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Potent block of volume-activated chloride currents in endothelial cells by the uncharged form of quinine and quinidine

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1 The effects of quinine and quinidine on the volume-activated chloride current ($I_{Cl(vol)}$) in cultured endothelial cells from bovine pulmonary artery were studied by use of the whole-cell patch-clamp technique.

2 At pH 7.4 both quinine and quinidine induced a fast and reversible block of $I_{Cl(vol)}$ with K_i values of 20 ± 4 μM and 30 ± 10 μM, respectively.

3 The blocking efficiency of both drugs increased dramatically with increasing extracellular pH, indicating that the blockade is mediated by the uncharged form of quinine and quinidine.

4 These results suggest a hydrophobic interaction with high affinity between volume-activated chloride channels and uncharged quinine and quinidine within the membrane bilayer of endothelial cells.

Keywords: Endothelium; patch-clamp; volume-activated chloride current; quinine; quinidine

Introduction

Volume increase activates in many non-excitable cells a Cl^- current ($I_{Cl(vol)}$) which has been described in detail elsewhere (Nilius *et al.*, 1994a,b,c). These chloride channels are involved in the control of various cell functions, including cell volume regulation (Sarkadi & Parker, 1991), pH control (Hoffmann & Simonsen, 1989; Völkl *et al.*, 1994), and transport of organic osmolytes (Kirk & Kirk, 1993). In endothelial cells they may also act as mechano-sensors (Oike *et al.*, 1994). Recent studies have shown an inhibition of cell proliferation by blockers of $I_{Cl(vol)}$ (Schumacher *et al.*, 1995; Voets *et al.*, 1995) and associated the presence of volume-sensitive chloride channels with cervical carcinogenesis (Chou *et al.*, 1995), indicating a possible role for this current in mitogenesis.

In this study we tested the effect of the antimalarial drug quinine and the antiarrhythmic drug quinidine on the volume-activated chloride current in endothelial cells. Both drugs have been widely used to inhibit a variety of potassium channels (see Kuriyama *et al.*, 1995), including volume-activated potassium channels (Sandford *et al.*, 1992; Nilius *et al.*, 1995).

Methods

We used endothelial cells from an established cell line from bovine pulmonary artery (cell line CPAE, ATCC CCL-209). Cells were grown in Medium 199, detached by exposure to 0.05% trypsin in a Ca^{2+}- and Mg^{2+}-free solution, reseeded on gelatin coated cover slips, and kept in culture for two to four days before use. We only used non-confluent cells in our experiments. Whole-cell membrane currents were measured in ruptured patches. All experiments were performed at room temperature (20-23°C). Currents were monitored with an EPC-7 (List Electronic, Germany) patch clamp amplifier and sampled at 4 ms intervals (2048 points per record, filtered at 100 Hz). The holding potential was 0 mV. The following voltage protocol was used: a step to −80 mV for 0.6 s, followed by a step to −150 mV for 0.2 s and a 2.6 s linear voltage ramp to +100 mV. This protocol was repeated every 15 s. The average current during the successive voltage steps to −80 mV was used to reconstruct the time course of the volume-activated current. At this potential, near the K^+ equilibrium potential, the contribution of K^+ currents is minimal. The current-voltage relationships were derived from the current recorded during the voltage ramp. Data were analysed in

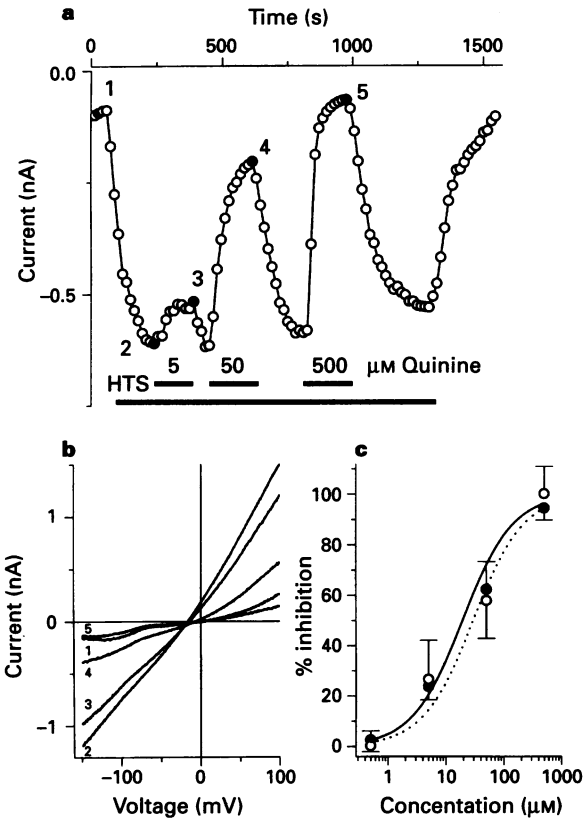

Figure 1 Inhibition of $I_{Cl(vol)}$ by quinine and quinidine. (a) Time course of activation of $I_{Cl(vol)}$ during superfusion with hypotonic solution (HTS), reversible inhibition of the current with different concentrations of quinine and deactivation of the current after returning to isotonic solution. Data were obtained from the voltage step to −80 mV. (b) Current-voltage relationships obtained from voltage ramps at the times indicated in (a). Note that the HTS-induced current reverses very close to the calculated equilibrium potential for chloride (−24 mV). (c) Dose-response curve for inhibition of $I_{Cl(vol)}$ by quinine (●) and quinidine (○). Each data point represents observations from at least 4 cells. The solid line (quinine) and the dotted line (quinidine) represent the best fits obtained with equation [1].

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Origin (MicroCal Software, Inc.). Pooled data are given as the mean ± s.e.mean.

The standard extracellular solution was a Krebs solution, containing (in mM): NaCl 150, KCl 6, $MgCl_2$ 1, $CaCl_2$ 1.5, glucose 10, HEPES 10, titrated with NaOH to pH 7.4. The osmolarity, as measured with a vapour pressure osmometer (Wescor 5500, Schlag, Gladbach, Germany), was 320 ± 5 mOsm. Hypotonic solutions contained NaCl 96, KCl 6, $MgCl_2$ 1, $CaCl_2$ 1.5, glucose 10, HEPES 10, and were titrated with NaOH to pH 6, pH 7.4 or pH 8.9. The osmolarity of these solutions was 240 ± 5 mOsm (~25% hypotonicity). The pipette solution contained (in mM): KCl 40, K-aspartate 100, $MgCl_2$ 1, EGTA 0.5, Na_2ATP 4, HEPES 10, titrated with KOH to pH 7.2. Quinine hydrochloride and quinidine hydrochloride monohydrate were purchased from Sigma.

Results

Volume-activated chloride currents were activated by replacing the external isotonic Krebs solution by a 25% hypotonic solution. This current has been previously described in endothelial cells (Nilius *et al.*, 1994a,b; Szücs *et al.*, 1996). Figure 1a shows an experiment in which different concentrations of quinine were added to the external solution during a maintained superfusion with hypotonic solution. Quinine induced a fast and concentration-dependent block of $I_{Cl(vol)}$, which was almost complete with 500 μM. This block was reversible and recovery of the current upon washout was fast. Current-voltage relationships before and after addition of the different drug concentrations (Figure 1b) show that $I_{Cl(vol)}$ is equally inhibited at positive and negative potentials (a voltage-independent block). Very similar results were obtained with quinidine (not shown). The concentration-dependence of the inhibition of $I_{Cl(vol)}$, measured at −80 mV, by both drugs is represented in a dose-response curve (Figure 1c). Data points were fitted with the equation:

$$\% \text{ inhibition} = \frac{100}{1 + K_i/C} \quad [1]$$

in which C is the drug concentration and K_i represents the drug concentration needed for half maximal block. The estimated values of K_i were 20 ± 4 μM (quinine) and 30 ± 10 μM (quinidine).

At pH 7.4, both quinine and its stereoisomer quinidine occur mainly with a single positive charge, due to a ternary amine group with a pK_a value of 10. In order to investigate if their inhibitory effects on $I_{Cl(vol)}$ are mediated by the positively charged or the neutral form, we performed experiments in which the pH of the hypotonic solution was increased to 8.9 or decreased to 6.0 during drug application. Short (1–2 min) changes in the pH of the external solution alone did not significantly affect the amplitude of currents measured in isotonic or hypotonic conditions (not shown). Figure 2 shows the results of a set of experiments in which the pH of the external solution was changed during application of different concentrations of quinine or quinidine. It is obvious that the inhibitory effect of both drugs was markedly reduced at lower pH (Figure 2a,b), and potentiated at higher pH (Figure 2c,d). These differences could be explained by assuming that only the uncharged form of quinine and quinidine exert a blocking effect. To calculate the uncharged concentrations we used the equation:

$$\log\left(\frac{U}{C}\right) = \text{pH} - pK_a \quad [2]$$

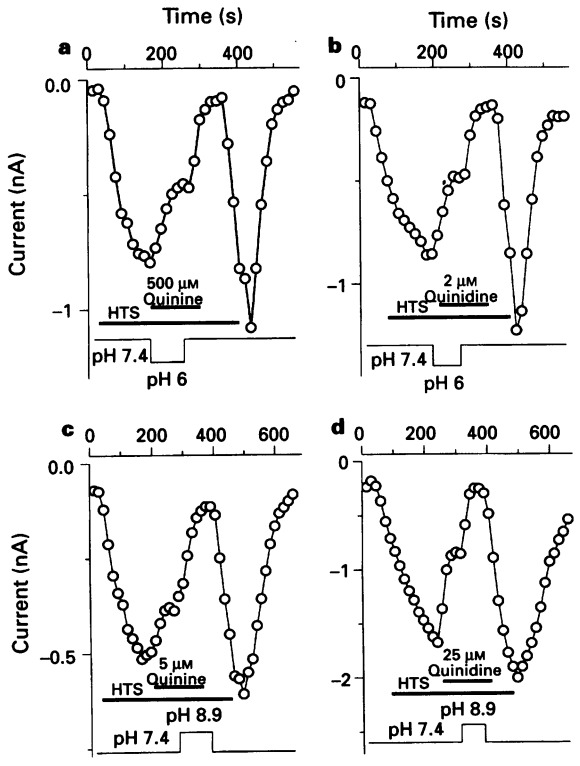

Figure 2 Time course of the current at −80 mV showing the effect of decreased (a,b) and increased (c,d) pH on the block of $I_{Cl(vol)}$ by quinine (a,c) and quinidine (b,d).

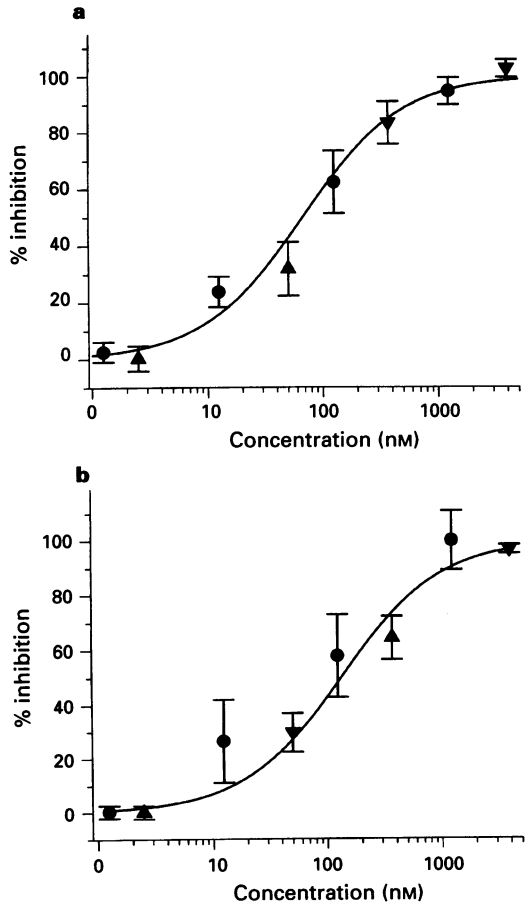

Figure 3 Dose-response curves for the inhibition $I_{Cl(vol)}$ by the uncharged form of quinine (a) and quinidine (b). Data points obtained at pH 6 (▲), pH 7.4 (●) and pH 8.9 (▼) were fit by use of equation [1]. The uncharged concentrations were calculated with equation [2]. Each data point represents observations from at least 4 cells.

where U is the concentration of uncharged drug, and C the concentration of charged drug in the solution. From this equation, it can be calculated that at pH 6 only 0.01% of quinine or quinidine is in its neutral form, compared with 0.25% at pH 7.4 and 8% at pH 8.9. Figure 3 shows the inhibition of $I_{Cl(vol)}$ by quinine and quinidine at the various pH values as a function of the uncharged concentration of the drugs. The pooled data points obtained at the different pH values for quinine (Figure 3a) and quinidine (Figure 3b) could both be fitted by equation [1], with K_i values of 67 ± 13 nM and 135 ± 24 nM for, respectively, quinine and quinidine.

Discussion

The molecular and functional analysis of volume-activated chloride channels is still hampered by the non-availability of sufficiently sensitive and selective pharmacological tools as modulators of these channels (see Nilius *et al.*, 1996). Such tools might be of special interest for endothelial cells, as blockers of volume-activated chloride currents have been shown to inhibit endothelial proliferation (Voets *et al.*, 1995). In breast cancer the inhibition of endothelial growth leads to tumour regression, by reducing vascularization and thus impairing tumour perfusion (Furman Haran *et al.*, 1994).

In previous studies it has been shown that quinine or quinidine in concentrations up to 1 mM inhibit volume-activated chloride channels by only 10 to 30% (Banderali & Roy, 1992; Verdon *et al.*, 1995; Nilius *et al.*, 1995). In the endothelial cells used in our experiments both drugs were able to block $I_{Cl(vol)}$ completely. These differences in sensitivity might be an indication for the presence in these cells of different types of volume-activated chloride currents, and quinine or quinidine could be used to discriminate between these types.

Our results prove that the inhibition $I_{Cl(vol)}$ is mediated by the uncharged form of quinine and quinidine. A possible explanation could be that the block is due to an intracellular action following permeation of the uncharged form through the cell membrane, as has been proposed for chromones (Heinke *et al.*, 1995). However, if uncharged quinine or quinidine enters the cell, it would be immediately ionised (the internal solution is at pH 7.2) and the subsequent washout would be rather slow. As reversal of the block was always fast and complete, it seems more likely that neutral quinine and quinidine induce their high affinity block by a hydrophobic interaction with the channel protein(s) within the membrane bilayer. The voltage-independent nature of the block supports this hypothesis.

In conclusion, volume-activated chloride currents in endothelial cells are efficiently blocked by the uncharged forms of quinine and quinidine. Given that these uncharged molecules exert their inhibitory effect in the nanomolar range, quinine-like structures might provide a basis for the development of novel modulatory compounds for volume-activated chloride currents, with an even higher affinity and specificity.

References

BANDERALI, U. & ROY, G. (1992). Activation of K^+ and Cl^- channels in MDCK cells during volume regulation in hypotonic media. *J. Membr. Biol.*, **126**, 219–234.

CHOU, C.Y., SHEN, M.R. & WU, S.N. (1995). Volume-sensitive chloride channels associated with human cervical carcinogenesis. *Cancer Res.*, **55**, 6077–6083.

FURMAN HARAN, E., MARETZEK, A.F., GOLDBERG, I., HOROWITZ, A. & DEGANI, H. (1994). Tamoxifen enhances cell death in implanted MCF7 breast cancer by inhibiting endothelium growth. *Cancer Res.*, **54**, 5511–5514.

HEINKE, S., SZÜCS, G., NORRIS, A., DROOGMANS, G. & NILIUS, B. (1995). Inhibition of volume-activated chloride channels in endothelial cells by chromones. *Br. J. Pharmacol.*, **115**, 1393–1398.

HOFFMANN, E.K. & SIMONSEN, L.O. (1989). Membrane mechanisms in volume and pH regulation in vertebrate cells. *Physiol. Rev.*, **69**, 315–382.

KIRK, K. & KIRK, J. (1993). Volume-regulatory taurine release from a human lung cancer cell line. Evidence for amino acid transport via a volume-activated chloride channel. *FEBS Lett.*, **336**, 153–158.

KURIYAMA, H., KITAMURA, K. & NABATA, H. (1995). Pharmacological and physiological significance of ion channels and factors that modulate them in vascular tissues. *Pharmacol. Rev.*, **47**, 387–573.

NILIUS, B., EGGERMONT, J., VOETS, T. & DROOGMANS, G. (1996). Volume-activated Cl^--channels. *Gen. Pharmacol.*, (in press).

NILIUS, B., OIKE, M., ZAHRADNIK, I. & DROOGMANS, G. (1994a). Activation of a Cl^- current by hypotonic volume increase in human endothelial cells. *J. Gen. Physiol.*, **103**, 787–805.

NILIUS, B., SEHRER, J., DE SMET, P., VAN DRIESSCHE, W. & DROOGMANS, G. (1995). Volume regulation in a toad epithelial cell line: role of K^+ and Cl^- channels. *J. Physiol.*, **487**, 367–378.

NILIUS, B., SEHRER, J. & DROOGMANS, G. (1994b). Permeation properties and modulation of volume-activated Cl^--currents in human endothelial cells. *Br. J. Pharmacol.*, **12**, 1049–1056.

NILIUS, B., SEHRER, J., VIANA, F., DE GREEF C, RAEYMAEKERS, L., EGGERMONT, J. & DROOGMANS, G. (1994c). Volume-activated Cl^- currents in different mammalian non-excitable cell types. *Pflügers Arch.*, **428**, 364–371.

OIKE, M., DROOGMANS, G. & NILIUS, B. (1994). Mechanosensitive Ca^{2+} transients in endothelial cells from human umbilical vein. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 2940–2944.

SANDFORD, C.A., SWEIRY, J.H. & JENKINSON, D.H. (1992). Properties of a cell volume-sensitive potassium conductance in isolated guinea-pig and rat hepatocytes. *J. Physiol.*, **447**, 133–148.

SARKADI, B. & PARKER, J.C. (1991). Activation of ion transport pathways by changes in cell volume. *Biochim. Biophys. Acta*, **1071**, 407–427.

SCHUMACHER, P.A., SAKELLAROPOULOS, G., PHIPPS, D.J. & SCHLICHTER, L.C. (1995). Small-conductance chloride channels in human peripheral T lymphocytes. *J. Membr. Biol.*, **145**, 217–232.

SZÜCS, G., BUYSSE, G., EGGERMONT, J., DROOGMANS, G. & NILIUS, B. (1996). Characterisation of volume-activated chloride currents in endothelial cells from bovine pulmonary artery. *J. Membr. Biol.*, **147**, 189–197.

VERDON, B., WINPENNY, J.P., WHITFIELD, K.J., ARGENT, B.E. & GRAY, M.A. (1995). Volume-activated chloride currents in pancreatic duct cells. *J. Membr. Biol.*, **147**, 173–183.

VOETS, T., SZÜCS, G., DROOGMANS, G. & NILIUS, B. (1995). Blockers of volume-activated Cl^- currents inhibit endothelial cell proliferation. *Pflügers Arch.*, **431**, 132–134.

VÖLKL, H., BUSCH, G.L., HÄUSSINGER, D. & LANG, F. (1994). Alkalinization of acidic cellular compartments following cell swelling. *FEBS Lett.*, **338**, 27–30.

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