (Fig. 2). For the caecum and the proximal colon, the permeability of vesicles prepared from the apical membranes was very low. The permeabilities of the basolateral vesicles from the caecum

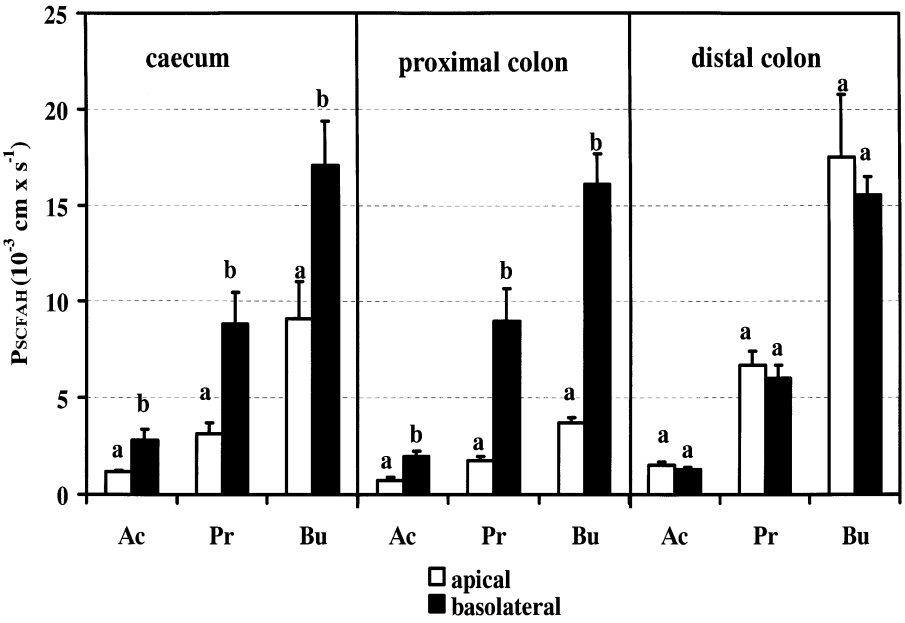

Fig. 2. Permeability coefficients (P_{SCFAH}) of the protonated form of acetic acid (Ac), propionic acid (Pr) and butyric acid (Bu) in native vesicles prepared from highly purified apical (□) and basolateral (■) membranes from the caecum, the proximal and the distal colon. Values are means ± S.D., at least $n = 5$, with each n representing an average of three individual determinations. Different indices (a, b) indicate significant differences between native vesicles prepared from apical and basolateral membranes.

are two to three times higher, and those from the proximal colon, three to five times higher than the corresponding apical membranes. For the distal colon, however, native vesicles from apical and basolateral membranes did not differ markedly in their permeability properties. The permeabilities of the basolateral vesicles were similar in all three segments of the large intestine.

3.2. Permeability properties of lipid vesicles

As with the findings in native vesicles, the permeability of the lipid vesicles from lipid extracts of the three epithelia for SCFAH increased significantly in all segments of the guinea pig large intestine with increasing chain length of the respective SCFA (acetic acid < propionic acid < butyric acid)

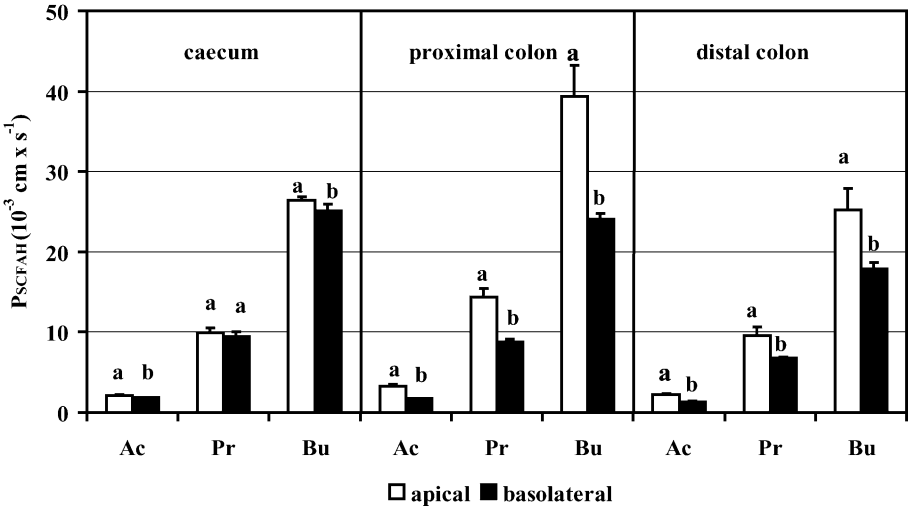

Fig. 3. Permeability coefficients (P_{SCFAH}) of the protonated form of acetic acid (Ac), propionic acid (Pr) and butyric acid (Bu) for vesicles obtained from lipid extracts of apical (□) and basolateral (■) membranes from the caecum, the proximal and the distal colon of guinea pigs. Values are means ± S.D., at least $n = 4$, thereby each n represents an average of three individual determinations. Different indices (a, b) symbolize significant differences between vesicles prepared from apical and basolateral lipid extracts.

Table 1
Content of cholesterol and protein of the purified epithelial membranes

	Caecum		Proximal colon		Distal colon	
	Apical	Basolateral	Apical	Basolateral	Apical	Basolateral
Cholesterol (μmol/μmol phospholipid)	0.57 ± 0.17^{a}	0.53 ± 0.08^{a}	0.29 ± 0.04^{b}	0.64 ± 0.02^{a}	0.25 ± 0.06^{b}	0.66 ± 0.05^{a}
Protein (mg/μmol phospholipid)	8.28 ± 0.95^{a}	2.60 ± 0.15^{b}	5.55 ± 0.19^{c}	3.57 ± 0.81^{b}	6.16 ± 0.63^{c}	2.90 ± 0.15^{b}

In the case of cholesterol, $n=5$; in the case of protein, $n=5$ for caecal epithelia and $n=3$ for colonic epithelia. Different indices (a, b, c) symbolize significant differences within the rows. The cholesterol to phospholipid ratios were calculated by assuming an average molar weight of phospholipid of 730 g/mol.

(Fig. 3). There are marked segmental differences in the permeabilities of native (Fig. 2) and lipid vesicles (Fig. 3). Most prominent are the much higher permeabilities of the lipid vesicles from the apical membranes: for the caecum, the permeability of lipid vesicles is 2–3-fold that of native vesicles; the difference is 5–10-fold for the proximal colon and 1.4-fold for the distal colon). For the basolateral membranes, on the other hand, there are only minor or no differences in permeability between native and lipid vesicles.

The permeabilities of the lipid vesicles from the apical membranes are similar to those from the basolateral membranes in the caecum; they are higher than those from the basolateral membranes in the proximal (60–90%) and in the distal colon (40–60%). Only for the distal colon are the proportions of the permeabilities for Ac, Pr, and Bu similar for the apical and the basolateral membranes of native and lipid vesicles. Due to the high permeabilities of the lipid vesicles from the apical membranes of the proximal colon and of the caecum, the respective proportions of the three SCFA are totally different.

For the interpretation of the observed differences in permeabilities we analyzed the composition of the membranes with respect to fatty acid pattern and the cholesterol and protein contents. The phospholipid classes of the membranes have been reported previously [14].

3.3. Membrane composition

3.3.1. Cholesterol content

The cholesterol contents of the basolateral membranes of the proximal and distal colon are more than twice as high as those of the corresponding apical membranes (Table 1). Such differences were not found in the caecum, where apical and basolateral membranes have approximately the same, relatively high, cholesterol content.

For the native vesicles, there was no relationship detectable between membrane permeabilities and cholesterol content. However, for the vesicles prepared from the lipid extracts of the membranes, P_{SCFAH} decreased with increasing cholesterol content of the respective membranes with the exception of the apical lipid vesicles of the distal colon, where the permeability was relatively low at a low cholesterol content. The correlation could be improved by adding P_{Bu} data from cholesterol-free lipid vesicles prepared from phosphatidylcholine (egg yolk), where P_{Bu} was $55.7 \pm 6.3 \times 10^{-3}$ cm s^{-1} ($n=6$).

3.3.2. Fatty acid pattern

Remarkable differences in fatty acid pattern were found in the comparison of apical membrane phospholipids of all three intestinal segments. Stearic acid (C18:0) and linoleic acid (C18:2) contents were found to be highest in the distal colon and lowest in the caecum (Fig. 4A). Reversed values

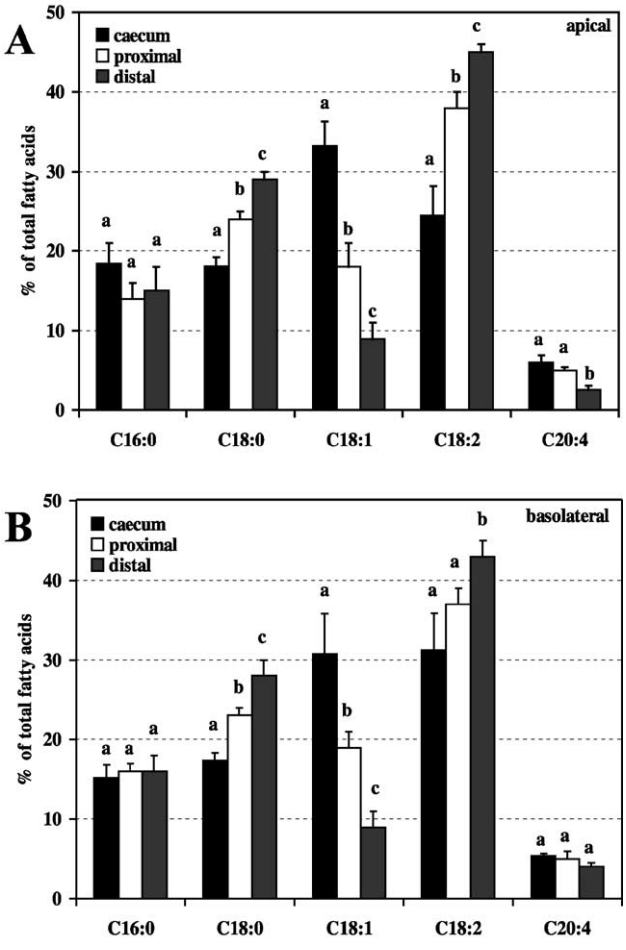

Fig. 4. Fatty acid pattern of the phospholipid extracts as percentage of total fatty acids (w/w) of purified apical (A) and basolateral (B) membranes of the caecum, the proximal and distal colon. Values are means ± S.D., $n=3$ for colonic membranes, $n=4$ for the apical and $n=5$ for the basolateral fractions of the caecum. There are significant differences between apical and basolateral membrane fractions for linoleic acid ($P<0.05$) in the caecum and for arachidonic acid ($P<0.04$) in the distal colon. Different indices (a, b, c) indicate significant differences within the three epithelia for their respective type of fatty acid; palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and arachidonic acid (C20:4).

were obtained for oleic acid (C18:1). In the apical membrane of the caecum, 33% of total fatty acids are C18:1, while C18:1 accounts for only 9% of total fatty acids in the distal colon.

As shown in Fig. 4B the fatty acid pattern of the basolateral membrane phospholipids did not significantly differ from that of the corresponding apical membrane phospholipids of the three segments, with two exceptions. First, in the caecal segment, the basolateral membrane phospholipids had a higher linoleic acid content (31% of total fatty acids) than the corresponding apical membrane (25%). Second, the amount of arachidonic acid (C20:4) differs significantly in the distal colon. Here, the basolateral membrane phospholipids have a higher C20:4 (4%) content than the apical fraction (2.6%).

3.3.3. Protein content

In all three epithelia, the amount of membrane protein (mg protein/μmol phospholipid) is significantly higher in the apical membranes than in the corresponding basolateral membranes (Table 1). Protein content is highest in the caecal apical membranes, 3.2 times that of the basolateral membranes (proximal colon 1.6, distal colon 2.1).

It is interesting to note that for the basolateral membranes, where the protein content is relatively low, the permeabilities for SCFAH are rather similar in all segments of the large intestine; this applies to both the native vesicles and the lipid vesicles. Furthermore, in the apical membranes of the proximal colon and the caecum, where the protein content is high, P_{SCFAH} is low for the native vesicles. This is not the case for the apical membranes of the distal colon.

4. Discussion

The aim of this study was to estimate differences in the permeability of SCFAH of the apical and the basolateral membranes in the caecum, the proximal and the distal colon of the guinea pig. Furthermore, we postulated that membrane composition may correlate with differences in membrane permeability.

4.1. Permeabilities of vesicles prepared from apical and from basolateral membranes (native vesicles) of caecal and colonic segments from the guinea pig

There are significant differences in the permeability between vesicles prepared from the apical and the basolateral membranes in the caecum and the proximal colon on the one hand, and in the distal colon on the other. First of all, native vesicles from apical membranes of the *caecum* and the *proximal colon* of the guinea pig are considerably less permeable than the corresponding basolateral fractions (Fig. 2). These findings are in agreement with the interpretation of results from recent unidirectional flux studies [1,12], from in situ perfused segments of the large intestine [22], and from investigations using perfused crypts isolated from rabbit proximal colon [23]. The low permeabilities in our study strongly suggest that the apical membrane of the caecum and the proximal colon of the guinea pig is an effective barrier for the permeation of small uncharged molecules and weak electrolytes. Low permeabilities of apical membranes have also been reported for epithelial apical membranes in the stomach and kidney [24–28].

Native vesicles prepared from apical and basolateral membranes from *the distal colon* of the guinea pig have similar permeabilities for SCFAH. The permeabilities of the apical membranes of the distal colon are higher than those of the caecum and the proximal colon (Fig. 2). This indicates that the apical membrane of the distal colon does not contribute a specific barrier function as proposed for the caecum and the proximal colon. The higher permeability of the apical membrane of the distal colon is in accordance with the higher mucosal-to-serosal fluxes of SCFA in the distal colon of the guinea pig [1,12]. SCFA are mainly produced by the microbial breakdown of undigested carbohydrates in the caecum but also in the proximal colon. The moderate barrier function of the apical membranes in this section of the hindgut prevents uncontrolled absorption of these SCFA at too high a rate. In the distal colon, on the other hand, the SCFA that still remain in gut contents can be absorbed and utilized.

SCFA are absorbed across the hindgut epithelium both in the protonized and in the ionized forms [1,2]. There has been controversial discussion on the proportions in which these two forms contribute to the absorption [3,5,8,29]. In the context of our study it is important to note that only the non-ionic diffusion in the protonized state is recorded in the stopped flow experiments for the following reasons. An $SCFA^-–HCO_3^-$ exchanger has been postulated for the transport of $SCFA^-$ anions [6–9]. For the rat distal colon, it has been shown that a $SCFA^-–HCO_3^-$ exchanger is present in both the apical and the basolateral membrane of the enterocytes [8,29]. However, our estimated permeabilities cannot be attributed to a $SCFA^-–HCO_3^-$ exchanger [6–9] since our experiments were performed in the absence of bicarbonate. Any bicarbonate exchange activity would be silent. Furthermore, carrier-mediated $SCFA^-$-uptake via bicarbonate exchange occurs within minutes, as has been shown by Mascolo et al. [8] in uptake experiments using apical membrane vesicles of the rat distal colon. But the non-ionic diffusion of SCFAH occurs within milliseconds. Therefore, any impact of carrier-mediated $SCFA^-$-uptake upon the measurement of non-ionic diffusion of SCFAH can be excluded under our experimental conditions.

4.2. Known membrane compositions do not sufficiently explain observed differences in permeability

It is well-established that cholesterol is one of the membrane components which have an impact upon membrane fluidity, resulting in diminished membrane perme-

ability [30]. In our studies with the native vesicles, it was therefore surprising that the cholesterol content of the membranes did not correlate negatively with the estimated permeabilities. The cholesterol content of the apical membranes is even lower in the proximal colon than in the corresponding basolateral membranes, while it is similar in the caecum. In the distal colon, where permeabilities are similar, the cholesterol content of the basolateral membranes is 2.6 times higher than in the apical membranes. Nor can the high barrier function of the apical membranes be explained by their fatty acid pattern (Fig. 4). The fatty acid compositions of apical and basolateral fractions are almost identical in both segments.

The high protein content (Table 1) of the apical membranes of the caecum and the proximal colon may be an indication for a causal involvement in the high barrier function against non-ionic diffusion of protonated SCFA. Integral membrane proteins and the extracellular matrix of the apical membranes might reduce apparent permeability due to unstirred layer effects. In this context, it is interesting to consider that apical membranes from the mammalian bladder, which exhibit a lower permeability than the basolateral membranes, contain large amounts of structural proteins called uroplakins, which appear to form paracrystalline arrays in the outer leaflet of the apical membrane bilayer [31–33]. The importance of membrane proteins in limiting the permeability is indicated by the high permeability of the protein-free lipid vesicles prepared from the apical membranes of the caecum and the proximal colon.

In the distal colon, unlike the caecum and proximal colon of the guinea pig, SCFAH permeabilities of the basolateral and the corresponding apical native vesicles are similar, although the latter has a protein content twice as high (Table 1). This observation suggests that the protein content of the membranes cannot be exclusively responsible for the differences in the barrier function of the apical and the basolateral sides. There must be further factors that facilitate or limit SCFAH fluxes across these membranes. For instance, it could be speculated that the proteins of the apical membrane of the distal colon show different hydrophobicities than the proteins of the other membranes, resulting in the inability of the proteins to form hydrogen bonds with the phospholipids and/or to partially penetrate the lipid bilayer and destabilize the phospholipid acyl chains, so that a more loosely packed membrane may develop [34,35]. Such molecular aspects could be responsible for increasing membrane fluidity and consequently for the higher permeability of the apical membrane of the distal colon for the diffusion of SCFAH.

A further explanation for the observed barrier function of the apical membranes of the caecum and the proximal colon may be the role of bilayer asymmetry in regulating membrane permeability. It is well known that glycosphingolipids and sphingomyelin are present at high concentrations in the outer leaflet of the epithelial apical membranes, and that cholesterol associates closely due to van der Waals' forces and hydrogen binding with these molecules. These interactions induce tight packing in the bilayer, resulting in diminished membrane permeability [35]. Recently, such aspects of lipid composition and bilayer asymmetry in respect to barrier function were studied by examining the permeabilities of liposomes that model individual leaflets of the apical membrane of a barrier epithelium (Madin–Darby canine kidney type 1 cells) [36]. The results of these investigations have shown that although the barrier resides in the exofacial leaflet and both sphingomyelin and glycosphingolipids play a role in reducing membrane permeability, an absolute requirement for cholesterol remains to mediate this effect.

4.3. Permeabilities of vesicles prepared from lipid extracts from apical and from basolateral membranes (lipid vesicles) of caecal and colonic segments from the guinea pig

The contradictory results regarding the composition of the native membranes and their permeability has led to studies on the permeability of protein-free vesicle prepared from lipid extracts from apical and basolateral membranes. First of all, it is important to note that the permeabilities estimated for the lipid vesicles do not correspond to those deduced from unidirectional flux studies and from absorption data from in situ perfused intestinal segments [1,22]. Generally, the SCFAH permeabilities for lipid vesicles derived from the apical membrane fractions of the caecum and the proximal colon are significantly higher than those observed in native vesicles. Similar observations have also been made by Lande et al. [21], who compared permeabilities to water, protons and several small non-electrolytes of vesicles prepared from apical membranes of gastric parietal cells and from lipids quantitatively extracted from these membranes. Our findings indicate that in native membranes, the permeability is determined by additional factors no longer present in the exclusive lipid structure. Those factors include the absence of membrane proteins and the loss of native membrane bilayer asymmetry that accompanies extraction and reconstitution of the lipid vesicles.

However, in these lipid vesicles there was the expected negative correlation with the membrane cholesterol content. Indeed, with the exception of the apical membrane of the distal colon, for all other lipid vesicles, SCFAH permeabilities decreased with increasing cholesterol content. This confirms the results of earlier studies, where lowered fluidity and increased cholesterol content correlate with a decrease of urea influx in unilamellar vesicles of different composition [30]. However, there must also be additional factors that determine the permeability in these lipid vesicles. Although lipid vesicles from the apical membranes of the proximal and distal colon have similarly low cholesterol contents, they differ in their SCFAH permeability properties (Fig. 3). The SCFAH permeability for the lipid vesicles from the apical membranes of the proximal colon was approximately 1.5 times higher than in the distal colon. The observed differences in fatty acid pattern between these

two lipid vesicles might contribute to the difference in their permeability (Fig. 4). The apical membrane of the distal colon has both a higher concentration of saturated fatty acids and a lower concentration of unsaturated fatty acids than the apical membrane of the proximal segment. Both factors, higher saturated fatty acids and lower unsaturated fatty acids, enhance the cholesterol effect [37].

In addition to the different fatty acid pattern, other factors seem to be involved, as well, such as the phosphatidylethanolamine (PE) content. The apical membrane of the distal colon contains more PE than the corresponding membrane of the proximal segment [14]. Taking into account the fact that cholesterol is unable to form hydrogen bonds with glycerophospholipids such as PE [35,38,39], which leads to a more loosely packed bilayer, one can speculate that a higher content of PE would lead to a less well-ordered membrane with a higher permeability than a membrane system with lower PE content. Therefore, the higher PE content could be responsible for the reduced barrier function of the apical membrane of the distal colon compared with the apical membrane systems of the proximal colon and the caecum. Another possible factor affecting permeability might be a difference in glycosphingolipids, which have not yet been quantified in these membranes.

The most important aspect of our findings is that in the guinea pig proximal colon and caecum, the apical membranes are an effective barrier against the rapid diffusion of non-ionic, protonated SCFA. The permeabilities of the basolateral membranes are much higher than those of the apical membranes. In contrast, the apical membranes and their respective basolateral membranes in the distal colon show similar permeabilities. The membrane compositions studied thus far do not provide a sufficient explanation for the differences in permeability observed. Only in vesicles prepared from lipid extracts is cholesterol the main modulator of the barrier function for non-ionic diffusion of SCFAH. In native vesicles, the permeability for SCFAH does not correlate to cholesterol content, where membrane proteins and other factors such as bilayer asymmetry seem to be involved.

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