Journal of Physiology (1989), **408**, *pp.* 333–343
With 7 text-figures
Printed in Great Britain

EFFECT OF EXTRACELLULAR ADENOSINE TRIPHOSPHATE ON ELECTRICAL PROPERTIES OF SUBCONFLUENT MADIN–DARBY CANINE KIDNEY CELLS

BY A. JUNGWIRTH, F. LANG AND M. PAULMICHL
From the Institute for Physiology, University of Innsbruck, Austria

(*Received 29 February 1988*)

SUMMARY

1. The present study has been performed to test for an influence of extracellular ATP on the potential differences across the cell membrane (PD) in subconfluent MDCK cells utilizing conventional microelectrodes.

2. In the absence of ATP, the mean measured PD was $-47{\cdot}5 \pm 0{\cdot}3$ mV ($\pm$S.E.M., $n = 320$). Application of 10 μmol/l ATP leads to rapid (< 2 s) hyperpolarization of the cell membrane by $-18{\cdot}5 \pm 0{\cdot}4$ mV ($n = 221$), reduction of input resistance by 14 ± 1 MΩ ($n = 106$) and increase of the sensitivity of PD to alterations of extracellular potassium.

3. The concentration needed for half-maximal effect ($K_{\frac{1}{2}}$) of ATP is $\approx 0{\cdot}5$ μmol/l. ATP-γ-S ($K_{\frac{1}{2}} \approx 0{\cdot}4$ μmol/l) and ADP ($K_{\frac{1}{2}} \approx 0{\cdot}9$ μmol/l) are similarly effective, whereas up to 1 mmol/l AMP or adenosine does not significantly alter PD. Application of 10 μmol/l theophylline, 1 μmol/l phentolamine and 10 μmol/l indomethacin does not blunt the hyperpolarizing effect of ATP.

4. The ATP-induced hyperpolarization is completely abolished in the presence of 1 mmol/l quinidine but only incompletely by 0·1 mmol/l quinidine or 1 mmol/l barium. In calcium-free extracellular fluid (1 mmol/l EDTA added) PD is $-18{\cdot}5 \pm 1{\cdot}7$ mV ($n = 18$). With reduced extracellular calcium, the hyperpolarizing effect of ATP is blunted ($-12{\cdot}3 \pm 1{\cdot}6$ mV, $n = 18$) and only transient.

5. In conclusion, ATP hyperpolarizes MDCK cells by increasing the potassium conductance. The activation of potassium channels requires calcium.

INTRODUCTION

Despite the increasing recognition of ATP as a neurotransmitter (Drury & Szent-Györgyi, 1929; Burnstock, 1976, 1981; Gordon, 1986), only little is known about the potential role of extracellular ATP in epithelial transport regulation. In rat jejunum 1 mmol/l ATP has been shown to increase transepithelial potential difference, an effect which has been attributed possibly to altered energy supply (Kohn, Newey & Smyth, 1970); in parotid acinar cells ATP has been shown to increase intracellular calcium activity, to enhance rubidium efflux and to stimulate amylase secretion (Gallacher, 1982; McMillian, Soltoff, Cantley & Talamo, 1987); in Madin–Darby

canine kidney (MDCK) cells ATP has been shown to stimulate chloride secretion (Simmons, 1981*a*).

MDCK cells are a permanent cell line from a dog kidney (Madin & Darby, 1958). If grown to confluency, MDCK cells exhibit transepithelial transport of fluid and solutes (Cereijido, Ehrenfeld, Meza & Martinez-Palomo, 1980; Simmons, 1981*a*, 1982). Transport systems at the apical cell membrane include sodium–hydrogen ion exchange (Rindler, Taub & Saier, 1979; Rindler & Saier, 1981) and chloride conductance (Kolb, Brown & Murer, 1985), transport systems at the basolateral cell membrane, potassium conductance (Aiton, Brown, Ogden & Simmons, 1982; Brown & Simmons, 1982), NaCl–KCl co-transport (Aiton, Chipperfield, Lamb, Ogden & Simmons, 1981) and sodium, potassium-ATPase (Cereijido *et al.* 1980; Aiton *et al.* 1982). The same transport systems are expressed in subconfluent MDCK cells (Paulmichl, Gstraunthaler & Lang, 1985; Lang, Defregger & Paulmichl, 1986*a*; Paulmichl, Friedrich & Lang, 1986*b*).

The present study was performed to test for an effect of ATP on the cell membrane potential in subconfluent MDCK cells and possibly to identify the ionic mechanism of stimulated chloride transport in MDCK cells.

METHODS

The techniques employed have been described in previous papers in detail (Paulmichl *et al.* 1985). In short, MDCK cells from the American Type Culture Collection (Madin & Darby, 1958; Gstraunthaler, 1988) 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, equilibrated with 95% humidified air and 5% carbon dioxide at 37 °C. After growth to confluency monolayers were dispersed by incubation in a calcium- and magnesium-free, trypsin–EDTA-containing balanced salt solution (pH 7·4), 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 which allowed 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, at pH 7·4. The solutions were equilibrated with 5% carbon dioxide and 95% air (pH 7·4) and kept at 37 °C. Where indicated, KCl was increased to 10, 20 or 40 mmol/l replacing equal amounts of NaCl, or calcium omitted and 1 mmol/l EDTA added. These latter solutions had calcium activities of less than 1 nmol/l. α-Adenosine, AMP, ADP, ATP, theophylline, indomethacin, quinidine, phentolamine and ouabain (all from Sigma, Munich, FRG) and ATP-γ-S (Fluka, Buchs, Switzerland) were added at the concentrations specified.

Measurements of potential difference across the cell membrane (PD) were made using conventional microelectrodes (tip diameter $< 0{\cdot}5$ 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, FRG) and connected with a high-input-impedance electrometer (FD 223, WPI, Hamden, CT, USA). Measurements were made using an Ag–AgCl reference 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, FRG), using a piezostepper (PM 20 N, Frankenberger, 8034 Germering, FRG) mounted on a Leitz micromanipulator (Leitz, Wetzlar, FRG). Measurements were performed on a vibration-damped table. The potential differences were recorded on a chart recorder (Linseis, Selb, FRG). 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 manoeuvres 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 MΩ) before and after intracellular recording.

The transference number for potassium (tk = slope potassium conductance/slope cell membrane conductance) was calculated from Helman & Thompson (1982):

$$tk = (\mathrm{dPD}/61{\cdot}5\ \mathrm{mV})/\log(5{\cdot}4/20),$$

where dPD is the depolarization following increase of extracellular potassium concentration from 5·4 to 20 mmol/l. This concentration step has been chosen to minimize errors arising from potential-sensitive conductances as discussed in detail previously (Völkl & Lang, 1988).

Data are given as arithmetic means ± standard error of the mean (S.E.M.). Statistical analysis was made by paired t test, where applicable. The number of experiments (n) denotes the number of cells. For each series cells have been studied from at least five culture dishes. Statistically significant differences were assumed at $P < 0{\cdot}05$.

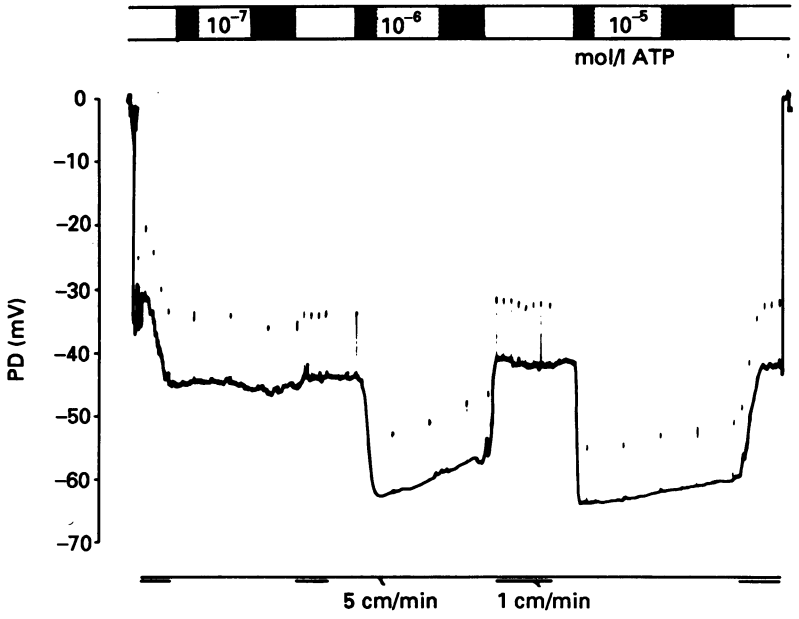

Fig. 1. Effect of 0·1, 1 and 10 μmol/l adenosine triphosphate (ATP) on the potential difference (PD) across the cell membrane (original tracing).

RESULTS

In the absence of ATP the potential difference across the cell membrane (PD) was −47·5 ± 0·3 mV (n = 320). Impalement leads to a reversible increase of microelectrode input resistance by 66 ± 3 MΩ (n = 188).

Application of 10 μmol/l ATP leads to a rapid (< 2 s) hyperpolarization of the cell membrane (Figs 1, 3, 6 and 7) by −18·5 ± 0·4 mV (n = 221) to −65·9 ± 0·4 mV (n = 221) and reduces the input resistance by 14 ± 1 MΩ (n = 106). The concentration needed for half-maximal hyperpolarization ($K_{\frac{1}{2}}$, determined from interpolation) of ATP is ≈ 0·5 μmol/l (Fig. 2). ATP-γ-S ($K_{\frac{1}{2}} \approx 0{\cdot}4$ μmol/l) and ADP ($K_{\frac{1}{2}} \approx 0{\cdot}9$ μmol/l) are similarly effective, whereas up to 1 mmol/l AMP and adenosine do not significantly alter PD (+0·1 ± 0·2 mV, n = 8 and 0·0 ± 0·1 mV, n = 9 respectively, Fig. 2).

Increase of extracellular potassium concentration from 5·4 to 20 or 40 mmol/l depolarizes the cell membrane by $+12{\cdot}4 \pm 0{\cdot}6$ mV ($n = 34$) or $+22{\cdot}5 \pm 1{\cdot}4$ mV ($n = 34$), respectively, in the absence of ATP, and by $+23{\cdot}2 \pm 0{\cdot}5$ mV ($n = 10$) or $+41{\cdot}8 \pm 0{\cdot}6$ mV ($n = 14$), respectively, in the presence of ATP (Fig. 3). 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

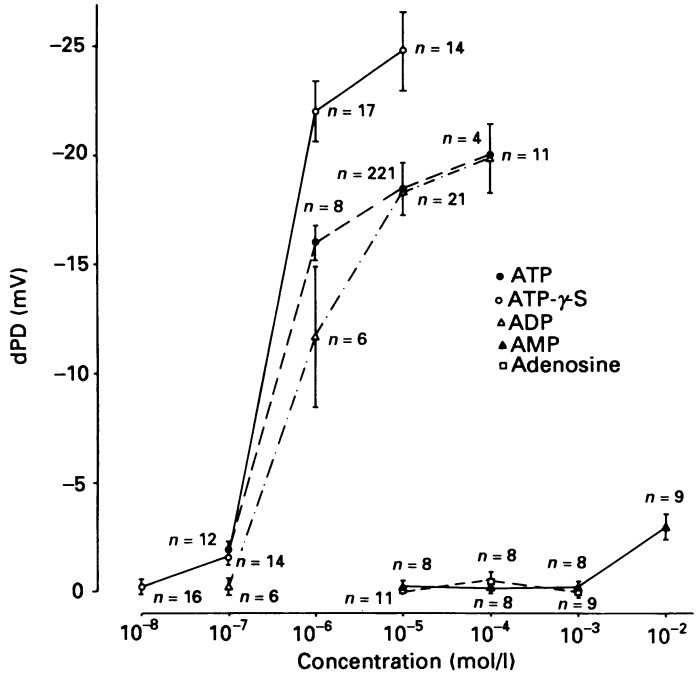

Fig. 2. Dose–response curve: the hyperpolarization of the cell membrane (dPD) following application of ATP-γ-S, ATP, ADP, AMP and adenosine at the respective concentrations (mean values ± S.E.M., n = number of cells tested).

(see Methods). ATP increases *tk* significantly from $0{\cdot}36 \pm 0{\cdot}02$ ($n = 33$) to $0{\cdot}66 \pm 0{\cdot}02$ ($n = 10$). Increase of extracellular potassium concentration from 5·4 to 10 mmol/l depolarizes the cell membrane by $+5{\cdot}0 \pm 0{\cdot}4$ mV ($n = 12$). The presence of barium (1 mmol/l) depolarizes the cell membrane to $-29{\cdot}7 \pm 0{\cdot}9$ mV ($n = 20$, Fig. 4) by a reduction in the potassium conductance: in the presence of barium step increase of extracellular potassium from 5·4 to 10 mmol/l does not significantly alter PD ($-0{\cdot}2 \pm 0{\cdot}3$ mV, $n = 6$), i.e. *tk* is virtually abolished (Fig. 4).

In the presence of 1 mmol/l barium, ATP leads to a transient hyperpolarization to $-60{\cdot}7 \pm 1{\cdot}7$ mV ($n = 20$) followed by a decline to $-39{\cdot}5 \pm 3{\cdot}6$ mV ($n = 5$) after ≈ 2 min (Figs 4 and 5). Despite the reduction in the apparent potassium conductance observed in the presence of barium, ATP may still induce a marked hyperpolarization of the membrane by an increase in the apparent potassium conductance: increase of extracellular potassium concentration from 5·4 to 10 mmol/l leads to a depolarization of the cell membrane by $+7{\cdot}1 \pm 0{\cdot}6$ mV ($n = 10$) during the transient hyper-

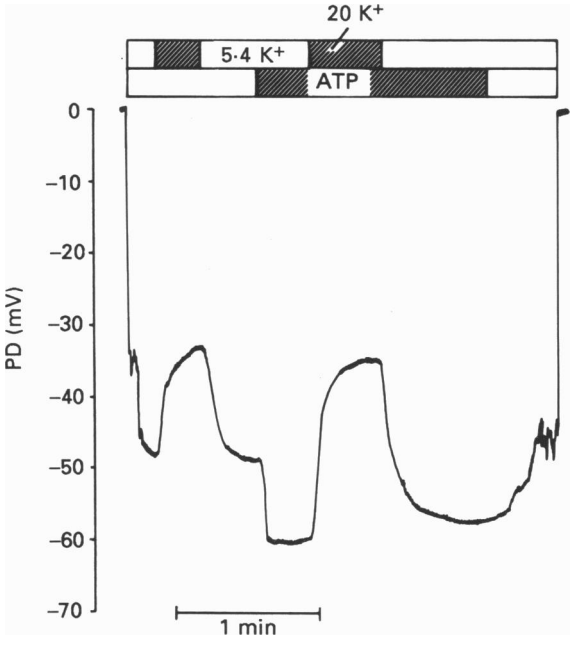

Fig. 3. Effect of increasing extracellular potassium concentration from 5·4 (5·4 K^+) to 20 (20 K^+) mmol/l on the potential difference across the cell membrane (PD) both in the absence and presence of 10 μmol/l ATP (original tracing).

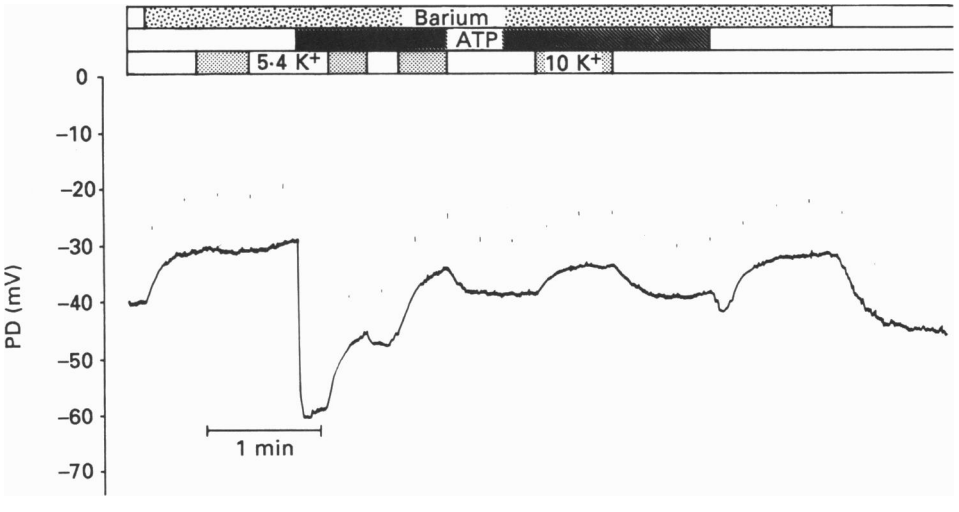

Fig. 4. Effect of 10 μmol/l ATP and of increasing extracellular potassium concentration from 5·4 to 10 mmol/l on the potential difference across the cell membrane (PD) in the presence of 1 mmol/l barium (original tracing).

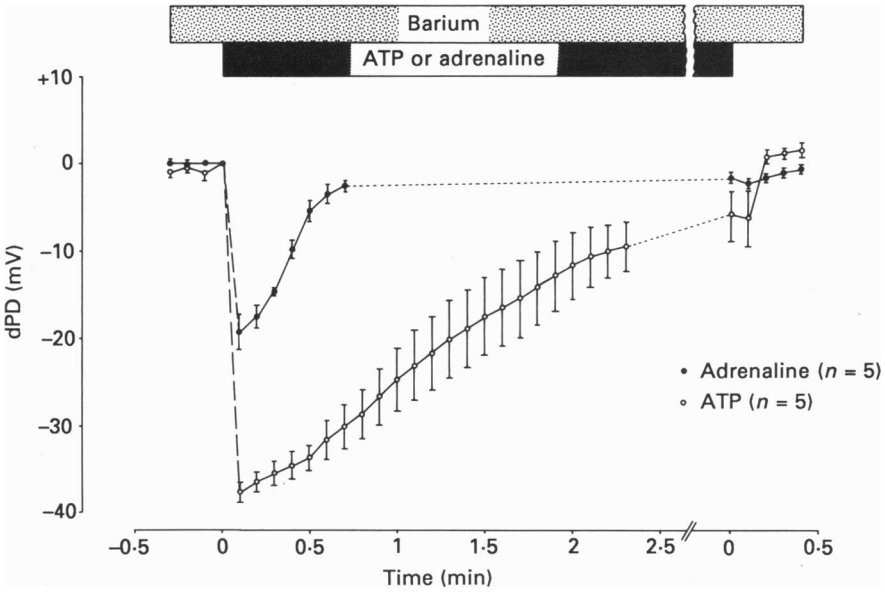

Fig. 5. Effect of 10 μmol/l ATP and of 1 μmol/l adrenaline on the potential difference across the cell membrane (PD) in the presence of 1 mmol/l barium (mean values $\pm$ S.E.M., n = number of cells tested).

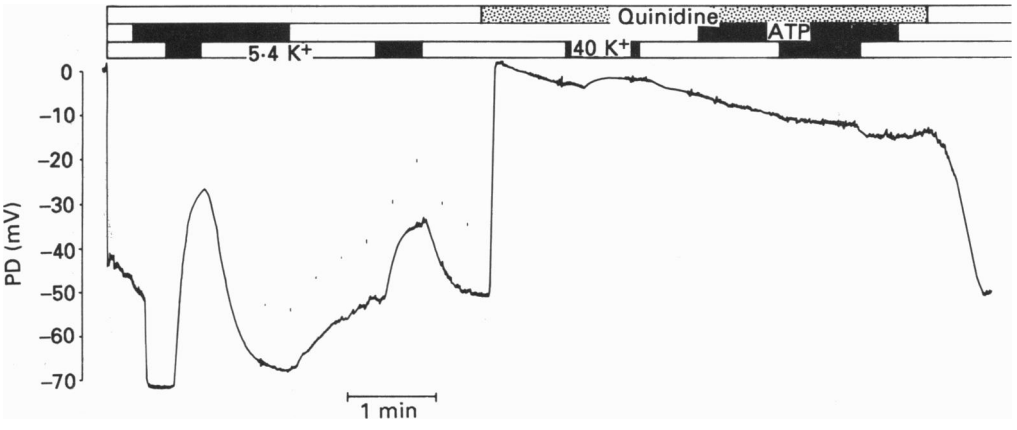

Fig. 6. Effect of 10 μmol/l ATP and of increasing extracellular potassium concentration from 5·4 to 40 mmol/l on the potential difference across the cell membrane (PD) in the presence of 1 mmol/l quinidine (original tracing).

polarization and by $+4{\cdot}3 \pm 1{\cdot}0$ mV ($n = 7$) during the sustained hyperpolarization (Fig. 4). Apparently the potassium channels activated by ATP are in large part subsequently blocked by barium. Removal of ATP in the continued presence of barium depolarizes the cell membrane by $+6{\cdot}7 \pm 3{\cdot}5$ mV ($n = 5$) to $-31{\cdot}4 \pm 1{\cdot}6$ mV ($n = 5$, Fig. 5). As apparent from Fig. 5, the hyperpolarization following application of ATP in the presence of barium is clearly more sustained than the hyperpolarization following application of adrenaline in the presence of barium. Application of

10 μmol/l theophylline, 1 μmol/l phentolamine and 10 μmol/l indomethacin do not significantly alter PD ($+0{\cdot}4 \pm 0{\cdot}4$ mV, $n = 7$, $-0{\cdot}3 \pm 0{\cdot}2$ mV, $n = 11$, and $-0{\cdot}2 \pm 0{\cdot}3$ mV, $n = 7$, respectively) and do not significantly modify the hyperpolarizing effect of ATP ($-18{\cdot}3 \pm 1{\cdot}0$ mV, $n = 7$, $-21{\cdot}0 \pm 1{\cdot}4$ mV, $n = 11$, and $-25{\cdot}4 \pm 2{\cdot}6$ mV, $n = 7$, respectively).

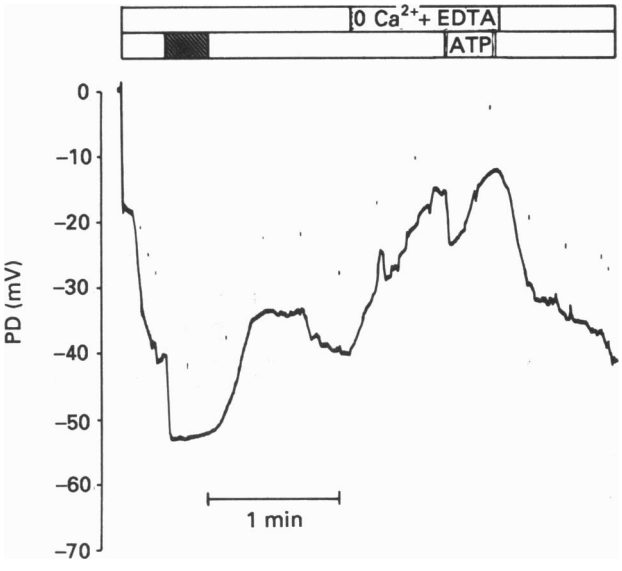

Fig. 7. Effect of 10 μmol/l ATP on the potential difference across the cell membrane (PD) in the nominal absence of calcium and presence of 1 mmol/l EDTA (original tracing).

Quinidine at 0·1 mmol/l depolarizes the cell membrane to $-23{\cdot}5 \pm 3{\cdot}0$ mV ($n = 24$), but does not block the ATP-induced hyperpolarization ($-22{\cdot}7 \pm 4{\cdot}3$ mV, $n = 19$). Quinidine at 1 mmol/l depolarizes the cell membrane to $-6{\cdot}9 \pm 2{\cdot}7$ mV ($n = 8$) and completely abolishes the hyperpolarizing effect of ATP (Fig. 6). Reduction of extracellular calcium activity to $\approx$ 10 nmol/l (calcium omitted and 1 mmol/l EDTA added) depolarizes the cell membrane to $-18{\cdot}5 \pm 1{\cdot}7$ mV ($n = 18$). With reduced extracellular calcium, the hyperpolarizing effect of ATP is blunted ($-12{\cdot}3 \pm 1{\cdot}6$ mV, $n = 18$) and only transient (Fig. 7). A similar blunting of the ATP effect is observed, if calcium is omitted without addition of 1 mmol/l EDTA.

Ouabain depolarizes the cell membrane by $+4{\cdot}8 \pm 1{\cdot}3$ mV ($n = 10$) within 72 s. Subsequent application of ATP hyperpolarizes the cell membrane by $-29{\cdot}9 \pm 1{\cdot}9$ mV ($n = 10$).

DISCUSSION

The present study illustrates that subconfluent MDCK cells are sensitive to extracellular ATP. The efficacy of ATP-γ-S indicates that metabolism of ATP is not required for its effect. Since the effect of ATP cannot be mimicked by adenosine (Fig. 2), it appears that the hyperpolarizing effect of ATP in MDCK cells is mediated

by P_2 receptors. Accordingly, the effect of ATP is not affected by theophylline, which has been shown to interfere with P_1 purinergic receptors (Burnstock, 1981).

The ATP-induced hyperpolarization is the result of enhanced potassium conductance, as reflected by the increased potassium selectivity and the reduced input resistance. Apparently the ATP-induced hyperpolarization does not require stimulation of sodium, potassium-ATPase since ouabain does not blunt the effect of ATP. The stimulation of potassium conductance by extracellular ATP contrasts with the inhibitory effect of intracellular ATP on potassium channels in a variety of tissues (Romero, 1978; Noma, 1983; Trube & Hescheler, 1984; Findlay, Dunne & Petersen, 1985; Arkhammar, Nilsson, Rorsman & Berggren, 1987; Findlay, 1987; Horie, Irisawa & Noma, 1987; Stanfield, 1987; Sturgess, Hales & Ashford, 1987). The question of whether intracellular ATP similarly regulates potassium channels in epithelia cannot be answered with certainty at present (Lang, Messner & Rehwald, 1986*b*). Increased potassium permeability of the cell membrane in response to extracellular ATP has been reported for fibroblasts (Okada, Yada, Ohno-Shosaku, Oiki, Ueda & Machida, 1984) and activation of potassium channels by ATP has been observed in cultured myoblasts and myotubes (Kolb & Wakelam, 1983).

The hyperpolarization due to enhanced potassium conductance may contribute to the ATP stimulation of chloride secretion (Simmons, 1981*a*) by increasing the driving force for chloride exit across the luminal cell membrane. Whether ATP stimulates in addition a chloride channel cannot be answered from these studies. In MDCK cells chloride channels are stimulated by cyclic AMP (Lang *et al.* 1986*a*; Lang, Paulmichl, Defregger, Gstraunthaler, Pfaller & Deetjen, 1987) and ATP has been shown to stimulate cyclic AMP generation in some tissues such as intestinal cells (Korman, Lemp, Jackson & Gardner, 1982), adipocytes (Crooke, Allan, Pattinson & Sneyd, 1980) and fibroblasts (Westcott, Engelhard & Storm, 1979). Any stimulation of chloride channels may have been masked by the marked stimulation of potassium channels. In the case of adrenaline, the effects can be dissociated since stimulation of chloride conductance is mediated by β-receptors (Lang *et al.* 1986*a*; 1987). Isoprenaline leads to a slight depolarization and an increase of chloride selectivity of the cell membrane. Following application of adrenaline, stimulation of potassium channels by far outcasts the stimulation of chloride channels and the cell membrane approaches the equilibrium potential for potassium (Paulmichl, Defregger & Lang, 1986*a*).

The hyperpolarizing effect of ATP is blunted and only transient in the nominal absence of extracellular calcium. Similarly to ATP, both adrenaline (Paulmichl *et al.* 1986*a*) and bradykinin (Paulmichl, Friedrich & Lang, 1987) hyperpolarize the cell membrane by stimulation of potassium conductance. The effect of these hormones is similarly transient but it is not blunted in the nominal absence of extracellular calcium. Possibly ATP is less efficient in recruiting intracellular calcium than adrenaline or bradykinin. In any case release of intracellular calcium alone is not likely to account for the sustained hyperpolarization. Rather calcium entry must occur in addition across the plasma membrane and, in fact, an ATP-stimulated calcium channel has been identified recently in smooth muscle (Benham & Tsien, 1987).

The effect of ATP is blocked by 1 mmol/l quinidine, but apparently only in part

by 1 mmol/l barium. Prior studies revealed that the potassium channels activated by adrenaline are, with some delay, completely blocked by barium. The comparison of the effects of ATP and adrenaline reveals that in the presence of barium the ATP-induced hyperpolarization is clearly more sustained than the effect of adrenaline. Thus, in contrast to adrenaline, ATP hyperpolarizes the cell membrane in part via a mechanism which is not sensitive to barium, even after some delay. This observation may again point to a difference in signal transduction of the two hormones.

The observation that ATP-induced hyperpolarization was not blunted by indomethacin is compatible with prostaglandin-dependent stimulation by ATP of short-circuit current (SCC) in confluent MDCK cells (Simmons, 1981*b*). Possibly prostaglandins stimulate chloride conductance at the luminal cell membrane and activation of basolateral potassium channels by ATP increases SCC only if luminal chloride channels are patent.

In conclusion, ATP hyperpolarizes MDCK cells by activation of potassium channels. The effect is mediated by P_2 receptors and depends on calcium. By increasing the driving force for chloride exit, the hyperpolarization could lead to stimulation of chloride secretion.

The authors greatfully acknowledge the valuable discussion with Professor Dr P. Deetjen and Dr F. Friedrich, the skilled technical assistance of G. Siber and S. David, and excellent mechanical and electronic support by K. H. Streicher and Ing. M. Hirsch. This study has been supported by the Fonds zur Förderung der wissenschaftlichen Forschung, Grant Nr. P5813 and P6792M.

REFERENCES

AITON, J. F., BROWN, C. D. A., OGDEN, P. & SIMMONS, N. L. (1982). K^+ transport in 'tight' epithelial monolayers of MDCK cells. *Journal of Membrane Biology* **65**, 99–109.

AITON, J. F., CHIPPERFIELD, A. R., LAMB, J. F., OGDEN, P. & SIMMONS, N. L. (1981). Occurrence of passive furosemide-sensitive transmembrane potassium transport in cultured cells. *Biochimica et biophysica acta* **646**, 389–398.

ARKHAMMAR, P., NILSSON, T., RORSMAN, P. & BERGGREN, P.-O. (1987). Inhibition of ATP-regulated K^+ channels precedes depolarization-induced increase in cytoplasmic free Ca^{2+} concentration in pancreatic β-cells. *Journal of Biological Chemistry* **262**, 5448–5454.

BENHAM, C. D. & TSIEN, R. W. (1987). A novel receptor-operated Ca^{2+}-permeable channel activated by ATP in smooth muscle. *Nature* **328**, 275–278.

BROWN, C. D. A. & SIMMONS, N. L. (1982). K^+ transport in 'tight' epithelial monolayers of MDCK-cells: Evidence for a calcium-activated K^+ channel. *Biochimica et biophysica acta* **690**, 95–105.

BURNSTOCK, G. (1976). Purinergic receptors. *Journal of Theoretical Biology* **62**, 491–503.

BURNSTOCK, G. (1981). Neurotransmitters and trophic factors in the autonomic nervous system. *Journal of Physiology* **313**, 1–35.

CEREIJIDO, M., EHRENFELD, J., MEZA, I. & MARTINEZ-PALOMO, A. (1980). Structural and functional membrane polarity in cultured monolayers of MDCK cells. *Journal of Membrane Biology* **52**, 147–159.

CROOKE, M. J., ALLAN, E., PATTINSON, N. & SNEYD, J. G. T. (1980). Formation of cyclic AMP from exogenous ATP by isolated hepatocytes and adipocytes. *Biochimica et biophysica acta* **631**, 28–39.

DRURY, A. N. & SZENT-GYÖRGYI, A. (1929). The physiological activity of adenine compounds with special reference to their action upon mammalian heart. *Journal of Physiology* **68**, 213–237.

FINDLAY, I., DUNNE, M. J. & PETERSEN, O. H. (1985). ATP-sensitive inward rectifier and voltage- and calcium-activated K^+ channels in cultured pancreatic islet cells. *Journal of Membrane Biology* **88**, 165–172.

FINDLAY, I. (1987). The effects of magnesium upon adenosine triphosphate-sensitive potassium channels in a rat insulin-secreting cell line. *Journal of Physiology* **391**, 611–629.

GALLACHER, D. V. (1982). Are there purinergic receptors on parotid acinar cells? *Nature* **296**, 83–86.

GORDON, J. L. (1986). Extracellular ATP: effects, sources and fate. *Biochemical Journal* **233**, 309–319.

GSTRAUNTHALER, G. (1988). Epithelial cells in tissue culture. *Renal Physiology* **11**, 1–32.

HELMAN, S. I. & THOMPSON, S. M. (1982). Interpretation and use of electrical equivalent circuits in studies of epithelial tissues. *American Journal of Physiology* **243**, F519–531.

HORIE, M., IRISAWA, H. & NOMA, A. (1987). Voltage-dependent magnesium block of adenosine-triphosphate-sensitive potassium channel in guinea-pig ventricular cells. *Journal of Physiology* **387**, 251–272.

KOHN, P. G., NEWEY, H. & SMYTH, D. H. (1970). The effect of adenosine triphosphate on the transmural potential in rat small intestine. *Journal of Physiology* **208**, 203–220.

KOLB, H.-B., BROWN, C. D. A. & MURER, H. (1985). Identification of a voltage-dependent anion channel in the apical membrane of a Cl^- secretory epithelium (MDCK). *Pflügers Archiv* **403**, 262–265.

KOLB, H.-A. & WAKELAM, M. J. D. (1983). Transmitter-like action of ATP on patched membranes of cultured myoblasts and myotubes. *Nature* **303**, 621–623.

KORMAN, L. Y., LEMP, G. F., JACKSON, M. J. & GARDNER, J. D. (1982). Mechanism of action of ATP on intestinal epithelial cells cyclic AMP-mediated stimulation of active ion transport. *Biochimica et biophysica acta* **721**, 47–54.

LANG, F., DEFREGGER, M. & PAULMICHL, M. (1986*a*). Apparent chloride conductance of subconfluent Madin Darby canine kidney cells. *Pflügers Archiv* **407**, 158–162.

LANG, F., MESSNER, G. & REHWALD, W. (1986*b*). Electrophysiology of sodium-coupled transport in proximal renal tubules. *American Journal of Physiology* **250**, F953–962.

LANG, F., PAULMICHL, M., DEFREGGER, M., GSTRAUNTHALER, G., PFALLER, W. & DEETJEN, P. (1987). Transport systems in cultured epithelial kidney cells (MDCK) and their modulation by diuretics and hormones. In *Diuretics* II: *Chemistry, Pharmacology, and Clinical Applications*, ed. PUSCHETT, J. B. & GREENBERG, A., pp. 107–120. Amsterdam: Elsevier.

MCMILLIAN, M. K., SOLTOFF, S. P., CANTLEY, L. C. & TALAMO, B. R. (1987). Extracellular ATP elevates intracellular free calcium in rat parotid acinar cells. *Biochemical and Biophysical Research Communications* **149**, 523–530.

MADIN, S. H. & DARBY, N. B. (1958). As catalogued in *American Type Culture Collection Catalogue of strains* **2**, 574–576.

NOMA, A. (1983). ATP-regulated K^+ channels in cardiac muscle. *Nature* **305**, 147–148.

OKADA, Y., YADA, T., OHNO-SHOSAKU, T., OIKI, S., UEDA, S. & MACHIDA, K. (1984). Exogenous ATP induces electrical membrane responses in fibroblasts. *Experimental Cell Research* **152**, 552–557.

PAULMICHL, M., DEFREGGER, M. & LANG, F. (1986*a*). Effects of epinephrine on electrical properties of Madin-Darby canine kidney cells. *Pflügers Archiv* **406**, 367–371.

PAULMICHL, M., FRIEDRICH, F. & LANG, F. (1986*b*). Electrical properties of Madin-Darby-canine-kidney cells: effects of extracellular sodium and calcium. *Pflügers Archiv* **407**, 258–263.

PAULMICHL, M., FRIEDRICH, F. & LANG, F. (1987). Effects of bradykinin on electrical properties of Madin–Darby canine kidney epithelioid cells. *Pflügers Archiv* **408**, 408–413.

PAULMICHL, M., GSTRAUNTHALER, G. & LANG, F. (1985). Electrical properties of Madin–Darby canine kidney cells. Effects of extracellular potassium and bicarbonate. *Pflügers Archiv* **405**, 102–107.

RINDLER, M. J. & SAIER JR, M. H. (1981). Evidence for Na^+/H^+ antiport in cultured dog kidney cells (MDCK). *Journal of Biological Chemistry* **256**, 10820–10825.

RINDLER, M. J., TAUB, M. & SAIER JR, M. H. (1979). Uptake of $^{22}Na^+$ by cultured dog kidney cells (MDCK). *Journal of Biological Chemistry* **254**, 11431–11439.

ROMERO, P. J. (1978). Is the Ca^{2+}-sensitive K^+ channel under metabolic control in human red cells? *Biochimica et biophysica acta* **507**, 178–181.

SIMMONS, N. L. (1981*a*). Stimulation of Cl^- secretion by exogenous ATP in cultured MDCK epithelial monolayers. *Biochemica et biophysica acta* **646**, 231–242.

SIMMONS, N. L. (1981*b*). Identification of a purine (P_2) receptor linked to ion transport in a cultured renal (MDCK) epithelium. *British Journal of Pharmacology* **73**, 379–384.

SIMMONS, N. L. (1982). Cultured monolayers of MDCK cells: A novel model system for the study of epithelial development and function. *Journal of General Pharmacology* **13**, 287–291.

STANFIELD, P. R. (1987). Nucleotides such as ATP may control the activity of ion channels. *Trends in Neurosciences* **10**, 335–339.

STURGESS, N. C., HALES, C. N. & ASHFORD, M. L. J. (1987). Calcium and ATP regulate the activity of a non-selective cation channel in a rat insulinoma cell line. *Pflügers Archiv* **409**, 607–615.

TRUBE, G. & HESCHELER, J. (1984). Inward-rectifying channels in isolated patches of the heart cell membrane: ATP-dependence and comparison with cell-attached patches. *Pflügers Archiv* **401**, 178–184.

VÖLKL, H. & LANG, F. (1988). Electrophysiology of cell volume regulation in proximal tubules of the mouse kidney. *Pflügers Archiv* **411**, 514–519.

WESTCOTT, K. A., ENGELHARD, V. H. & STORM, D. R. (1979). Production of cyclic AMP from extracellular ATP by intact LM cells. *Biochimica et biophysica acta* **583**, 47–54.