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Effects of arylaminobenzoate-type chloride channel blockers on equivalent short-circuit current in rabbit colon *

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Abstract. Arylaminobenzoates were examined in rabbit colon mounted in an Ussing chamber. The open-circuit transepithelial voltage (V_{te}) and resistance (R_{te}) were measured and the equivalent short-circuit current ($I_{SC} = V_{te}/R_{te}$) was calculated. After serosal (s) and mucosal (m) addition of indomethacin (1 μmol/l) I_{SC} was -71 ± 11 ($n = 118$) μA/cm^2. Amiloride (0.1 mmol/l, m) inhibited this current and reversed the polarity to $+32 \pm 4$ ($n = 118$) μA/cm^2. In the presence of amiloride and indomethacin, prostaglandin E_2 (1 μmol/l, s), known to induce Cl^- secretion, generated an I_{SC} of -143 ± 8 ($n = 92$) μA/cm^2. The arylaminobenzoate and Cl^- channel blocker 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB) reduced I_{SC} reversibly with a half-maximal inhibition (IC_{50}) at approximately 0.35 mmol/l and 0.2 mmol/l for mucosal and serosal application respectively. To test whether the poor effect was caused by mucus covering the luminal surface, dose/response curves of the mucosal effect were repeated after several pretreatments. Acidic pH on the mucosal side reduced IC_{50} to approximately 0.1 mmol/l. A similar effect was observed after *N*-acetyl-L-cysteine (m) preincubation. Pretreatment with *N*-acetyl-L-cysteine (m) and carbachol (s), in order to exhaust mucus secretion, and L-homocysteine (m) were more effective and reduced IC_{50} to approximately 50 μmol/l. To test whether this effect of NPPB was caused by non-specific effects, the two enantiomers of 5-nitro-2-(+/−1-phenylethylamino)-benzoate were tested of which only the (+) form inhibited the Cl^- conductance in the thick ascending limb of the loop of Henle (TAL). In the present study the (+) enantiomer inhibited significantly more strongly than the (−) form. This suggests that the inhibitory effect of NPPB, even though it requires rather high concentrations, is probably due to Cl^- channel inhibition. For other arylaminobenzoates the sequence of potencies was different from that determined for the TAL. The present data indicate that substances that have been designed to block the Cl^- conductance of the TAL segment also inhibit reversibly but with much lower affinity the PGE_2-induced Cl^- secretion in rabbit colon.

Key words: Colon – Rabbit – NPPB – Chloride channel blockers – Chloride secretion – Secretory diarrhoea

Introduction

The Cl^- channel blockers of the arylaminobenzoate type have been developed for the thick ascending limb of the loop of Henle (TAL) [5, 10, 25]. Shortly after their description it was shown that these substances also block epithelial Cl^- channels in the rectal gland of *Squalus acanthias* [14], HT_{29} cells [2, 8, 11, 16], T_{84} cells [11], respiratory epithelial cells [20], colonocytes [7] and in many other tissues. These data suggest that these blockers might also inhibit the Cl^- secretion in the ileum and colon. In fact, the Cl^- channel blocker anthracene-9-carboxylate has been used in a previous study to inhibit Cl^- secretion [18]. If this approach was feasible, substances of this kind might eventually be used in the therapy of secretory diarrhoea. To examine this issue, we have carried out the present experiments. The aim was to induce Cl^- secretion in colon sheets mounted in an Ussing chamber. Once Cl^- secretion was established by addition of prostaglandin E_2 (PGE_2) to the serosal solution, putative Cl^- channel blockers were added to the mucosal fluid and their effect on the equivalent short-circuit current (I_{SC}) was examined. It is shown that several of the arylaminobenzoates inhibit colonic Cl^- secretion reversibly and dose-dependently.

Materials and methods

Rabbits of either sex (800–1200 g body weight) were killed by decapitation. The distal colon was removed immediately. After removal of the muscle layers and in-

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terstitial tissue a piece of mucosa with an area of 1.0 cm^2 or 0.5 cm^2 was mounted in an Ussing chamber [3, 17]. The solutions on both sides were continuously stirred by a bubble-lift system (Finsterle, MPI Frankfurt, FRG) ensuring constant circulation. The carbogen gas used for bubbling contained O_2 and CO_2 in a mixture of 95% and 5%. Two pairs of Ag/AgCl electrodes served for current injection and voltage (V_{te}, mV) measurements respectively. To estimate the tissue resistance (R_{te}, $\Omega\,cm^2$), rectangular current pulses (amplitude 100 μA, duration 100 ms) were injected (I_{inj}), and the voltage deflection (ΔV_{te}) caused by the tissue input resistance was measured. The chamber as well as the solutions were kept at a temperature of 37° C by means of water jackets.

The solutions on both sides of the epithelium were identical and consisted of (in mmol/l) NaCl 120, KCl 5, $NaHCO_3$ 25, $CaCl_2$ 1.3, $MgCl_2$ 1, an D-glucose 5. With the above carbogen gas mixture the pH of the solution was 7.4. The solution contained indomethacin at 1 μmol/l to prevent endogenous prostaglandin synthesis. Indomethacin was present in the mucosal and serosal solution. All drugs were added to the mucosal or serosal baths in small volumes after dissolving them in either 50 μl dimethylsulphoxide or control solution such that the ionic composition of the solution was not altered appreciably. The maximal concentration of dimethylsulphoxide was 3 ml/l, which concentration had no effect on I_{SC} in pilot experiments. The sources of the Cl^- channel blockers used and their synthesis have been described in a previous report [25]. All other compounds and chemicals were purchased from Merck (Darmstadt, FRG and Sigma (Deisenhofen, FRG). They were of the highest available grade of purity.

Protocol. The standard protocol consisted of an initial equilibration period of about 10 min, during which the voltage reached some negative value of around −3 to −15 mV. R_{te} was obtained by:

$$R_{te} = (\Delta V_{te} - \Delta V_{te}^{\circ}) \times A \times 1/I_{inj}\,[\Omega\,cm^2],$$

where ΔV_{te}° is the voltage deflection measured in the filled chamber before the tissue was mounted, and ΔV_{te} is the voltage deflection after mounting the tissue. Usually ΔV_{te}° was approximately 15% − 25% of ΔV_{te}. A is the area of the tissue in cm^2, and I_{inj} is the infected current in mA. R_{te} was around 150 $\Omega\,cm^2$. Pilot experiments ensured that the injected unipolar pulses had no influence on the measured parameters. From the open-circult voltage (V_{te}) and R_{te} the I_{SC} was calculated by Ohm's law:

$$I_{SC} = V_{te}/R_{te}\,(A/cm^2)$$

The I_{SC} of approximately −70 $\mu A/cm^2$, measured in the presence of indomethacin (serosal and mucosal solution), corresponded mostly to the rheogenic reabsorption of Na^+. In the next step amiloride was added to the mucosal bath at 0.1 mmol/l to block Na^+ reabsorption. Then PGE_2 (1μmol/l) was added to the serosal solution in the presence of amiloride in order to stimulate Cl^- secretion. Within some 3 − 10 min this induced an increase in V_{te} to some −8 mV to −20 mV and a small reduction in R_{te}. The mean I_{SC} was −150 $\mu A/cm^2$ during this period. After a further equilibration period of 10 min putative blockers were added to the mucosal or serosal bath. In another series of experiments the tissue was pretreated with sulphydryl reagents, carbachol etc. by adding these compounds to the mucosal or serosal solution in the beginning of the experiment. Details of these protocols will be given in the respective Results section.

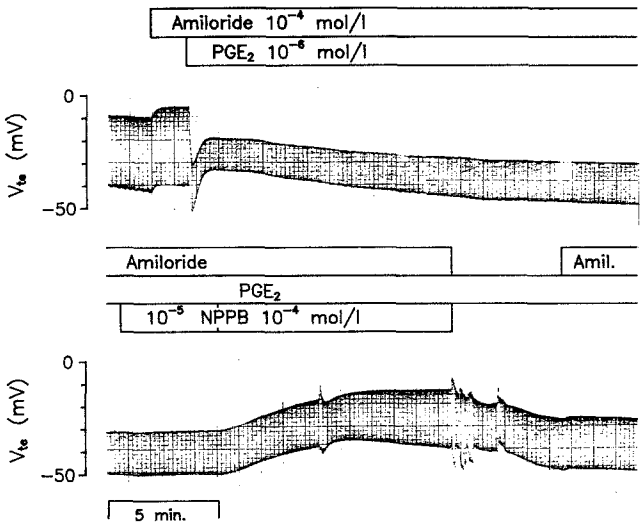


Fig. 1. Trace of an Ussing chamber experiment of rabbit distal colon. The tissue was pretreated with indomethycin (1 μmol/l, serosa and mucosa). The voltage (V_{te}) is plotted as a function of time. The voltage deflections correspond to the transepithelial resistance (R_{te}). Note that V_{te} is reduced and R_{te} slightly increased by amiloride (0.1 mmol/l, apical solution). PGE_2 generates an apically negative V_{te} and a reduction in R_{te}. 5-Nitro-2-(3-phenylpropylamino)-benzoate (NPPB) reduces V_{te} and increases R_{te}. These effects are reversible

Data are presented as means ± SEM (n), with n referring to the number of preparations. Paired t-test with a significance level of $P \leq 0.05$ was used.

Results

Figure 1 shows a typical experiment. Amiloride reduced V_{te} and increased R_{te} slightly. PGE_2 increased the apical negative voltage and reduced R_{te}. 5-Nitro-2-(3-phenylpropylamino)-benzoate (NPPB), added to the mucosal solution, reduced V_{te} and increased R_{te} with approximately the same time course. Its effect was reversible. For the entire series the following mean results were obtained. After equilibration with indomethacin V_{te} was −9.2 ± 0.9 mV (mucosa negative), R_{te} 150 ± 6 $\Omega\,cm^2$, and I_{SC} −71 ± 7 $\mu A/cm^2$ (n = 118). After adding 0.1 mmol/l amiloride to the mucosal solution V_{te} and I_{SC} changed polarity to positive values: +4.5 ± 0.5 mV and +32 ± 4 $\mu A/cm^2$, respectively. R_{te} increased significantly to 170 ± 6 $\Omega\,cm^2$ (n = 118). Stimulation of Cl^- secretion by application of 1 μmol/l PGE_2 serosally resulted again in a change of polarity of the transepithelial potential and of the short-circuit current to even higher values compared to the control conditions: V_{te} increased to −14.1 ± 0.7 mV and I_{SC} to −143 ± 8 (n = 42) $\mu A/cm^2$. R_{te} decreased to 105 ± 4 $\Omega\,cm^2$. All these differences were

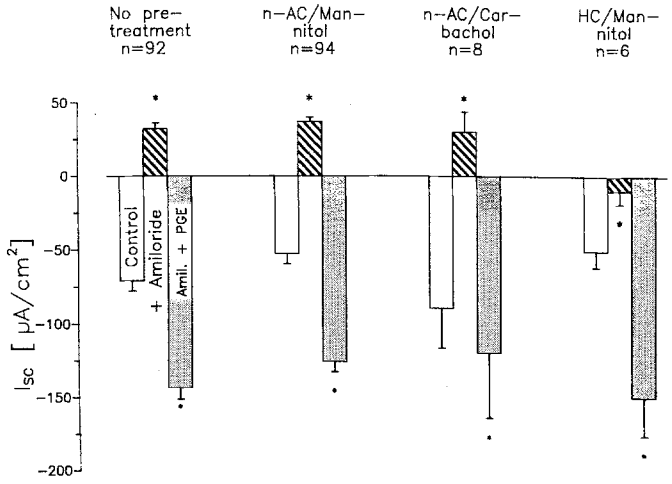


Fig. 2. The equivalent short-circuit current of rabbit distal colon after various pretreatments. The *left column* in each series corresponds to indomethacin (both sides, 1 μmol/l), the *middle column* to indomethacin and amiloride (mucosal side, 0.1 mmoll), and the *right column* corresponds to the addition of prostaglandin E_2 (1 μmol/l, serosal side). Mean values ± SEM. * Statistical significance. The first set of experiments corresponds to control conditions (no pretreatment), the second series represents pretreatment with *N*-acetyl-L-cysteine (apical side). The third series comprises the pretreatment with *N*-acetyl-L-cysteine (apical side) and carbachol (serosal side), and the fourth series pretreatment with L-homocysteine (apical side). In the second and fourth series mannitol (10 mmol/l) was added to the serosal side for osmotic balance. Note that the various mucolytic pretreatments do not reduce the PGE_2-stimulated equivalent short-circuit current

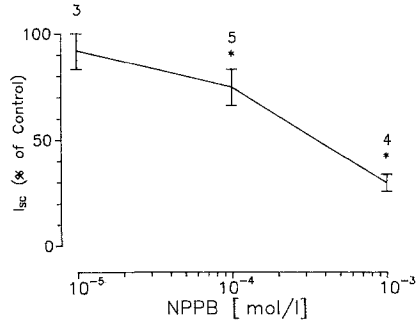


Fig. 3. Dose/response curve for NPPB on equivalent short-circuit current (I_{SC}) in rabbit distal colon. These preparations did not undergo any mucolytic treatment (cf. Fig. 2 and Table 1). I_{SC} is given as a percentage of control. NPPB was added to the mucosal side. NPPB has a significant effect at 0.1 mmol/l. The half maximal effect is achieved at approximately 0.35 mmol/l

statistically significant. These results are summarized in Fig. 2.

NPPB, added to the mucosal and serosal side of PGE_2 and amiloride-pretreated tissues, reduced V_{te} and I_{SC} dose-dependently. The effect of NPPB was, after careful rinsing, reversible. R_{te} was usually slightly increased by NPPB. This effect occurred with approximately the same time course as the reduction in V_{te}. It was small at 0.1 mmol/l and became statistically significant (+25 ± 8%, n = 4) at 0.1 mmol/l. When compared to amiloride the effect of NPPB was rather slow with a time constant of the order of several minutes. The dose/

Table 1. Effectiveness of 5-nitro-2-(3-phenylpropylamino)-benzoate after several pretreatments

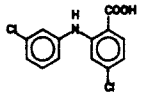
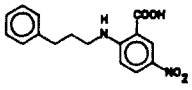
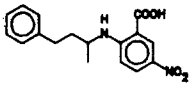
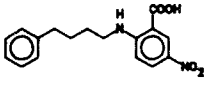
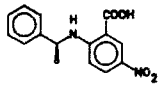
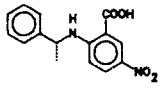
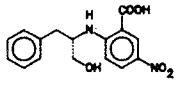
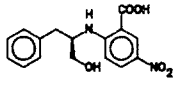
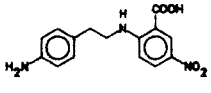
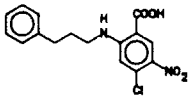
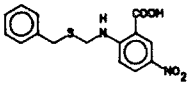
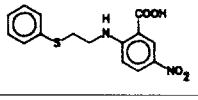
Pretreatment			
Mucosal	Serosal	IC_{50} (μmol/l)	n
Control	Control	350	5
pH 6.5	Control	100	3
N-Acetylcysteine (10 mmol/l)	Mannitol (10 mmol/l)	100	10
N-Acetylcysteine (10 mmol/l)	Carbachol (0.5 mmol/l)	94	6
Homocysteine (10 mmol/l)	Mannitol (10 mmol/l)	53	6
Protamine sulphate (50 mg/l)	Control	290	5

response relationship for the effect of NPPB on I_{SC} is shown in Fig. 3. It is evident from this figure that 0.1 mmol/l NPPB led to a small but significant inhibition of I_{SC}. The IC_{50} value of approximately 0.35 mmol/l is surprisingly high if one considers that the IC_{50} for inhibition of the I_{SC} in the thick ascending limb of the loop of Henle (TAL) was 80 nmol/l [25], and that an IC_{50} of around 0.1 μmol/l has been reported for the inhibition of the Cl^- channel in outside-out-oriented membrane patches of HT_{29} cells [24]. Also it was not expected that NPPB would act even better from the serosal side of the epithelium. In fact, NPPB at 0.1 mmol/l inhibited by 87 ± 5% from the serosal side but only 37 ± 6% from the mucosal side (n = 4). This was entirely unexpected since the Cl^- channels responsible for Cl^- secretion, are localized in the mucosal plasma membrane [13, 17]. Two explanations are at hand for this paradox: (a) NPPB acted nonspecifically, i.e. for instance by its protonophoric action on mitochondria [10, 25] or (b) NPPB, added to the mucosal side, had to permeate serial barriers such as the mucus. In the following we have addressed these hypotheses.

The first hypothesis was examined by using an enantiomeric pair of the Cl^- channel blockers 5-nitro-2-(1-phenylethylamino)-benzoate (nos. 150 and 151, Table 2), for which we have shown previously that only the (+) form acts in the TAL [25]. The rationale for the use of these enantiomers is that the effect on the Cl^- conductance is stereospecific, whilst the protonophoric effect of these compounds [10, 25] and probably also their effect on cyclooxygenase or other pathways [1, 23] might not be. As can be seen in Table 2, the effect of the (+) form was significantly stronger than that of the (−) form. The IC_{50} was > 1 mmol/l for the (−) form (n = 6), but only about 0.33 mmol/l for the (+) form. These data indicate that the inhibitory effect of NPPB is, at least in part, due to an action on the Cl^- channel.

To examine the second hypothesis we pretreated the mucosal surface of the tissue in order to reduce the mucus [18] to facilitate the permeation of NPPB to the apical cell surface. None of these manœuvres had any deleteri-

Table 2. Inhibitory effect of arylaminobenzoates on equivalent short-circuit current of rabbit distal colon mucosa. The number and the data for cortical thick ascending limb (cTAL) refer to the list of compounds published by Wangemann et al. [25]. The last four compounds of the table have not yet been published. Data for these compuonds in the cTAL have been obtained in the authors laboratory. The numbers in parentheses below the IC_{50} values refer to the numbers of experiments

No.	Formula	Chemical name	Colon IC_{50} (mucosa) (mol/l)	cTAL IC_{50} (serosa) (mol/l)
131		3,5-Dichlorodiphenyl-amine-2-carboxylic acid	5.3×10^{-4} (6)	1.6×10^{-5}
144		5-Nitro-2-(3-phenylpropyl-amino)-benzoic acid	3.5×10^{-4} (5)	8.0×10^{-8}
156		5-Nitro-2-(4-phenyl-2-propylamino)-benzoic acid	1.4×10^{-4} (5)	5.0×10^{-8}
145		5-Nitro-2-(4-phenylbutyl-amino)-benzoic acid	2.8×10^{-4} (6)	1.8×10^{-6}
150		5-Nitro-2-(*R*-(−)-1-phenyl-ethylamino)-benzoic acid	3.5×10^{-4} (9)	1.6×10^{-6}
151		5-Nitro-2-(*S*-(+)-1-phenyl-ethylamino)-benzoic acid	$>10^{-3}$ (6)	$>10^{-4}$
153		5-Nitro-2-(*S*-(−)-3-phenyl-propanol-2-amino)-benzoic acid	$>10^{-3}$ (3)	$>10^{-4}$
152		5-Nitro-2-(*R*-(+)-3-phenyl-propanol-2-amino)-benzoic acid	$>10^{-3}$ (3)	1.3×10^{-5}
219		2-(2-(4-Aminophenylethyl)-amino)-5-nitro-benzoic acid	$>10^{-3}$ (3)	6.0×10^{-5} (8)
222		4-Chloro-5-nitro-2-(3-phe-nylpropylamino)-benzoic acid	$>10^{-3}$ (3)	4.0×10^{-7} (5)
283		2-(2-Benzylthioethyl-amino-5-nitro-benzoic acid	7.5×10^{-5} (3)	2.5×10^{-6} (4)
284		5-Nitro-2-(2-phenylthio-ethyl-amino)benzoic acid	7.5×10^{-5} (6)	1.5×10^{-6} (5)

ous effect on the baseline I_{SC} values of this preparation. This is shown in Fig. 2. In a first series NPPB was added to an acidic mucosal solution (pH 6.5). Since the pK_a values of these blockers are in the range of 3 – 5 [25] eight times more blocker should be non-ionized at a pH of 6.4, and this non-ionized moiety might be able to penetrate the mucus layer better than the anionic form. In fact, as is apparent from Table 1, the dose/response curve was shifted to the left considerably by this manœuvre resulting in an IC_{50} of about 0.1 mmol/l as compared to about

0.35 mmol/l for the solution with a pH of 7.4. Next we tried to reduce the number of negative fixed charges of the mucus by protamine sulphate (50 mg/l, mucosal side). This had very little influence on the IC_{50} of NPPB (Table 1). The exposure of the mucosa to 10 mmol/l sulphydryl reagent *N*-acetyl-L-cysteine on the mucosal side and mannitol (10 mmol/l) on the serosal side (for osmotic equilibrium) reduced the IC_{50} for NPPB slightly to around 0.1 mmol/l (Table 1). L-Homocysteine (10 mmol/l, mucosal side) with mannitol (10 mmol/l, serosal side) had an even stronger effect and reduced the IC_{50} to about 53 μmol/l. The same was observed when the mucosa was pre-exposed to *N*-acetyl-L-cysteine (10 mmol/l, mucosal side) together with carbachol (0.5 mmol/l, serosal side). The latter compound was used in order to exhaust mucus secretion by the goblet cells. These data indicate that at least part of the problem of a poor sensitivity of the colonic mucosa towards NPPB can be explained by the mucus layer. Obviously the mucus layer with its fixed negative charges impedes the access of the negatively charged NPPB to its target side in the apical cell membrane of Cl^--secreting cells.

In a last series of experiments several compounds related to NPPB, which are known to block the Cl^- conductance in the TAL, were tested. The results of these experiments are summarized in Table 2. These compounds inhibited the I_{SC} of distal colonic mucosa with IC_{50} values between about 70 μmol/l and ≥ 1 mmol/l. For all compounds it could be shown that mucolytic treatment resulted in some increase in their effect.

Discussion

Cl^- secretion in the distal colon

The preparation used in this study shows negative equivalent short-circuit currents after pretreatment with indomethacin, amiloride and PGE_2. Although we have not identified the ions responsible for these negative currents in the present study, it appears very likely that the current in the presence of indomethacin alone is caused by Na^+ reabsorption. This is supported by our finding that this current can be blocked or even reversed by amiloride [3, 17]. Previous studies using comparable preparations have accumulated evidence that the current induced by PGE_2 is mostly due to Cl^- secretion [3, 6, 17]. In the present study, instead of measuring the short-circuit current, we have decided to measure the open-circuit potential difference and to estimate the apparent transepithelial resistance by current injection. We are aware that our estimates of the transepithelial resistance and hence of the calculated equivalent short-circuit current may be in error because of complicating serial resistors, but our aim was to establish a bioassay system rather than to measure accurately the true short-circuit current across this preparation [22]. Still the I_{SC} data obtained in this study compare favourably to these of previous reports [3, 6, 17, 19]. Our preparations were stable some 10–20 min after the addition of PGE_2 and could be used for some 2 h.

Effect of NPPB

The main effects of NPPB were a dose-dependent reduction of V_{te} and a small increase in R_{te}. Both changes occurred with approximately the same time course. These data are in accordance with previous studies in which similar results have been reported for diphenylamine-2-carboxylate [15], a rather poor Cl^- channel inhibitor in our previous studies in the TAL [5, 25] and for NPPB [6]. This action of NPPB is fully compatible with its known effect of blocking a Cl^- conductance in thick ascending limb cells [25]. Two quantitative aspects, however, deserve further comments: (a) why should the IC_{50} in the colon without any mucolytic treatment be only 0.35 mmol/l, whilst that in the thick ascending limb is 80 nmol/l [25]; and (b) why should NPPB act in the colonic preparation slightly more effectively from the blood side, whereas NPPB had a strongly sided effect in the thick ascending limb?

Regarding the first point one might argue that NPPB has difficulties in reaching its target, namely the Cl^- channel in the colon, but has no problem in the TAL, where the basal cell pole is freely accessible. Along these lines we suspected that the mucus cover in the colon may represent a barrier for NPPB. The mucus contains fixed negative charges and is rather hydrophilic. NPPB, on the other hand, carries a negative charge at physiological pH and is lipophilic in its non-ionized form. Along these lines it was of interest to note that the positively charged amiloride, when added to the mucosal solution, acted much faster. We have tried several manœuvres in an attempt to reduce the mucus layer (carbachol-induced exhaustion of mucus secretion): destroying the mucus crosslinking (by *N*-acetyl-L-cysteine and L-homocysteine) and reducing the anionic moiety of NPPB (pH 6.5), and we found that some of these manœuvres were effective inasmuch as they shifted the dose/response curve for NPPB and related blockers to the left. Therefore, the mucus barrier may in part be responsible for the high IC_{50} value found in this preparation. On the other hand, even with all our efforts we were unable to reduce IC_{50} for NPPB to below 50 μmol/l. This may indicate that, apart from mucus, there may exist other barriers preventing NPPB from reaching its target. The geometry of colonic crypts may be part of this problem, with most of the Cl^- secretion being localized in the crypt [26].

Furthermore, we cannot exclude a priori the possibility that the Cl^- channels responsible for Cl^- secretion in the crypt cells may be much less sensitive to NPPB than are the Cl^- channels responsible for Cl^- reabsorption in the TAL [25]. Along these lines it has been reported that fairly high NPPB concentrations are required to block the intermediate conductance outward rectifying Cl^- channels in inside-out-oriented membrane patches in T_{84} and HT_{29} colonic carcinoma cell lines as well as in respiratory epithelial cells [2, 8–10, 12, 16, 20, 24]. Our most recent findings in these cells, however, indicate that the dose/response curves for NPPB and related macromolecules (i.e. NPPB coupled to dextran or polyethyleneglycol with a molecular mass of 3 kDa) are shifted to the left if these compounds are added to the outer surface of

these Cl^- channels in outside-out-oriented patches [24]. Therefore, it appears likely that the outwardly rectifying Cl^- channels of these cells have a sensitivity towards NPPB very similar to that of the Cl^- conductance in TAL [25]. This still does not exclude the possibility that Cl^- secretion in colonocytes occurs at least in part through yet another Cl^- channel, which is much less sensitive to NPPB. The most recent and unpublished voltage and current measurements from our laboratory in HT_{29} cells support this view. Therefore, regarding this point we conclude that (a) NPPB has difficulties in reaching its target in the colonic crypt and (b) that the Cl^- channels responsible for Cl^- secretion in the colon may be less sensitive to NPPB than those of the TAL.

Regarding the second point of the symmetrical effects of NPPB in our colonic preparation, one has again to consider that such seemingly symmetrical responses could be generated by the dilemma of apical diffusion barriers (cf. above) and by a partial disruption of epithelial integrity. In addition, one should consider that compounds such as NPPB might have other effects, which eventually also reduce the equivalent short-circuit current and, hence, may be misinterpreted as an effect on the Cl^- conductance. Such side-effects comprise: (a) a direct inhibition of a non-selective cation channel or other channels [21] (and H. Gögelein, personal communication); (b) an inhibition of band-3-mediated anion exchange [4]; (c) the inhibition of cyclooxygenase [1, 23]; and (d) a protonophoric effect on mitochondria [10]. The effect in other ion channels has been examined in several other preparations in our laboratory. We confirm that NPPB and even more so diphenylamine-2-carboxylate block non-selective cation channels, but we have never noted an effect in several K^+ channels (collecting duct cells, TAL, respiratory epithelial cells, smooth muscle cells). The protonophoric effect of NPPB has been noted in our original study as a depolarization observed with NPPB concentrations of 10 μmol/l or larger [25]. It may be caused simply by the fact that NPPB in its non-ionized form may shuttle protons across the inner mitochondrial membrane. Side-effects such as this should be similar for all of these compounds and especially the latter effect should not be stereoselective. Along these lines, the present data indicate that non-specific effects cannot account for all of the observed inhibition by NPPB because the (−) enantiomer of one such compound (no. 150, Table 2) was significantly more effective than the (+) enantiomer (no. 151, Table 2). For this latter compound, and for the non-specific inhibitory effects concentrations of more than 1 mmol/l are required for half-maximal inhibition. Therefore, we conclude that at least part of the effect of NPPB is due to its inhibitory action on Cl^- channels.

Compounds related to NPPB

In the present study we have included several of the more potent Cl^- conductance blockers examined in our previous study in the TAL [25] and we confirm that NPPB is one of the most potent compounds. However, in the colon, unlike in the TAL, other substances such as nos. 156, 283, and 284 are equally potent or even more potent than NPPB. Furthermore, a correlation between the IC_{50} values determined here for the colon and the IC_{50} values measured in the TAL [25] was not possible. At this stage we have no way to decide whether this discrepancy reflects differences between these compounds in their ability to reach their target or whether these findings reflect properties of two different Cl^- conductances. Further studies with isolated colonocytes are needed to clarify this point.

In conclusion the present study indicates that NPPB and related compounds reduce the equivalent short-circuit current of rabbit colon. This effect requires some 50 μmol/l NPPB even with mucolytic pretreatment. The effect is at least in part due to the inhibition of a Cl^- current across the apical surface.

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