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Effect of acetylcholine on electrical properties of subconfluent Madin Darby canine kidney cells

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To elucidate the effects of acetylcholine on the electrical properties of incompletely confluent Madin Darby canine kidney (MDCK) cells continuous measurements of the potential difference across the cell membrane (PD) were made with conventional microelectrodes during rapid changes of extracellular fluid composition. During control conditions PD averages -48.9 ± 1.0 mV ($n = 51$). 1 μmol/l acetylcholine leads to a sustained but reversible hyperpolarization of the cell membrane by -17.9 ± 0.7 mV ($n = 51$). Half-maximal effect is observed at some 100 nmol/l. 1 μmol/l atropine does not significantly alter the potential difference across the cell membrane, but abolishes reversibly the hyperpolarizing effect of acetylcholine. Increase of extracellular potassium concentration from 5.4 mmol/l to 20 mmol/l depolarizes the cell membrane by $+12.1 \pm 1.1$ mV ($n = 12$) in the absence and by $+25.7 \pm 0.9$ mV ($n = 12$) in the presence of acetylcholine. Within 80 s removal of extracellular calcium leads to a depolarization of the cell membrane by $+16.2 \pm 3.2$ mV ($n = 9$). In the nominal absence of extracellular calcium acetylcholine leads to a transient hyperpolarization by -13.8 ± 1.8 mV ($n = 9$), which can be elicited only once. In conclusion, acetylcholine hyperpolarizes the plasma membrane of MDCK cells by calcium-dependent enhancement of potassium conductance.

Introduction

Madin Darby canine kidney (MDCK) cells are a permanent cell line of a dog kidney [13,38,40,52,57]. If grown to confluency, MDCK cells exhibit transepithelial transport of fluid and solutes [7,20,27,56]. Transport across the apical cell membrane is accomplished by a sodium/hydrogen ion exchanger [41,42] and a chloride conductance [17], transport across the basolateral cell membrane by a potassium conductance [1,4], a NaCl/KCl cotransport [25,43] and a sodium/potassium-ATPase [1,7]. Accordingly, the monolayers are capable to both, sodium reabsorption [7,27,40,56] and chloride secretion [3,50].

The same transport systems are expressed in subconfluent MDCK-cells: In those cells we have identified potassium conductance [30], chloride conductance [19], sodium/hydrogen ion exchange [32], NaCl/KCl cotransport [19], sodium/potassium-ATPase [30] and sodium/calcium ion exchange [32].

The potassium conductance is stimulated by intracellular calcium in both, confluent [4] and subconfluent [18,32] MDCK cells. Epinephrine [31] and bradykinin [33] stimulate potassium con-

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ductance apparently by increasing intracellular calcium activity. Accordingly, the hormones hyperpolarize the plasma membrane of MDCK cells.

The present study has been performed to test, if the potential difference across the cell membrane of MDCK cells is similarly sensitive to acetylcholine and if so, to identify the mechanisms accounting for the observed alterations of cell membrane potential.

Methods

The techniques employed have been described in previous papers in detail [30]. In short, MDCK cells from the American Type Culture Collection [21] were used from passage 70 to 90. Serial cultures were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin [12,54], equilibrated with 95% humidified air and 5% carbondioxide at 37° C. After growing to confluency monolayers were dispersed by incubation in a calcium- and magnesium-free, trypsin-EDTA containing balanced salt solution (pH 7.4) [51] plated on sterile cover glasses and incubated again in the same medium as above for at least 48 h. Cover glasses with incompletely confluent cell layers were mounted in a perfusion chamber allowing for rapid fluid exchange (chamber volume 0.1 ml, perfusion rate 20 ml/min).

Extracellular perfusates were composed of (in mmol/l): NaCl 114, KCl 5.4, $MgCl_2$ 0.8, $CaCl_2$ 1.2, Na_2HPO_4/NaH_2PO_4 1.2 (4 : 1), $NaHCO_3$ 20, gluconate 5.5. The solutions were equilibrated with 5% CO_2 and 95% air (pH 7.4) and kept at 37° C. Where indicated, KCl was increased to 20 or 40 mmol/l, respectively, replacing equal amounts of NaCl, NaCl replaced by sodium gluconate or calcium omitted (with or without addition of 1 mmol/l EDTA). The latter solutions had calcium activities of less than 1 nmol/l. Acetylcholine (Sigma, Munich, F.R.G.) was added at concentrations ranging from 10 nmol/l to 100 μmol/l, as specified.

Measurements of potential difference across the cell membrane (PD) were made using conventional microelectrodes (tip diameter < 0.5 μm, input resistance 100–200 MΩ, tip potential < 5 mV), back filled with 1 mol/l KCl. The microelectrodes were made by pulling filament containing borosilicate tubes (o.d. 1 mm, i.d. 0.5 mm, Hilgenberg, Malsfeld, F.R.G.) and connected with a high input impedance electrometer (FD 223, W.P.I., Hamden, CT, U.S.A.). Measurements were made versus an Ag/AgCl electrode connected with the bath via a flowing 3 mol/l KCl-agar bridge. Impalements were made under an inverted phase-contrast microscope (Invertoscop ID Zeiss, F.R.G.), using a piezostepper (PM 20 N, Frankenberger, 8034 Germering, F.R.G.) mounted on a Leitz micromanipulator (Leitz, Wetzlar, F.R.G.). Measurements were performed on a vibration-damped table. The potential differences were recorded on a chart recorder (Linseis, Selb., F.R.G.). To determine the resistance of the microelectrodes before, during and after micropuncture, square wave pulses up to 50 pA were injected by a stimulator (Grass Instr., U.S.A.) and the voltage deflection was used to calculate the respective resistance. Experimental maneuvers were performed only, if the impalements resulted in rapid establishment of PD readings above -40 mV, stable (± 2 mV) for at least 30 s, and if electrode resistance and tip potential were similar (± 2 mV, ± 10 MΩ) before and after intracellular recording.

The transference number for potassium (tk = slope potassium conductance/slope cell membrane conductance) was calculated from (Ref. 14):

$$tk = (dPD/61.5\ mV)\ lg\ 5.4/20$$

where dPD is the depolarization following increase of extracellular potassium concentration from 5.4 to 20 mmol/l.

The data are given as arithmetic means $\pm$ standard error (S.E.). Statistical analysis was made by the paired t-test, where applicable. Statistically significant differences were assumed at $P < 0.05$.

Results

During control conditions, the potential difference across the cell membrane (PD) averages -48.9 ± 1.0 mV ($n = 51$). Impalement leads to a reversible increase of microelectrode input resistance by 56.8 ± 3.1 MΩ ($n = 51$).

1 μmol/l acetylcholine leads to a sustained but reversible hyperpolarization of the cell membrane

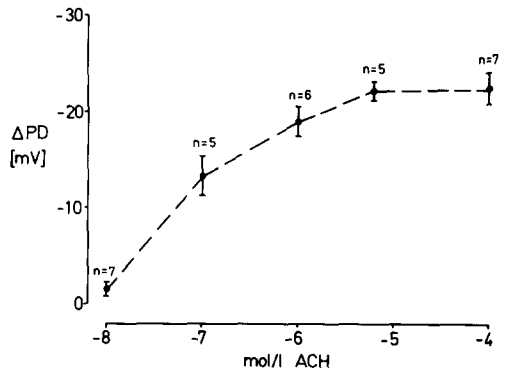

Fig. 1. Effect of 10 nmol/l, 100 nmol/l, 1 μmol/l, 10 μmol/l and 100 μmol/l acetylcholine (ACH) on the potential difference (ΔPD) across the cell membrane (mean values ± S.E., n = number of cells tested).

by -17.9 ± 0.7 mV ($n = 51$, Figs. 2, 3). The concentration needed to elicit half-maximal hyperpolarization is some 100 nmol/l (Fig. 1). During the acetylcholine induced hyperpolarization the input resistance decreases by 11.4 ± 1.6 MΩ ($n = 51$). Shortly following removal of acetylcholine, input resistance increases significantly and approaches a value 8.8 ± 1.9 MΩ ($n = 51$) greater than before application of the hormone.

1 μmol/l atropine does not significantly alter the potential difference across the cell membrane ($+0.7 \pm 0.9$ mV, $n = 6$, Fig. 2), but virtually abolishes the hyperpolarizing effect of acetylcholine ($+0.3 \pm 0.8$ mV, $n = 6$). The effect of atropine is reversible (Fig. 2).

Increase of extracellular potassium concentration from 5.4 mmol/l to 20 mmol/l depolarizes the cell membrane by $+12.1 \pm 1.1$ mV ($n = 12$, Fig. 3). Thus, an apparent transference number for potassium (tk = potassium conductance over cell membrane conductance) is calculated approaching 0.35 ± 0.03 ($n = 12$). In the presence of acetylcholine, increase of extracellular potassium concentration from 5.4 to 20 mmol/l depolarizes the cell membrane by $+25.7 \pm 0.9$ mV ($n = 12$). Thus, apparent tk is increased to 0.73 ± 0.03 ($n = 12$).

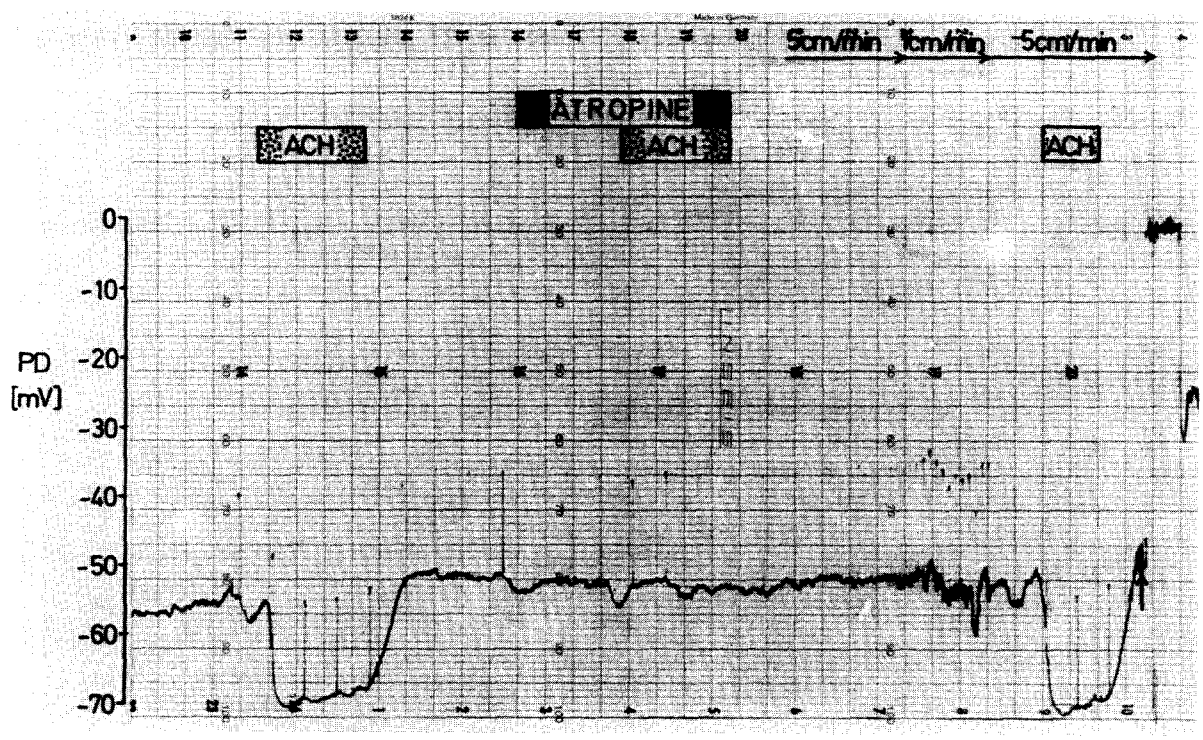

Fig. 2. Effect of 1 μmol/l acetylcholine on the potential difference (PD) across th cell membrane both, in the absence and presence of 1 μmol/l atropine (original tracing). (Arrow indicates withdrawal of microelectrode.)

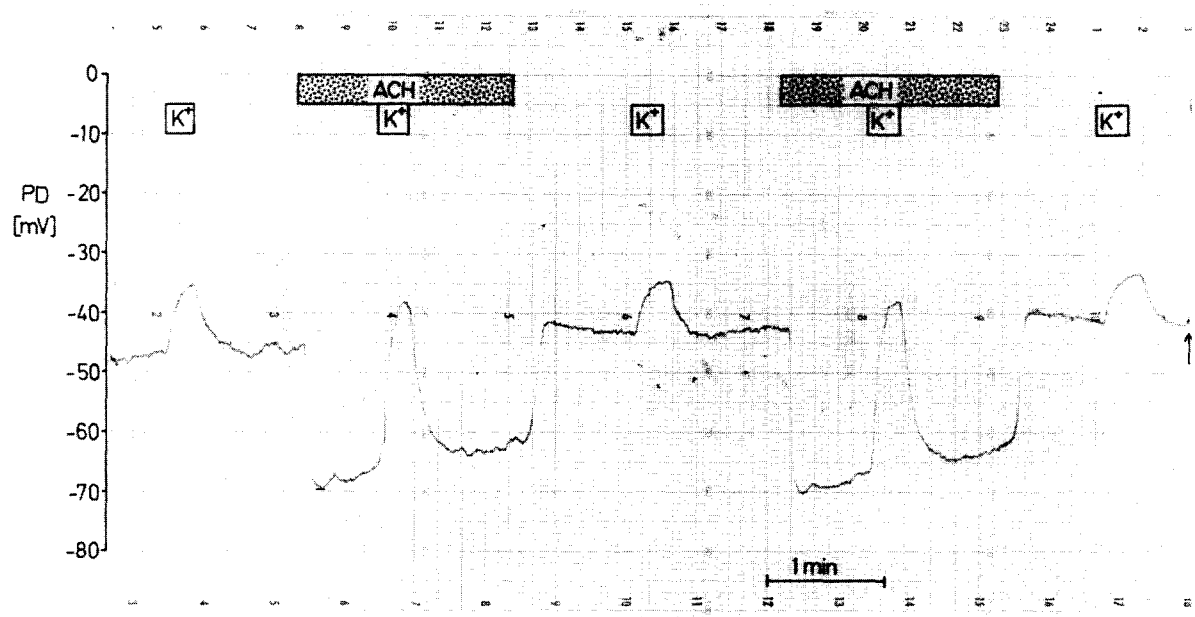

Fig. 3. Effect of increasing extracellular potassium concentration on the potential difference across the cell membrane (PD) both, in the absence and presence of 1 μmol/l acetylcholine (original tracing). (Arrow indicates withdrawal of microelectrode.)

Increase of extracellular potassium concentration to 40 mmol/l depolarizes the cell membrane to -23.4 ± 0.8 mV ($n = 7$, Fig. 4). Subsequent application of acetylcholine does not significantly alter PD (-0.6 ± 0.7 mV) but reduces input resistance by 17 ± 4 MΩ ($n = 7$). Fig. 5 shows the

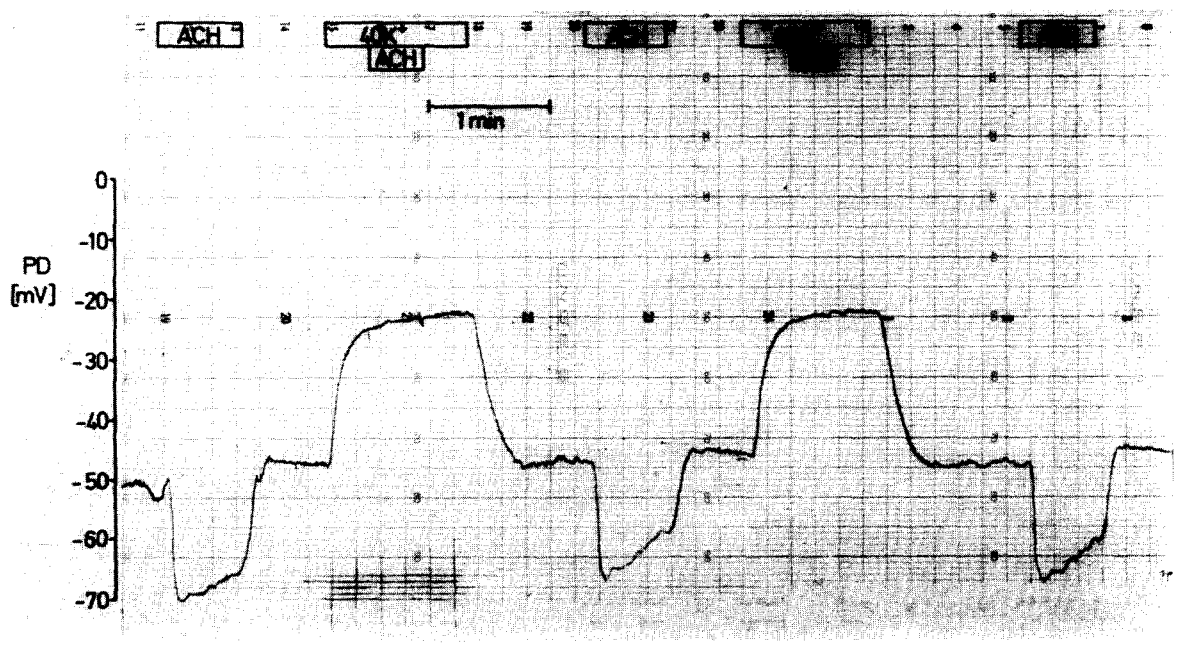

Fig. 4. Effect on the potential difference across the cell membrane (PD) of increasing extracellular potassium concentration to 40 mmol/l and of 1 mmol/l acetylcholine in the presence of 40 mmol/l extracellular potassium concentration (original tracing).

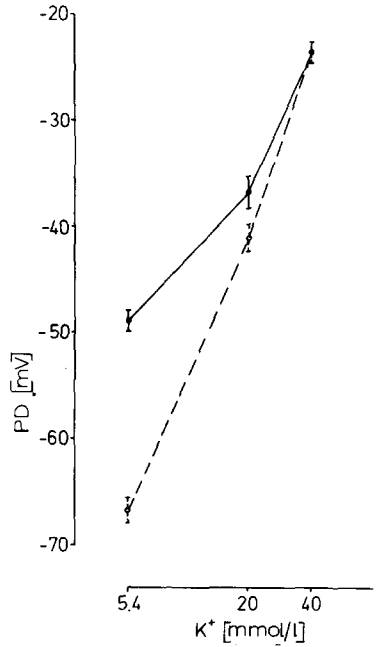

Fig. 5. Influence of extracellular potassium concentration ($[K^+]$) on the potential difference across the cell membrane (PD) both, in the presence (open symbols) and absence (closed symbols) of 1 μmol/l acetylcholine (arithmetic means ± S.E.M.).

dependence of PD on extracellular potassium concentration both in the absence and presence of acetylcholine.

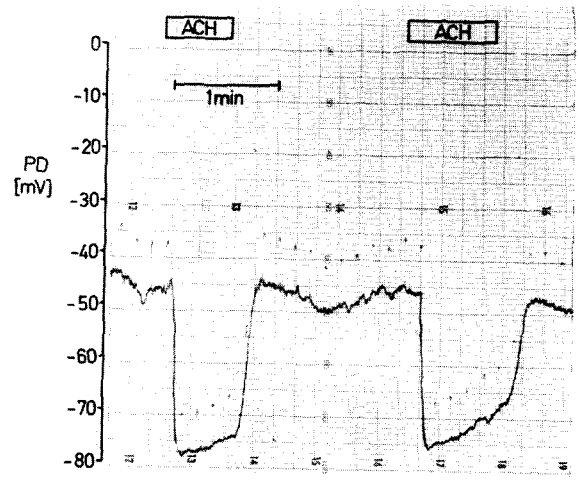

Fig. 6. Effect of 1 μmol/l acetylcholine on the potential difference across the cell membrane (PD) at low extracellular chloride concentration.

If extracellular NaCl is replaced by sodium-gluconate, acetylcholine still elicits a marked hyperpolarization by -21.9 ± 1.9 mV, $n = 7$ (Fig. 6).

Removal of extracellular calcium and addition of 1 mmol/l EDTA lead to a depolarization of the cell membrane by $+24.8 \pm 1.9$ mV within 100 s ($n = 11$). Acetylcholine applied at this point leads to a variable, transient hyperpolarization by -7.2

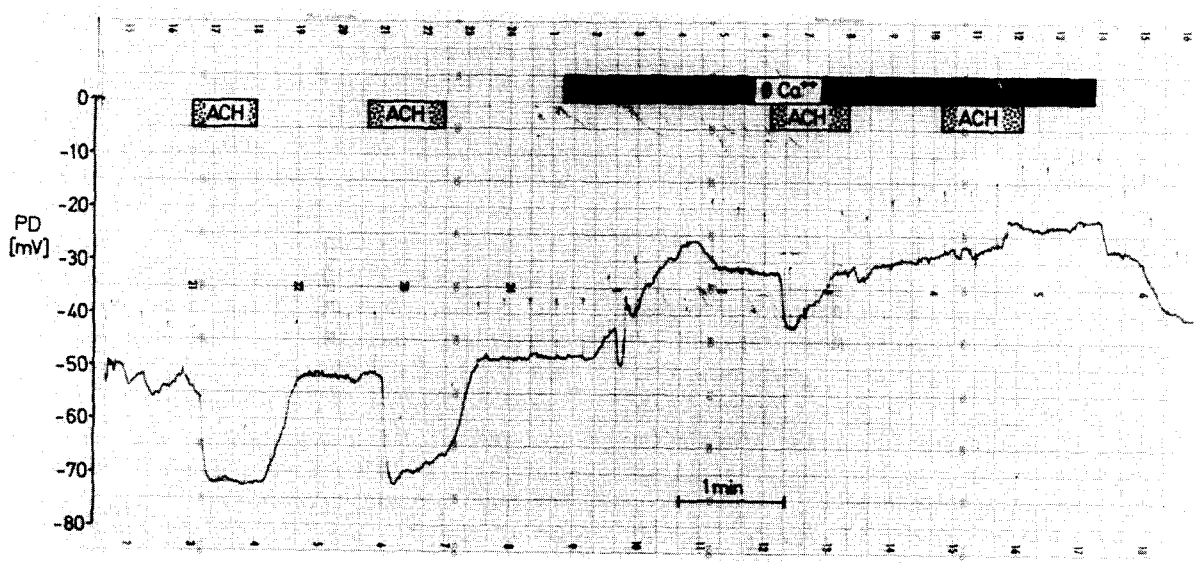

Fig. 7. Effect of 1 μmol/l acetylcholine on the potential difference (PD) across the cell membrane in the nominal absence of extracellular calcium and presence of 1 mmol/l EDTA (original tracing).

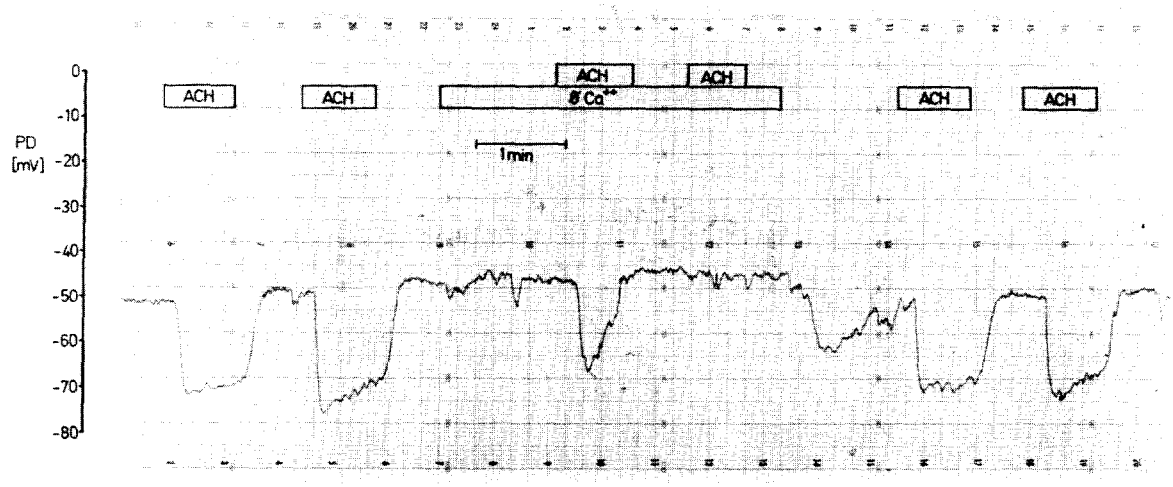

Fig. 8. Effect of 1 μmol/l acetylcholine on the potential difference (PD) across the cell membrane in the nominal absence of extracellular calcium.

± 1.5 mV ($n = 11$, Fig. 7), which, however, cannot be elicited in every cell. A second application of acetylcholine in the nominal absence of extracellular calcium and presence of 1 mmol/l EDTA does not significantly alter the potential difference across the cell membrane (+0.8 ± 0.4 mV, $n = 7$). Readdition of extracellular calcium, however, restores the effect of acetylcholine after some delay. If calcium is omitted without addition of EDTA, the cell membrane depolarizes by +16.2 ± 3.2 mV, $n = 9$ (Fig. 8) within 80 s. In the nominal absence of calcium, the hyperpolarizing effect of acetylcholine (−13.8 ± 1.8 mV, $n = 9$) is again transient and almost abolished at second application of the hormone (−1.1 ± 0.3 mV, $n = 9$) in continued absence of extracellular calcium.

Discussion

Previous observations in our laboratory revealed the sensitivity of cell membrane potential of MDCK cells to the hormones epinephrine [31] and bradykinin [33]. The hormones hyperpolarize the cell membrane of MDCK cells by stimulation of potassium conductance. Potassium channels in MDCK cells proved calcium sensitive in tracer studies [4], conventional electrophysiology [32] and patch-clamp studies [18]. On the other hand, the effect of the hormones proved dependent on the presence of calcium: If extracellular calcium was removed, the effect of either hormone was transient and could be elicited only once. We have thus concluded that the epinephrine induced sustained hyperpolarization was largely dependent on extracellular calcium but that at least part of the initial hyperpolarization of bradykinin and epinephrine was elicited by recruitment of calcium from intracellular stores.

The present study illustrates that MDCK cells are similarly sensitive to acetylcholine and that the effect of this hormone is similarly dependent on extracellular calcium.

Acetylcholine has been shown to activate potassium channels in other epithelia such as lacrimal glands [10,15,16,34,55], pancreas acinar cells [24,35] and parotid salivary gland [37]. The effect of acetylcholine on either potassium conductance or enzyme secretion was found to depend on calcium [22,47,49], to be paralleled by increase of intracellular calcium [26,29,36], to be mimicked by intracellular injection of calcium [15], and to be blunted by intracellular Ca-chelators [9]. It has been shown that calcium is recruited from both intracellular stores and extracellular fluid [6,8]. The origin of intracellular calcium is believed to be rough endoplasmic reticulum [5,39]. In a number of epithelia acetylcholine has been shown to stimulate chloride secretion in part by activation of chloride channels [11,33,28,44,45]. Any stimulation of chloride channels in our preparation may

be masked by the marked stimulation of potassium channels. In case of epinephrine, the effects can be dissociated, since stimulation of chloride conductance is mediated by β-receptors [19]. Accordingly, the application of isoproterenol leads to a shallow depolarization and an increase of chloride selectivity of the cell membrane [19], whereas following application of epinephrine stimulation of potassium channels by far outcasts the stimulation of chloride channels and the cell membrane approaches the equilibrium potential for potassium [31]. The efficacy of acetylcholine at low extracellular chloride concentration indicates that chloride movements do not substantially contribute to the hyperpolarizing effect of acetylcholine.

Nothing is known about direct effects of acetylcholine on renal tubular electrolyte transport. In vivo, infusion of acetylcholine leads to marked natriuresis, an effect attributed mainly to the vasodilatory action of acetylcholine [2,53]. In toad urinary bladder, cholinergic agents reduce net transepithelial sodium transport, an effect seemingly not related to altered potassium conductance [46,48,58]. The present observation may stimulate studies on the effect of the hormone on isolated nephron segments.

In conclusion, acetylcholine hyperpolarizes subconfluent MDCK cells by activation of potassium channels. This effect depends on calcium.

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References

1 Aiton, J.F., Brown, C.D.A., Ogden, P. and Simmons, N.L. (1982) J. Membr. Biol. 65, 99–109.
2 Baer, P.G., Navar, L.G. and Guyton, A.C. (1970) Am. J. Physiol. 219, 619–625.
3 Brown, C.D.A. and Simmons, N.L. (1981) Biochim. Biophys. Acta 649, 427–435.
4 Brown, C.D.A. and Simmons, N.L. (1982) Biochim. Biophys. Acta 690, 95–105.
5 Brown, G.R., Richardson, A.E. and Dormer, R.L. (1987) Biochim. Biophys. Acta 902, 87–92.
6 Case, R.M. and Clausen, T. (1973) J. Physiol. 235, 75–102.
7 Cereijido, M., Ehrenfeld, J., Meza, I. and Martinez-Palomo, A. (1980) J. Membr. Biol. 52, 147–159.
8 Dormer, R.L., Poulsen, J.H., Licko, V. and Williams, J.A. (1981) Am. J. Physiol. 240, G38–G49.
9 Dormer, R.L. (1984) Biochem. Biophys. Res. Commun. 119, 876–883.
10 Findlay, I. (1984) J. Physiol. 350, 179–195.
11 Findlay, I. and Petersen, O.H. (1985) Pflügers Arch. 403, 328–330.
12 Gstraunthaler, G., Pfaller, W. and Kotanko, P. (1985) Am. J. Physiol. 248, F536–F544.
13 Handler, J.S., Perkins, F.M. and Johnson, J.P. (1980) Am. J. Physiol. 238, F1–F9.
14 Helman, S.I. and Thompson, S.M. (1982) Am. J. Physiol. 243, F519–F531.
15 Iwatsuki, N. and Petersen, O.H. (1978) Pflügers Arch. 377, 185–187.
16 Iwatsuki, N. and Petersen, O.H. (1978) J. Physiol. 275, 507–520.
17 Kolb, H.A., Brown, C.D.A. and Murer, H. (1985) Pflügers Arch. 403, 262–265.
18 Kolb, A.-H., Paulmichl, M. and Lang, F. (1987) Pflügers Arch. 408, 584–591.
19 Lang, F., Defregger, M. and Paulmichl, M. (1986) Pflügers Arch. 407, 158–162.
20 Lever, J.E. (1979) J. Supramol. Struct. 12, 259–272.
21 Madin, S.H. and Darby, N.B. (1958) American Type Culture Collection catalogue of strains 2, 574–576.
22 Martinez, J.R. and Quissell, D.O. (1976) J. Pharmacol. Exp. Ther. 199, 518–525.
23 Martinez, J.R. and Cassity, N. (1985) Pflügers Arch. 403, 50–54.
24 Maruyama, Y., Petersen, O.H., Flanagan, P. and Pearson, G.T. (1983) Nature 305, 228–232.
25 McRoberts, J.A., Erlinger, S., Rindler, M.J. and Saier, M.H. Jr. (1982) J. Biol. Chem. 257, 2260–2266.
26 Merritt, J.E. and Rubin, R.P. (1985) Biochem. J. 230, 151–159.
27 Misfeldt, D.S., Hamamoto, S.T. and Pitelka, D.R. (1976) Proc. Natl. Acad. Sci. USA 73, 1212–1216.
28 Nauntofte, B. and Poulsen, J.H. (1986) Am. J. Physiol. 251, C175–C185.
29 Ochs, D.L., Korenbrot, J.I. and Williams, J.A. (1983) Biochem. Biophys. Res. Commun. 117, 122–128.
30 Paulmichl, M., Gstraunthaler, G. and Lang, F. (1985) Pflügers Arch. 405, 102–107.
31 Paulmichl, M., Defregger, M. and Lang, F. (1986) Pflügers Arch. 406, 367–371.
32 Paulmichl, M., Friedrich, F. and Lang, F. (1986) Pflügers Arch. 407, 258–263.

33 Paulmichl, M., Friedrich, F. and Lang, F. (1987) Pflügers Arch. 408, 408–413.
34 Pearson, G.T. and Peterson, O.H. (1984) Pflügers Arch. 400, 51–59.
35 Petersen, O.H. (1982) Biochim. Biophys. Acta 694, 163–184.
36 Powers, R.E., Johnson, P.C., Houlihan, M.J., Saluja, A.K. and Steer, M.L. (1985) Am. J. Physiol. 248, C535–C541.
37 Putney, J.W.Jr. (1983) Cell Calcium 4, 439–449.
38 Richardson, J.C.W., Scalera, V. and Simmons, N.L. (1981) Biochim. Biophys. Acta 673, 26–36.
39 Richardson, A.E. and Dormer, R.L. (1984) Biochem. J. 219, 679–685.
40 Rindler, M.J., Chuman, L.M., Shaffer, L. and Saier, M.H.Jr. (1979) J. Cell. Biol. 81, 635–648.
41 Rindler, M.J., Taub, M. and Saier, M.H.Jr. (1979) J. Biol. Chem. 254, 11431–11439.
42 Rindler, M.J. and Saier, M.H.Jr. (1981) J. Biol. Chem. 256, 10820–10825.
43 Saier, M.H.Jr. and Boyden, D.A. (1984) Mol. Cell. Biochem. 59, 11–32.
44 Saito, Y., Ozawa, T., Hayashi, H. and Nishiyama, A. (1985) Pflügers Arch. 405, 108–111.
45 Saito, Y., Ozawa, T., Hayashi, H. and Nishiyama, A. (1987) Pflügers Arch. 409, 280–288.
46 Sahib, M.K., Schwartz, J.H. and Handler, J.S. (1978) Am. J. Physiol. 235, F586–F591.
47 Scheele, G. and Haymovits, A. (1979) J. Biol. Chem. 254, 10346–10353.
48 Schilb, T.P. (1969) Am. J. Physiol. 216, 514–520.
49 Schulz, I. (1980) Am. J. Physiol. 239, G335–G347.
50 Simmons, N.L. (1981) Biochim. Biophys. Acta 646, 231–242.
51 Simmons, N.L. (1981) Biochim. Biophys. Acta 646, 243–250.
52 Simmons, N.L. (1982) Gen. Pharmacol. 13, 287–291.
53 Stein, J.H., Reineck, J.H., Osgood, R.W. and Ferris, T.F. (1971) Am. J. Physiol. 220, 227–232.
54 Taub, M., Chuman, L., Saier, M.H.Jr. and Sato, G. (1979) Proc. Natl. Acad. Sci. USA 76, 3338–3342.
55 Trautmann, A. and Marty, A. (1984) Proc. Natl. Acad. Sci. USA 81, 611–615.
56 Valentich, J.D., Tchao, R. and Leighton, J. (1979) J. Cell. Physiol. 100, 291–304.
57 Valentich, J.D. (1981) Ann. N.Y. Acad. Sci. 372, 384–405.
58 Wiesmann, W., Sinha, S., Yates, J. and Klahr, S. (1978) Am. J. Physiol. 235, F564–F569.