*Biochimica et Biophysica Acta*, 943 (1988) 471–476
Elsevier



BBA 74122

# Effects of extracellular nucleotides on electrical properties of subconfluent Madin Darby canine kidney cells

F. Lang [a], B. Plöckinger [a], D. Häussinger [b], M. Paulmichl [a]

[a] *Institute for Physiology, University of Innsbruck, Innsbruck (Austria) and* [b] *Medizinische Klinik, Universität Freiburg, Freiburg (F.R.G.)*

(Received 10 May 1988)

Key words: Nucleotide; Membrane potential; Microelectrode; Potassium conductance; (MDCK cell); (Dog kidney)

**ATP and ADP but not AMP lead to sustained hyperpolarization of Madin Darby canine kidney (MDCK) cells. The present study has been performed to test for an influence of other nucleotides on the potential difference across the cell membrane (PD) in subconfluent MDCK cells. PD has been continuously monitored with conventional microelectrodes during rapid exchange of extracellular fluid. Application of 1 μmol/l UTP leads to a rapid (< 2 s) hyperpolarization of the cell membrane by $-17.0 \pm 0.4$ mV (from $-50.1 \pm 0.6$ mV), a reduction of cell membrane resistance and an increase of the sensitivity of PD to alterations of extracellular potassium. The concentration needed for half maximal effect of UTP is ≈ 0.2 μmol/l. ITP is similarly effective, whereas UDP, GTP and GDP are less effective. Up to 1 mmol/l UMP, GMP, TTP or CTP do not significantly alter PD. In calcium-free extracellular fluid the hyperpolarizing effect of UTP is blunted ($-11.6 \pm 2.3$ mV) and only transient. In conclusion, UTP similar to purine triphosphates hyperpolarizes MDCK cells by increasing the potassium conductance. The activation of potassium channels requires calcium, which is apparently recruited from both intra- and extracellular sources.**

## Introduction

In the past, purines such as ATP have been recognized as transmitters in a variety of tissues [3,4,11,17]. Reports on effects of purines on epithelia, however, are scarce: Extracellular application of ATP has been shown to increase transepithelial potential difference in rat jejunum [26] and to enhance rubidium efflux as well as amylase secretion in parotid acinar cells [15].

In Madin Darby canine kidney (MDCK) cells, a permanent cell line from a dog kidney [16,18,42,43,47,50], transepithelial chloride secretion is stimulated by both, ATP and UTP [45,46], pointing to both purinergic and pyrimidinergic regulation of ion transport. In a separate study, we have shown that ATP leads to a sustained hyperpolarization of the cell membrane in subconfluent MDCK cells by calcium dependent enhancement of potassium conductance [25]. In isolated perfused liver, both, ATP and UTP stimulate potassium and calcium movements across the cell membranes. However, the effects of the two substances differ both quantitatively and qualitatively. Thus it was suggested that the two mediators may exert their effects via different cellular mechanisms [20].

The present study was performed to test for an effect of nucleotides on the cell membrane potential in subconfluent MDCK cells and to possibly disclose differences between their effects.

Correspondence: F. Lang, Institute for Physiology, University of Innsbruck, Fritz-Pregl-Strasse 3, A-6010 Innsbruck, Austria.

## Methods

The techniques employed have been described in previous papers in detail [35]. In short, MDCK cells from the American Type Culture Collection [18,32] 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 $\mu$g/ml streptomycin [49], 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) [46], plated on sterile cover glasses and incubated again in the same medium as above for at least 48 hours. 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): 114 NaCl, 5.4 KCl, 0.8 $MgCl_2$, 1.2 $CaCl_2$, 1.2 $Na_2HPO_4/NaH_2PO_4$ (4 : 1), 20 $NaHCO_3$, 5.5 glucose. The solutions were equilibrated with 5% carbondioxide and 95% air (pH 7.4) and kept at 37°C. Where indicated, KCl was increased to 20 mmol/l replacing equal amounts of NaCl, or calcium omitted. The nominally calcium-free solutions had calcium activities of less than 0.1 $\mu$mol/l, as verified with the use of a calcium selective electrode. UTP, UDP, UMP, ATP, ITP, GTP, GDP, GMP, TTP, CTP (all from Sigma, Munich, F.R.G.) were added at the concentrations, as specified.

Measurements of potential difference across the cell membrane (PD) were made using conventional microelectrodes (tip diameter < 0.5 $\mu$m, input resistance 100–200 M$\Omega$, 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, C.T., 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 and the voltage deflection was used to calculate the respective resistance. Experimental maneuvers were performed only, if the impalement 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 M$\Omega$) before and after intracellular recording. The transference number for potassium (tk = slope potassium conductance/ slope cell membrane conductance) was calculated from [21]:

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

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

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

## Results

In the absence of extracellular nucleotides the potential difference across the cell membrane (PD) approaches $-50.1 \pm 0.6$ mV ($n = 105$). Impalement leads to a reversible increase of microelectrode input resistance by $67.3 \pm 3.8$ M$\Omega$ ($n = 45$).

Application of 1 $\mu$mol/l UTP leads to a rapid (< 2 s), sustained, but fully reversible hyperpolarization of the cell membrane by $-17.0 \pm 0.4$ mV ($n = 87$) and reduces the input resistance by $35.1 \pm 4.9$ M$\Omega$ ($n = 41$). The concentration needed for half-maximal hyperpolarization ($K_{1/2}$) of UTP is approx. 0.2 $\mu$mol/l (Figs. 1, 4). Inosine triphosphate (ITP) is similarly effective (Figs. 2–4), whereas higher concentrations of UDP, GTP, GDP and TTP are required to elicit similar effects (Fig. 4). Up to 1 mmol/l CTP, UMP and GMP do not significantly alter PD ($-1.2 \pm 0.9$ mV, $n = 5$; $0.0 \pm 0.5$ mV, $n = 7$; $0.5 \pm 0.5$ mV, $n = 5$; respectively).

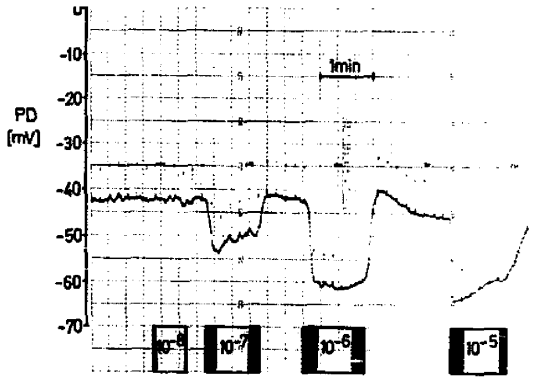


Fig. 1. Effect of 10 nmol/l, 100 nmol/l, 1 μmol/l and 10 μmol/l uridine triphosphate (UTP) on the potential difference (PD) across the cell membrane (original tracing).

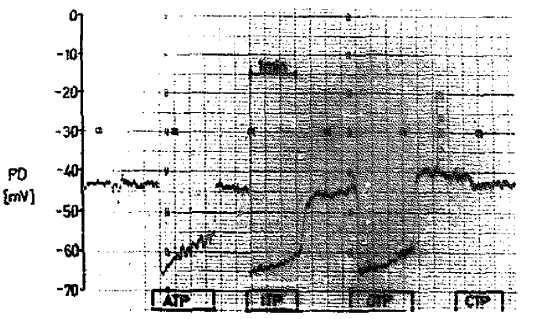


Fig. 3. Effect of 1 μmol/l ATP, ITP, GTP and CTP on the potential difference (PD) across the cell membrane (original tracing).

Increase of extracellular potassium concentration from 5.4 to 20 mmol/l depolarizes the cell membrane by $11.0 \pm 0.8$ mV ($n = 8$), in the absence and by $24.6 \pm 0.5$ mV ($n = 8$) in the presence of UTP (Fig. 5). The values allow calculation of the apparent transference numbers for potassium (tk), i.e. the apparent contribution of potassium conductance to the cell membrane conductance (see Methods). UTP increases tk from 0.31 $\pm$ 0.02 ($n = 8$) to $0.70 \pm 0.01$ ($n = 8$).

Reduction of extracellular calcium to less than 100 nmol/l leads to a depolarization of the cell membrane to $-22.6 \pm 4.3$ mV ($n = 7$). With reduced extracellular calcium, the hyperpolarizing effect of UTP is blunted ($-11.6 \pm 2.3$ mV, $n = 8$) and only transient (Fig. 6).

## Discussion

The present study shows that several nucleotides hyperpolarize the cell membrane of Madin Darby canine kidney (MDCK) cells by increasing the potassium conductance of the cell membrane. The increase of the potassium conductance is calcium dependent and is most likely mediated by increases of intracellular calcium activity.

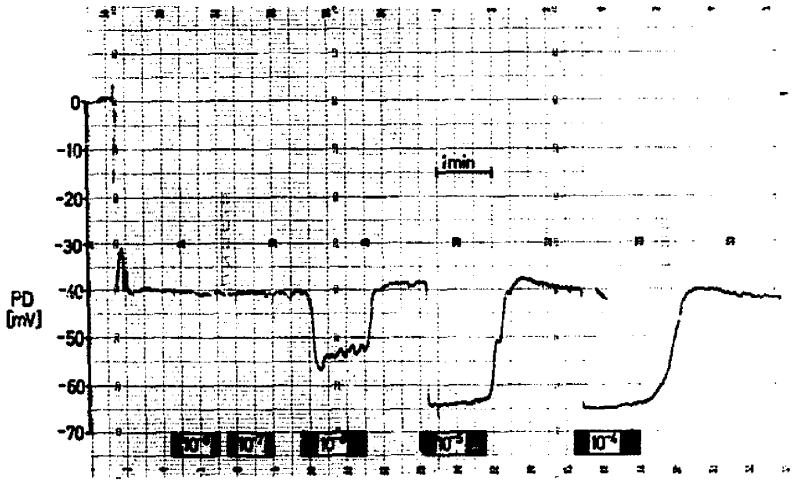


Fig. 2. Effect of 10 nmol/l, 100 nmol/l, 1 μmol/l, 10 μmol/l and 100 μmol/l inosine triphosphate (ITP) on the potential difference (PD) across the cell membrane (original tracing).

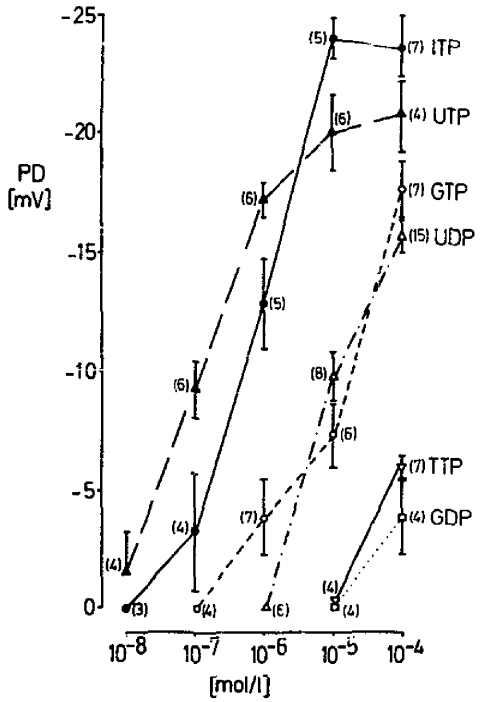


Fig. 4. Dose response curve of the nucleotides: The hyperpolarization of the cell membrane (dPD) following application of UTP, ITP, GTP, UDP and GTP at the respective concentrations (mean values ± S.E.; in parentheses, number of cells tested).

In previous studies, we have shown that potassium channels in subconfluent MDCK cells are activated by increases of intracellular calcium activity [14,27,37] and that a variety of hormones

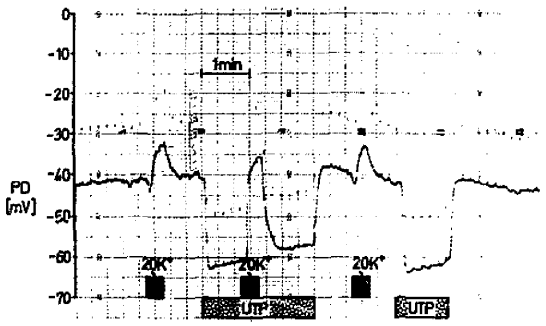


Fig. 5. Effect of increasing extracellular potassium concentration from 5.4 to 20 mmol/l on the potential difference across the cell membrane (PD) both, in the absence and presence of 1 μmol/l UTP (original tracing).

such as epinephrine [36], bradykinin [38], acetylcholine [29] and serotonin [39] activate the potassium channels at least in part by increasing intracellular calcium activity. ATP, similarly, activates potassium channels. Half-maximal effects are observed at 0.5 μmol/l ATP and 0.9 μmol/l ADP, whereas AMP does not significantly alter cell membrane potential [25]. The effects of UTP, UDP, ITP, GTP and GDP are indistinguishable from the effects of ATP (see Figs. 1–3). This does of course not rule out that the nucleotides exert their effects via different receptors.

Purine receptors have been subdivided in P1 receptors (A1 and A2), which are most sensitive to adenosine and in P2 receptors (P2x and P2y) most

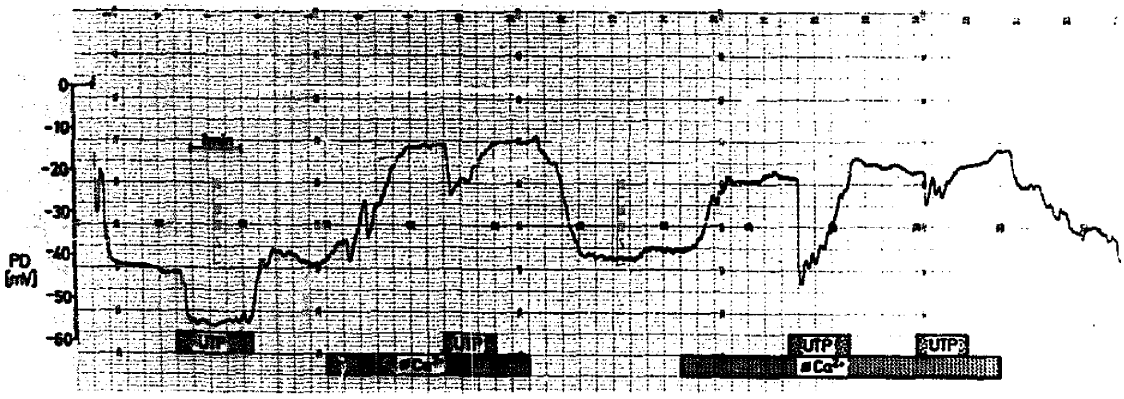


Fig. 6. Effect of 1 μmol/l UTP on the potential difference across the cell membrane (PD) in the nominal absence of extracellular calcium (original tracing).

sensitive to ATP [4,5,6]. Since the effect of ATP cannot be mimicked by adenosine [25], we have concluded that the hyperpolarizing effect of ATP in MDCK-cells is mediated by P2 receptors. In other tissues, two P2 receptor subtypes have been described [5,6,9,22,24,33,41], the one being linked to phospholipase C, the other to adenylate cyclase via an inhibitory guanine nucleotide regulatory protein [34].

In hepatocytes [2,8,23,48], perfused liver [7,20], Ehrlich ascites tumor cells [12,13,28], cardiac myocytes [10], endothelial cells [31], fibroblasts [40,51], thymocytes [40] and kidney tubules [44], ATP has been shown to increase intracellular calcium. In Ehrlich ascites tumor cells UTP, ITP and GTP proved similarly effective as ATP in raising intracellular calcium activity [12]. In those cells, the increase of intracellular calcium was only transient and probably due to release of calcium from intracellular stores. The transient hyperpolarization in the nominal absence of calcium indeed indicates that UTP similar to ATP is able to release intracellular calcium. In the presence of extracellular calcium, however, the hyperpolarizing effect of UTP, UDP, ATP, ADP, ITP, GTP and GDP is sustained, possibly pointing to additional entry of calcium from the extracellular space. An ATP-stimulated calcium channel has indeed been identified most recently in smooth muscle [1].

In conclusion, similar to ATP, other nucleotides such as UTP, UDP, ITP, GTP and GDP hyperpolarize MDCK-cells by activation of potassium channels. The effect depends on calcium. No differences can be observed among the effects of the nucleotides tested.

## Acknowledgements

The authors gratefully acknowledge the valuable discussion with Prof. Dr. P. Deetjen and Dr. A. Jungwirth, the skilled technical assistance of G. Siber, A. Grundnig and S. David, and the excellent mechanic and electronic support by K.H. Streicher and Ing. M. Hirsch. The study has been supported by the Fonds zur Förderung der wissenschaftlichen Forschung, Grant No. P5813 and P6792M.

## References

1 Benham, C.D. and Tsien, R.W. (1987) Nature 328, 275–278.
2 Burgess, G.M., Claret, M. and Jenkinson, D.H. (1981) J. Physiol. 317, 67–90.
3 Burnstock, G. (1976) J. Theor. Biol. 62, 491–503.
4 Burnstock, G. (1981) J. Physiol. 313, 1–35.
5 Burnstock, G. and Kennedy, C. (1985) Gen. Pharmacol. 16, 433–440.
6 Burnstock, G. and Warland, J.J.I. (1987) Br. J. Pharmacol. 90, 383–391.
7 Buxton, D.B., Robertson, S.M. and Olson, M.S. (1986) Biochem. J. 237, 773–780.
8 Charest, R., Blackmore, P.F. and Exton, J.H. (1985) J. Biol. Chem. 260, 15789–15794.
9 Cusack, N.J., Hourani, S.M.O., Loizou, G.D. and Welford, L.A. (1987) Br. J. Pharmacol. 90, 791–795.
10 De Young, M.B. and Scarpa, A. (1987) FEBS Lett. 223, 53–58.
11 Drury, A.N. and Szent-Györgyi, A. (1929) J. Physiol. 68, 213–237.
12 Dubyak, G.R. and De Young, M.B. (1985) J. Biol. Chem. 260, 10653–10661.
13 Dubyak, G.R. (1986) Arch. Biochem. Biophys. 245, 84–95.
14 Friedrich, F., Weiss, H., Paulmichl, M. and Lang, F. (1988) Pflügers Arch. 411, R70, 128.
15 Gallacher, D.V. (1982) Nature 296, 83–86.
16 Gaush, C.R., Hard, W.L. and Smith, T.F. (1966) Proc. Soc. Exp. Biol. Med. 122, 931–935.
17 Gordon, J.L. (1986) Biochem. J. 233, 309–319.
18 Gstraunthaler, G. (1988) Renal Physiol. 11, 1–32.
19 Handler, J.S., Perkins, F.M. and Johnson, J.P. (1980) Am. J. Physiol. 238, F1–F9.
20 Häussinger, D., Stehle, T. and Gerok, W. (1987) Eur. J. Biochem. 167, 65–71.
21 Helman, S.I. and Thompson, S.M. (1982) Am. J. Physiol. 243, F519–F531.
22 Hopwood, A.M. and Burnstock, G. (1987) Eur. J. Pharmacol. 136, 49–54.
23 Horstman, D.A., Tennes, K.A. and Putney, J.W., Jr. (1986) FEBS Lett. 204, 189–192.
24 Houston, D.A., Burnstock, G. and Vanhoutte, P.M. (1987) J. Pharmacol. Exp. Ther. 241, 501–506.
25 Jungwirth, A., Paulmichl, M. and Lang, F. (1988) Pflügers Arch. 411, R78, 145.
26 Kohn, P.G., Newey, H. and Smyth, D.H. (1970) J. Physiol. 208, 203–220.
27 Kolb, H.-A., Paulmichl, M. and Lang, F. (1987) Pflügers Arch. 408, 584–591.
28 Landry, Y. and Lehninger, A.L. (1976) Biochem. J. 158, 427–438.
29 Lang, F., Klotz, L. and Paulmichl, M. (1988) Biochim. Biophys. Acta 941, 217–224.
30 Lin, J., Krishnaraj, R. and Kemp, R.G. (1983) Fed. Proc. 42, 723.
31 Lückhoff, A. and Busse, R. (1986) J. Cell. Physiol. 126, 414–420.

32 Madin, S.H. and Darby, N.B. (1958) as catalogued in: American Type Culture Collection Catalogue of Strains 2, 574–576.
33 Needham, L., Cusack, N.J., Pearson, J.D. and Gordon, J.L. (1987) Eur. J. Pharmacol. 134, 199–209.
34 Okajima, F., Tokumitsu, Y., Kondo, Y. and Ui, M. (1987) J. Biol. Chem. 262, 13483–13490.
35 Paulmichl, M., Gstraunthaler, G. and Lang, F. (1985) Pflügers Arch. 405, 102–107.
36 Paulmichl, M., Defregger, M. and Lang, F. (1986) Pflügers Arch. 406, 367–371.
37 Paulmichl, M., Friedrich, F. and Lang, F .(1986) Pflügers Arch. 407, 258–263.
38 Paulmichl, M., Friedrich, F. and Lang, F. (1987) Pflügers Arch. 408, 408–413.
39 Paulmichl, M., Friedrich, F., Wöll, E., Weiss, H. and Lang, F. (1988) Pflügers Arch. 411, 394–400.
40 Perdue, J.F. (1971) J. Biol. Chem. 246, 6750–6759.
41 Reilly, W.M. and Burnstock, G. (1987) Eur. J. Pharmacol. 138, 319–325.
42 Richardson, J.C.W., Scalera, V. and Simmons, N.L. (1981) Biochim. Biophys. Acta 673, 26–36.
43 Rindler, M.J., Chuman, L.M., Shaffer, L. and Saier, M.H., Jr. (1979) J. Cell. Biol. 81, 635–648.
44 Rorive, G. and Kleinzeller, A. (1972) Biochim. Biophys. Acta 274, 226–239.
45 Simmons, N.L. (1981) Biochim. Biophys. Acta 646, 231–242.
46 Simmons, N.L. (1981) Biochim. Biophys. Acta 646, 243–250.
47 Simmons, N.L. (1982) J. Gen. Pharmacol. 13, 287–291.
48 Staddon, J.M. and McGivan, J.D. (1985) Eur. J. Biochem. 151, 567–572.
49 Taub, M., Chuman, L., Saier, M.H., Jr. and Sato, G. (1979) Proc. Nat. Acad. Sci. USA 76, 3338–3342.
50 Valentich, J.D. (1981) Ann. N.Y. Acad. Sci. 372, 384–405.
51 Weisman, G.A., De, B.K., Friedberg, I., Pritchard, R.S. and Heppel, L.A. (1984) J. Cell. Physiol. 119, 211–219.