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Quinine inhibits chloride and nonselective cation channels in isolated rat distal colon cells

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Isolated cells from rat distal colon were investigated with the patch-clamp technique. In cell-attached and cell-excised patches (inside-out) single chloride channels with outward-rectifying properties were observed. In excised patches the single-channel conductance g was 47 ± 5 pS at positive and 22 ± 2 pS at negative clamp potentials ($n = 6$). The Cl^- channel blocker 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB, 10 μM) induced fast closing events, whereas 10 μM of 3′,5-dichlorodiphenylamine-2-carboxylic acid (DCDPC) had no effect when applied to the cytosolic side. Quinine in the bath inhibited the Cl^- channel by reducing its single-channel amplitude and increased open channel noise. With 0.1 mM the current amplitude decreased by 54% and with 1 mM quinine by 67%. Ca^{2+}-dependent nonselective cation channels where observed after excision of the membrane patch. This channel was completely and reversibly inhibited by 100 μM DCDPC. Application of 1 mM quinine to the bath induced flickering and reduced the open-state probability from 0.94 to 0.44. In summary, besides its well established effects on K^+ channels, quinine also inhibits nonselective cation channels and chloride channels by inducing fast closing events.

Introduction

The alkaloid drugs quinine and quinidine are known to block K^+ channels in a number of tissues [1–13]. However, this blocking effect is not specific for K^+ channels. It was reported that quinidine also inhibits rapid inflow of Na^+ in cardiac muscle cells (for review, see Ref. 14). Moreover, it was observed that the drug inhibits nonselective cation channels in rat insolinoma cells [15] and in rat exocrine pancreatic cells [16]. Here we report that quinine blocks not only nonselective cation channels but also outward rectifying Cl^- channels in isolated rat distal colon cells.

It is assumed that cells in the distal colon have different transporting properties. Surface cells are thought to reabsorb NaCl and water, whereas crypt cells probably mediate salt and water secretion after stimulation with secretagogues [17,18]. Reconstitution of vesicles derived from epithelial cells of the colonic mucosa into planar lipid bilayers [19] as well as patch-clamp investigations in isolated cells of rat distal colon revealed Cl^- selective channels [20]. We used the latter preparation in order to investigate the action of inhibitors on Cl^- channels and on nonselective cation channels.

Methods and Materials

Cell isolation

Isolation of single colonocytes was similar as described by Morris, Gallacher and Lee [21]. Male Wistar rats (170–200 g), fed ad libitum, were killed by cervical dislocation. The distal colon just above the pelvic brim was dissected and inverted. The intestinal segment was rinsed in ice-cold NaCl-solution (in mM: 140 NaCl, 4.7 KCl, 1.3 $CaCl_2$, 1 $MgCl_2$, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes), pH 7.4), to which 1 mM dithiothreitol was added. Then the mucosal surface was distended by filling the ligated serosal sac with sodium-citrate solution (in mM: 27 sodium citrate, 96 NaCl, 1.5 KCl, 1.8 KH_2PO_4, 5.6 Na_2HPO_4, pH 7.4). After incubation in sodium-citrate solution for 10 min at 37 °C in a shaking water bath the incubation medium was replaced by $NaCl/Ca^{2+}$-free solution (in mM: 120 NaCl, 5 KCl, 1 $MgCl_2$, 10 glucose, 10 pyruvate, 10 ascorbate, 1.5 EDTA, 0.1% bovine serum albumin (BSA), 20 Hepes, pH 7.4). After 15 min at 37 °C epithelial cells were shaken free in a cold NaCl solution,

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to which 0.1% BSA was added. The cell suspension was passed through a 75 μm and then a 40 μm nylon mesh and was washed twice by centrifugation in NaCl solution + 0.1% BSA for 6 min at 850 rev/min. Isolated cells were stored on ice until use.

Data recording and analysis

Isolated colonocytes were transferred into a measuring chamber on the stage of an inverted microscope (Zeiss IM 35). The chamber was electrically heated at $35 \pm 1°$C. Inside-out oriented membrane patches [22] were investigated with a L/M EPC-7 patch clamp amplifier (List, Darmstadt, F.R.G.) which was remote controlled by an upgrade device [23]. Recording of single channels and data analysis was similar as described previously [16,24]. Briefly, patch pipettes were pulled from borosilicate glass capillaries with a wall thickness of 0.3 mm. Data were stored on a video recorder after the signal was digitized with a modified pulse code modulator (Sony PCM 501). The data were analyzed off-line with a computer system (LSI 11/23, Digital Equipment Corporation) with programmes written in Basic 23. After low-pass filtering (4 pole Bessel) with a -3 dB frequency of 0.2 kHz, data were sampled with a sample time of 1 ms and stored on hard disk. In the displayed current traces, single-channel currents carried by positive ions moving from the bath into the pipette are depicted as upward (positive) currents. The sign of the clamp potential refers to the bath side with respect to the pipette interior. All experiments were performed at $35 \pm 1°$C. The bathing solution was changed by gravimetrical flow and aspiration of the solution by an air-driven venturi. This simple device works with an air pressure of about 3 bar and is placed near the measuring chamber. The waste solution is dropping into a flask placed inside the Farady-cage. Therefore, no electrical noise is introduced by the suction system. The bathing solution could be changed continuously while data were recorded. In order to maintain temperature during bath perfusion, the inflowing solution was preheated electrically.

Materials

The substances 3',5-dichlorodiphenylamine-2-carboxylic acid (DCDPC) and 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) were obtained from Dr. H.C. Englert and Dr.H.J. Lang from the Hoechst AG (Frankfurt). Quinine was purchased from Sigma (Munich).

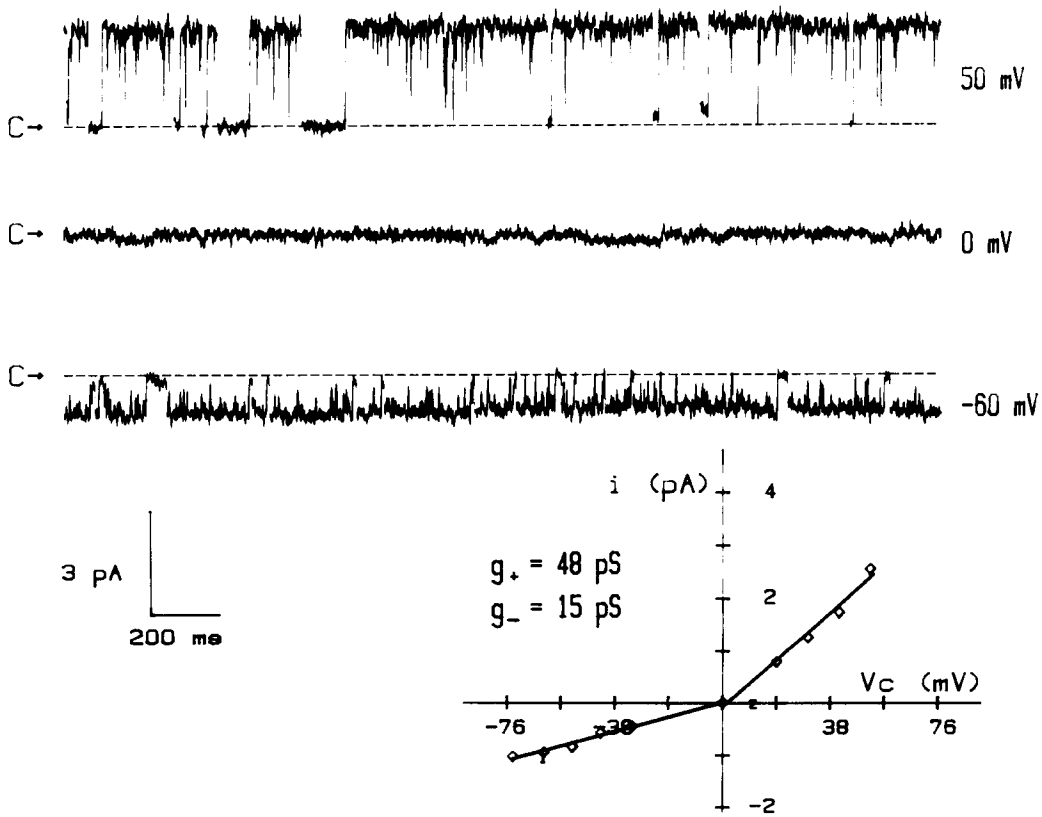


Fig. 1. Chloride channels in a cell-attached patch of an isolated rat distal colon cell. Pipette and bath contained NaCl-solution. C → and the dashed line denotes the closed state of the channel. At the right side of each current trace, the clamp potential (between pipette and bath) is indicated. The lower part demonstrates the respective current-voltage curve. Data points at positive and negative potentials were fitted separately by linear regression, yielding the indicated single channel conductances g_+ and g_-, respectively.

Results

Chloride channels

With NaCl-solution both in pipette and bath, outward rectifying Cl^- channels were observed both in cell-attached (Fig. 1) and in cell-excised patches. In the current-voltage (i/V) curve, data points at positive and at negative clamp potentials were fitted separately by

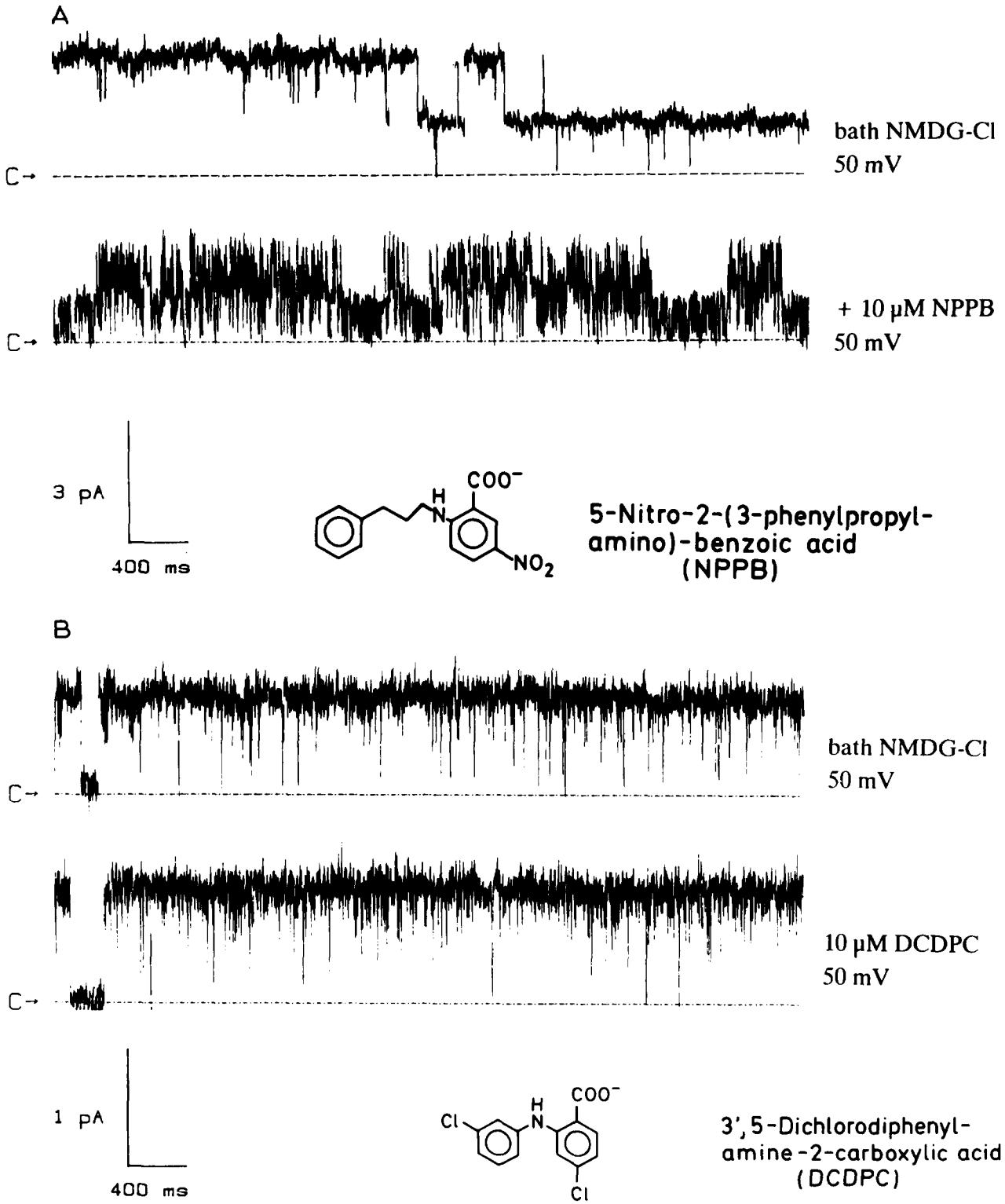


Fig. 2. Effect of NPPB (A) and of DCDPC (B) on single Cl^- channels in isolated patches with NaCl solution in pipette and NMDG-Cl solution in the bath. In (A) two channels are present in the patch, where NPPB induces flickering. 10 μM DCDPC has no significant effect. C → and the dashed line denote the closed state of the channels.

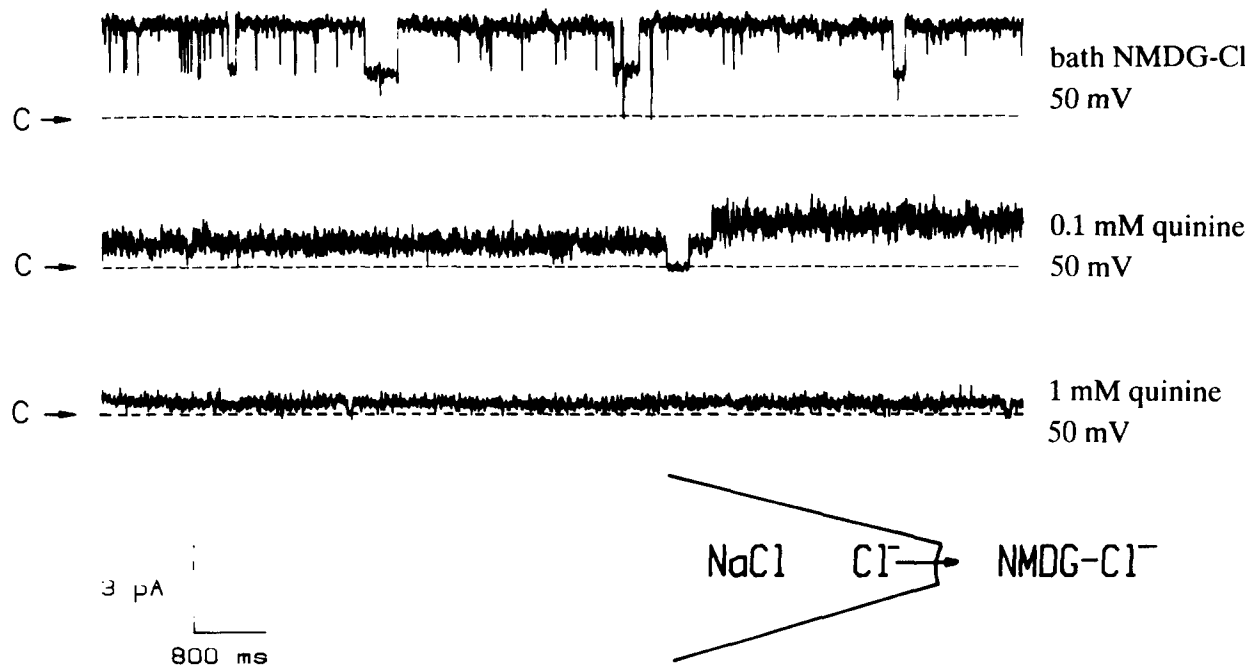


Fig. 3. Effect of quinine on single Cl^- channels in a cell-excised patch, with NaCl solution in the pipette and NMDG-Cl solution in the bath. C → and the dashed lines denote the closed state of the channel. Quinine, added to the bath, reduced the single channel amplitude and increased open channel noise. The inset shows a scheme of the patch pipette and solutions. The drugs were added to the bath solution.

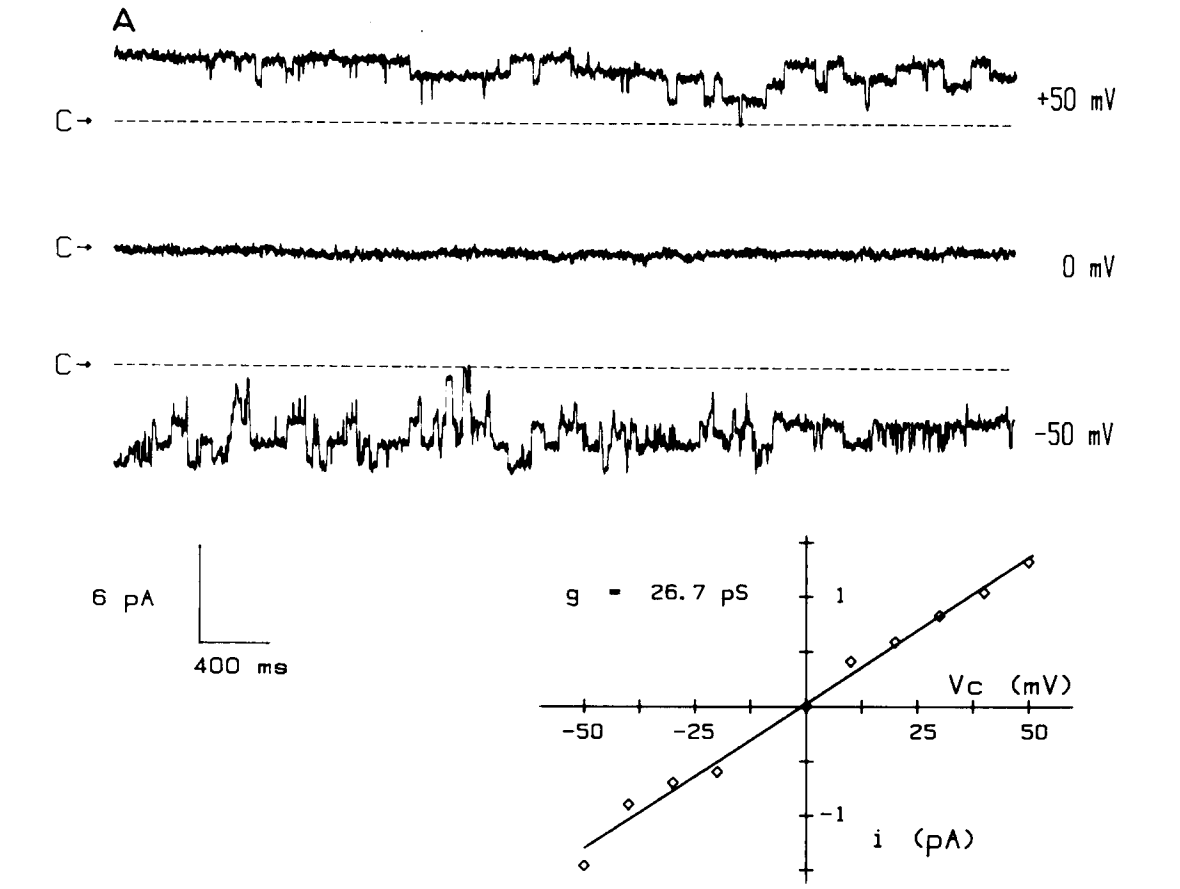
linear regression. The single-channel conductance (g) in cell-attached patches was 40 ± 6 pS at positive and 16 ± 3 pS at negative potentials ($n = 5$). In cell-excised patches g was 47 ± 5 pS at positive and 22 ± 2 pS at negative clamp potentials ($n = 6$). Changing the bath solution in cell-excised patches to a nominally Ca^{2+}-free NaCl-solution (no Ca^{2+} added, 5 mmol/l EGTA), did not affect single-channel conductance (seven observations, data not shown). When all Na^+ in the bath was replaced by *N*-methyl-D-glucamine, single chloride channels were also unaltered (11 observations, data not shown). In order to verify further that we deal with Cl^- channels, the Cl^- channel blocker 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) [25] was added to the bathing medium in cell-excised patches. As demonstrated in Fig. 2A, 10 μM of this substance induced pronounced flickering in this channel (three observations). These results are in agreement with observations made with single Cl^- channels in HT_{29} cells [26]. On the other hand, the substance 3',5-dichlorodiphenylamine-2-carboxylic acid (DCDPC) did not affect single Cl^- channel fluctuations, as shown in Fig. 2B (three observations). In both experiments demonstrated in Figs. 2A and 2B, the bathing medium always contained NMDG-Cl solution and currents at positive clamp voltages were investigated. This procedure acertains that we deal with Cl^- channels and not with nonselective cation channels (see below).

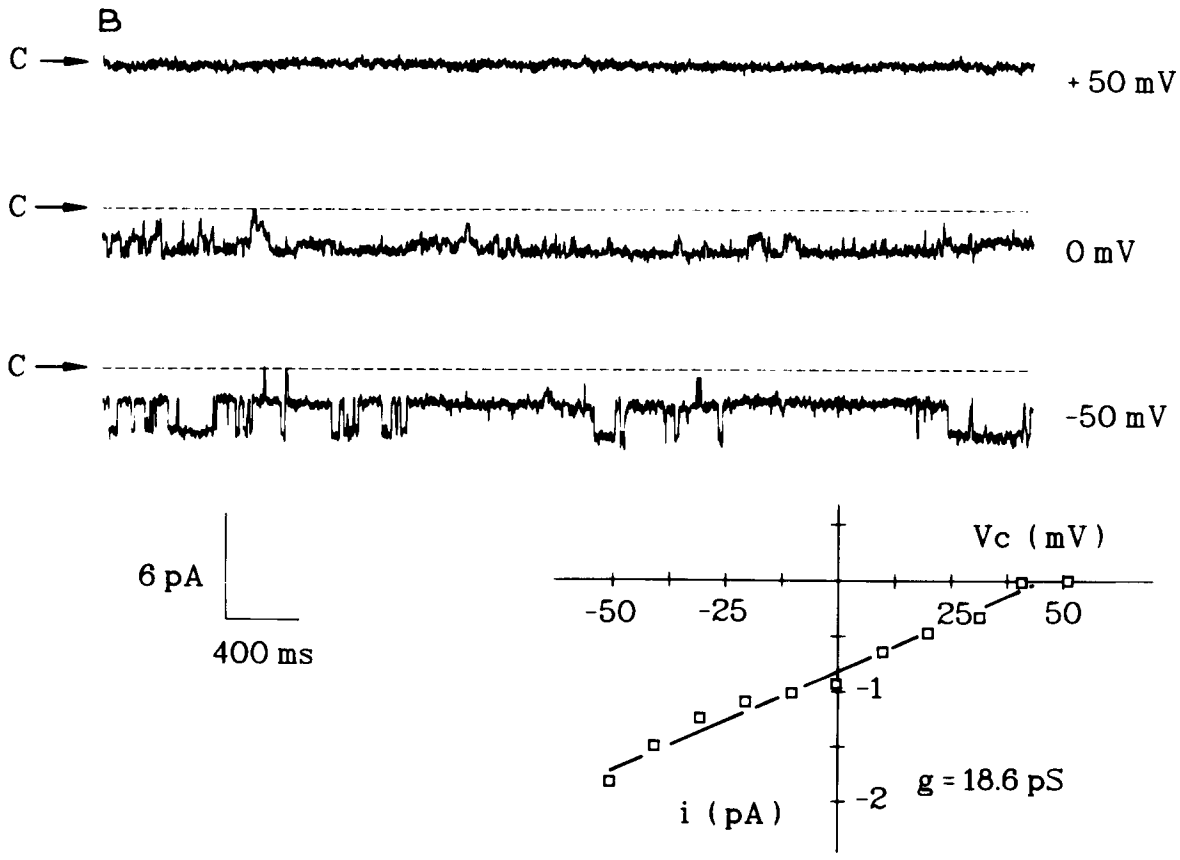
Fig. 3 demonstrates a typical experiment of the blocking effect of quinine on single Cl^- channels recorded in cell-excised patches, with NaCl-solution in the pipette and NMDG-Cl solution in the bath. Addition of 0.1 mM quinine to the bath reduced the single-channel amplitude by $54 \pm 8\%$ ($n = 3$) and increased the open-channel noise (middle trace in Fig. 3). Increasing the quinine concentration to 1 mM reduced the single-channel amplitude by $67 \pm 5\%$ ($n = 3$) (last trace in Fig. 3). As it can be detected in Fig. 3, the open-state probability of the channel apparently did not change in the presence of quinine.

Nonselective cation channels

Frequently, after excision of the membrane patch, a different channel type was observed. A typical experiment is demonstrated in Fig. 4A. The channel appeared mostly in multiples and had a single-channel conductance of 29 ± 2 pS ($n = 18$) with either NaCl or KCl solution in the pipette and NaCl solution in the bath. When the bathing solution was replaced by a NMDG-Cl solution, single-channel openings into the downward

Fig. 4. Nonselective cation channels in cell-excised patches of rat distal colon cells. (A) Pipette and bath contained NaCl solution. Multiples of three channels with equal amplitudes are discernible. The lower part demonstrates the i/V curve of one channel. (B) The pipette contains NaCl and the bath NMDG-Cl solution. The displacement of the i/V curve to the right demonstrates high selectivity of Na^+ against NMDG and no appreciable permeability for Cl^-.


A
C→
+50 mV
C→
0 mV
C→
-50 mV
6 pA
400 ms
g = 26.7 pS
1
Vc (mV)
-50
-25
25
50
-1
i (pA)


B
C
+ 50 mV
C
0 mV
C
-50 mV
6 pA
400 ms
Vc (mV)
-50
-25
25
50
-1
-2
g = 18.6 pS
i (pA)

direction appeared at 0 mV clamp potential and the i/V curve was displaced to the right by about 45 mV. This demonstrates that the channel is selective for cations and is not permeable for Cl^- ions. Since the single-channel amplitude was similar when all Na^+ in the pipette was replaced by K^+, we can denote this channel as nonselective cation channel. Omission of free Ca^{2+} from the bath solution inhibited single-channel activity completely and reversibly (seven observation, data not shown. Thus, this channel has similar properties as the nonselective cation channel observed in the basolateral membrane of rat exocrine pancreas [16,27].

Previously it was reported that the drug DCDPC inhibits the nonselective cation channel in rat exocrine pancreas [16]. Therefore, the action of this compound was investigated. As demonstrated in Fig. 5A, 10 μM of DCDPC decreased channel activity significantly and 100 μM of DCDPC inhibited completely. These block-

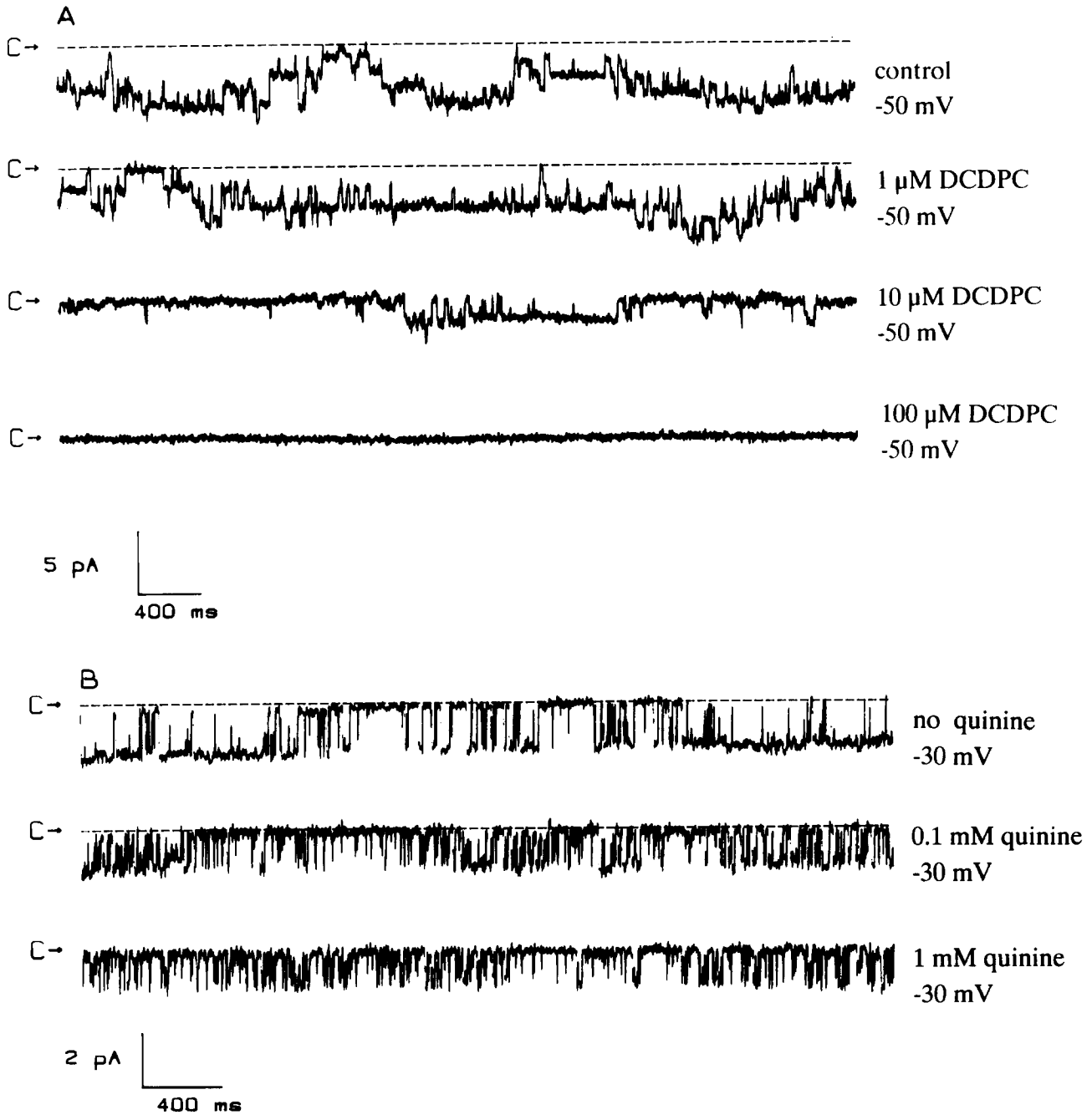


Fig. 5. (A) Effect of DCDPC in the bath on nonselective cation channels in a cell-excised patch. The pipette contains KCl solution and the bath NaCl solution. (B) Quinine in the bath induces flickering in nonselective cation channels in a cell-excised patch. Pipette NaCl solution, bath NMDG-Cl solution.

ing effects were reversible. 1 μM of DCDPC had no significant effect. Thus, the blocking effect of DCDPC is similar as observed in the exocrine pancreas. It was also reported that quinine inhibits nonselective cation channels [15,16]. Therefore, we added quinine to the bathing solution in cell-excised patches. Fig. 5B demonstrates that 0.1 mM quinine induced fast closing events and thereby reduced the open-state probability from 0.94 ± 0.5 ($n = 3$) under control conditions to 0.53 ± 0.14 ($n = 2$), whereas 1 mM quinine reduced P_0 to 0.44 ± 0.11 ($n = 3$).

Discussion

In the present study we investigated single ion channels in isolated cells from rat distal colon. As it can be assumed that these cells are no longer polarized, it remains undefined whether the recorded channels originated from the basolateral or from the apical cell membrane. Moreover it cannot be concluded whether we work with surface cells or with crypt cells. However, these isolated cells are a good tool to study properties of single ion channels, as shown in this study.

We demonstrated that outward rectifying chloride channels can be recorded in these cells. This channel has similar properties as previously described chloride channels from rat colonic enterocytes reconstituted into planar lipid bilayers [19], and chloride channels recorded in HT_{29} cells [28], and in airway epithelia [29,30] (for review, see Ref. 31). Recently, outward rectifying chloride channels were recorded in the apical membrane of intact rat distal colon cells [20]. In contrast to our results these authors recorded only nonrectifying Cl^- channels in isolated rat distal colon cells, whereas rectifying Cl^- channels were observed in the apical membrane of the intact epithelium. In the present paper we confirmed that NPPB is a blocker for Cl^- channels by inducing fast closing events [26]. The inhibition of Cl^- transport was recently demonstrated in experiments in Ussing chambers where NPPB inhibited the serosa to mucosa fluxes of Na^+ and Cl^- activated by forskolin [32]. As outward-rectifying Cl^- channels were mostly recorded in the apical membrane of Cl^- secreting epithelia, it is likely that the Cl^- channels reported in the present study originate from the apical membrane of crypt cells.

In addition to Cl^- channels we frequently observed nonselective cation channels in isolated rat distal colon cells. In a number of experiments both channel types occurred in the same membrane patch. The properties of the nonselective cation channel were similar to channels recorded in rat exocrine pancreatic cells [16,27]. Recently we could demonstrate that similar nonselective cation channels were activated by carbachol or by the Ca^{2+} ionophore A23187 in cell-attached patches of the basolateral membrane of intact crypt cells of the rat distal colon [33]. On the other hand, nonselective cation channels were also recorded in the apical membrane of surface cells in this preparation (own unpublished observations). Therefore, the origin of the nonselective cation channels described in the present study remains undefined.

The main observation of the present study is the blocking effect of quinine on chloride channels. Quinine is frequently used as a blocker of K^+ channels. In some tissues 100 μM of quinine inhibit K^+ channels completely [4,7,13], whereas only partial block is reported with this concentration of quinine for a number of other K^+ channels [2,3,6,8,11,12]. Besides its action on Na^+ inflow in cardiac muscle cells [14], quinine also affected the Na^+-H^+ exchanger in microvillus membrane vesicles of rabbit renal cortex [34] and the K^+-H^+ exchanger from respiring mitochondria [35]. Moreover, blocking effects of quinine were reported on Na^+ and Ca^{2+} inward currents in neurons of *Aplysia californica* [36], and on ouabain-sensitive and furosemide-sensitive K^+ fluxes and amiloride-sensitive Na^+ fluxes in Ehrlich ascites tumor cells [37]. As demonstrated previously [15,16] and in the present study, quinine blocks single nonselective cation channels when applied in the millimolar range. Thus, quinine exerts inhibitory effects on a number of transport processes and therefore appears to be a very unspecific blocker.

The mechanism of quinine block on transporting proteins remains still unclear. The induction of brief closing events (flickering) on single K^+ and nonselective cation channels indicates that the drug binds reversibly to a moiety of the channel protein, involved in the gating mechanism of the channel. In outward-rectifying Cl^- channels this binding-unbinding of quinine is probably so fast that the opening and closing of individual events can no longer be resolved by our electronic device so that an apparent decrease of the single-channel amplitude is observed. A similar decrease of single-channel amplitude occurs in the block of K^+ channels by tetraethylammonium [38].

It can be speculated that the manifold effects of quinine on transport proteins are due to a perturbation of the lipid phase. Indeed, it was observed that the drug perturbs the molecular organization of lipid bilayers of acidic phospholipids by increasing the order parameter of the region close to the polar surface and by decreasing the fluidity of the hydrocarbon core [39,40]. At present, effects of quinine originating from perturbed phospholipid environment on transport properties cannot be ruled out, although it seems unlikely that induction of flickering in single-channel experiments is merely due to alterations of phospholipid properties. Because of the manifold effects of quinine on cellular transport mechanisms, this drug should be used with great care in ion transport experiments.

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References

1 Van Driessche, W. and Hillyard, S.D. (1985) Pflügers Arch. 405, S77–S82.
2 Findlay, I., Dunne, M.J., Ullrich, S., Wollheim, C.B. and Petersen, O.H. (1985) FEBS Lett. 185, 4–8.
3 Iwatsuki, N. and Petersen, O.H. (1985) Biochim. Biophys. Acta 819, 249–257.
4 Richards, N.W. and Dawson, D.C. (1986) Am. J. Physiol. 251, C85–C89.
5 Guggino, S.E., Guggino, W.B., Green, N. and Sacktor, B. (1987) Am. J. Physiol. 252, C128–C137.
6 Gögelein, H., Greger, R. and Schlatter, E. (1987) Pflügers Arch. 409, 107–113.
7 Imaizumi, Y. and Giles, W.R. (1987) Am. J. Physiol. 253, H704–H708.
8 Glavinovic, M.I. and Trifaro, J.M. (1988) J. Physiol. 399, 139–152.
9 Merot, J., Bidet, M., Le Maout, S., Tauc, M. and Poujeol, P. (1989) Biochim. Biophys. Acta 978, 134–144.
10 Furukawa, T. Tsujimura, Y., Kitamura, K., Tanaka, H. and Habuchi, Y. (1989) J. Pharmacol. Exp. Therap. 251, 756–763.
11 Wong, B.S. (1989) Pflügers Arch. 414, 416–422.
12 Mancilla, E. and Rojas, E. (1990) FEBS Lett. 260, 105–108.
13 Bleich, M., Schlatter, E. and Greger, R. (1990) Pflügers Arch. 415, 449–460.
14 Hondeghem, L.M. and Katzung, B.G. (1977) Biochim. Biophys. Acta 472, 373–398.
15 Abramcheck, F.J., Van Driessche, W. and Helman, S.I. (1985) J. Gen. Physiol. 85, 555–582.
16 Sturgess, N.C., Hales, C.N. and Ashford, M.L.J. (1987) Pflügers Arch. 409, 607–615.
17 Gögelein, H. and Pfannmüller, B. (1989) Pflügers Arch. 413, 287–298.
18 Welsh, M.J., Smith, P.L., Fromm, M. and Frizzell, R.A. (1982) Science 218, 1219–1221.
19 Horvath, P.J., Ferriola, P.C., Weiser, M.M. and Duffey, M.E. (1986) Am. J. Physiol. 250, G185–G190.
20 Reinhardt, R., Bridges, R.J., Rummel, W. and Lindemann, B. (1987) J. Membr. Biol. 95, 47–54.
21 Diener, M., Rummel, W., Mestres, P. and Lindemann, B. (1989) J. Membr. Biol. 108, 21–30.
22 Morris, A.P., Gallacher, D.V. and Lee, J.A.C. (1986) FEBS Lett. 206, 87–92.
23 Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100.
24 Rohlicek, V., Fröbe, U., Gögelein, H. and Greger, R. (1989) Pflügers Arch. 413, 444–446.
25 Gögelein, H. and Greger, R. (1986) Pflügers Arch. 407, S142–S148.
26 Wangemann, P., Wittner, M., Di Stefano, A., Englert, H.C., Lang, H.J., Schlatter, E. and Greger, R. (1986) Pflügers Arch. 407, S128–S141.
27 Dreinhöfer, J., Gögelein, H. and Greger, R. (1988) Biochim. Biophys. Acta 946, 135–142.
28 Maruyama, Y. and Petersen, O.H. (1982) Nature 299, 159–161.
29 Hayslett, J.P., Gögelein, H., Kunzelmann, K. and Greger, R. (1987) Pflügers Arch. 410, 487–494.
30 Shoemaker, R.L., Frizzell, R.A., Dwyer, T.M. and Farley, J.M. (1986) Biochim. Biophys. Acta 858, 235–242.
31 Welsh, M.J. (1986) Pflügers Arch. 407, S116–S122.
32 Gögelein, H. (1988) Biochim. Biophys. Acta 947, 521–547.
33 Diener, M. and Rummel, W. (1989) Acta Physiol. Scand. 137, 215–222.
34 Siemer, C. and Gögelein, H. (1990) Pflügers Arch. 415, R46.
35 Mahnensmith, R.L. and Aronson, P.S. (1985) J. Biol. Chem. 260, 12586–12592.
36 Nakashima, R.A. and Garlid, K.D. (1982) J. Biol. Chem. 257, 9252–9254.
37 Hermann, A. and Gorman, A.L.F. (1984) J. Gen. Physiol. 83, 919–940.
38 Arruda, J.A.L. and Sabatini, S. (1980) J. Membr. Biol. 55, 141–147.
39 Smith, T.C. and Levison, C. (1989) Biochim. Biophys. Acta 978, 169–175.
40 Iwatsuki, N. and Petersen, O.H. (1985) J. Membr. Biol. 86, 139–144.
41 Surewicz, W.S. (1982) Biochim. Biophys. Acta 692, 315–318.
42 Needham, L., Dodd, N.J.F. and Houslay, M.D. (1987) Biochim. Biophys. Acta 899, 44–50.]