Pertussis toxin-dependent and -independent hormonal effects on cultured renal epithelioid cells

M. Paulmichl, E. Wöll and F. Lang

Institute for Physiology, University of Innsbruck, Innsbruck, Austria

The present study has been performed to test for the involvement of pertussis toxin-sensitive GTP-binding proteins (G-proteins) in the cellular transduction of hormone-induced activation of potassium channels. In Madin Darby canine kidney (MDCK) cells, a permanent cell line from dog kidney, epinephrine, acetylcholine, bradykinin, serotonin and ATP hyperpolarize the cell membrane by activation of potassium channels. In cells pretreated with pertussis toxin the hyperpolarizations elicited by either acetylcholine or serotonin are completely abolished; that following epinephrine is blunted and only transient. The hyperpolarizing effects of ATP or bradykinin are not affected by pertussis toxin. Thus, in MDCK cells both pertussis toxin-dependent and -independent mechanisms operate in parallel to enhance the potassium conductance of the cell membrane.

Membrane potential; Pertussis toxin; Epinephrine; Acetylcholine; Bradykinin; Serotonin; ATP; (MDCK cell)

1. INTRODUCTION

In a variety of epithelia, such as lacrimal glands [1,2], pancreas acinar cells [3], parotid salivary cells [4] and tracheal epithelium [5], stimulation of chloride secretion by hormones is paralleled by calcium-dependent stimulation of potassium conductance, which hyperpolarizes the cell membrane and thus increases the driving force for chloride exit across the luminal cell membrane. Madin Darby canine kidney (MDCK) cells, a permanent cell line from dog kidney, form monolayers displaying transepithelial chloride transport, which can be similarly stimulated by calcium-dependent activation of potassium conductance [6]. Subconfluent MDCK cells express the properties of confluent layers inasmuch as they display the same transport systems as confluent layers and prove very useful in the study of hormone-regulated transport systems. The cells respond with marked, fully reversible hyperpolarization of the cell membrane

following exposure to epinephrine [7], acetylcholine [8], bradykinin [9], serotonin [10] and ATP [11] due to activation of potassium channels. However, the hormones do not necessarily stimulate the same channels via identical intracellular mechanisms.

The present study has been performed to test for the involvement of pertussis toxin-sensitive G-proteins in potassium channel activation by these hormones.

2. MATERIALS AND METHODS

MDCK cells from the American Type Culture Collection were used from passage 90 to 100 [10]. 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 growing 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 into a perfusion chamber (volume 0.1 ml, perfusion rate 20 ml/min). Control perfusate was composed of (in mmol/l): 114 NaCl, 5.4 KCl, 0.8 $MgCl_2$, 1.2 $CaCl_2$, 0.8 Na_2HPO_4, 0.2 NaH_2PO_4, 20

Correspondence address: M. Paulmichl, Institute for Physiology, University of Innsbruck, Fritz-Pregl-Str. 3, A-6010 Innsbruck, Austria

NaHCO$_3$, 5.5 glucose, equilibrated with 5% carbon dioxide and 95% air (pH 7.4) and kept at 37°C. All chemicals were from Sigma (Munich, FRG). Measurements of the 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, 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, Oberkochen, FRG), using a piezostepper (PM 20 N, Frankenberger, Germering, FRG) mounted on a Leitz micromanipulator (Leitz, Wetzlar, FRG).

Epinephrine (1 μmol/l), acetylcholine (1 μmol/l), bradykinin (0.01 μmol/l), serotonin (1 μmol/l) and ATP (10 μmol/l) have been applied to untreated cells and to cells pretreated for more than 2 h with 1 mg/l islet-activating protein from the bacterium *Bordetella pertussis* (pertussis toxin = PTX), which ADP-ribosylates certain G-proteins [12].

3. RESULTS AND DISCUSSION

In untreated cells, each, epinephrine (1 μmol/l), acetylcholine (1 μmol/l), bradykinin (0.01 μmol/l), serotonin (1 μmol/l) and ATP (10 μmol/l) leads to a marked, fully reversible hyperpolarization of the cell membrane (fig.1). As reported previously [7–11], the effects of bradykinin and serotonin are transient, the effects of epinephrine, acetylcholine and ATP are sustained. The hyperpolarization is paralleled by a decrease of cell membrane resistance (table 1) and an increased sensitivity of the cell membrane potential to alterations of extracellular potassium concentration (table 1). Accordingly, the hyperpolarization is due to enhan-
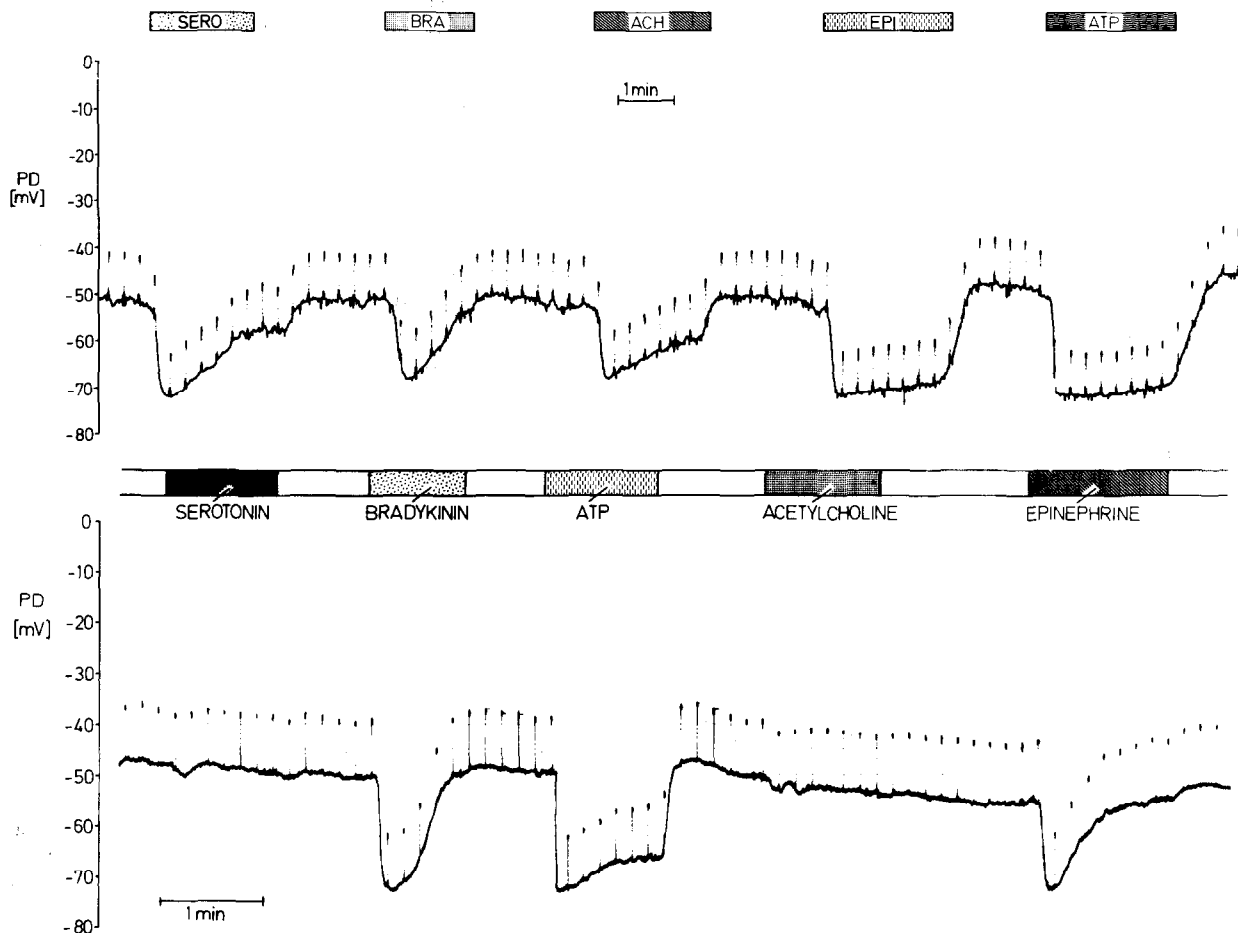

Fig.1. Original tracings showing the effects of 1 μmol/l serotonin, 0.01 μmol/l bradykinin, 10 μmol/l ATP, 1 μmol/l acetylcholine and 1 μmol/l epinephrine on the potential difference across the cell membrane (PD) in cells without (upper panel) and with (lower panel) 1 mg/l pertussis toxin pretreatment for more than 2 h.

Table 1

Hormone induced alteration of cell membrane potential (dPD) and cell membrane resistance (dR) in MDCK cells in the absence ($-$ PTX) and presence ($+$ PTX) of pertussis toxin; effect of the hormones on the transference number of the cell membrane for potassium (tk $=$ potassium conductance/total cell membrane conductance)

Hormones	$-$ PTX				$+$ PTX		
	dPD (mV)	dR (%)	tk	n	dPD (mV)	dR (%)	n
Serotonin	-14.8 ± 1.3	-33 ± 5	0.65 ± 0.02	12	-0.1 ± 0.4	-2 ± 8	11
ATP	-22.0 ± 2.6	-44 ± 10	0.80 ± 0.02	5	-20.0 ± 2.2	-68 ± 18	7
Bradykinin	-20.2 ± 2.2	-60 ± 16	0.79 ± 0.03	5	-18.6 ± 1.7	-58 ± 14	6
Epinephrine	-21.8 ± 1.9	-49 ± 7	0.76 ± 0.01	16	-9.1 ± 1.6	-28 ± 4	14
Acetylcholine	-16.2 ± 2.7	-30 ± 5	0.70 ± 0.03	5	$+0.3 \pm 0.4$	$+2 \pm 4$	7

tk is calculated from the depolarization of the cell membrane (dPD) following increase of extracellular potassium concentration from 5.4 to 20 mmol/l: tk $=$ dPD/[61.5 mV·lg (5.4/20)]. In the absence of hormones, tk amounts to 0.36 ± 0.03, $n = 22$ (mean values $\pm$ SE, $n =$ number of experiments)

cement of the potassium conductance of the cell membrane.

In the absence of hormones the potential difference across the cell membrane (PD) was not significantly different in untreated (PD $= -49.0 \pm$ 1.0 mV, $n = 44$) and PTX-treated cells (PD $= -49.8 \pm 0.9$ mV, $n = 43$).

As apparent from fig.1 and table 1, PTX does not significantly modify the hyperpolarizing effect of bradykinin or ATP. The effects of acetylcholine and of serotonin, however, are completely abolished by PTX. The effect of epinephrine is sustained in the absence but blunted and only transient in the presence of PTX. Thus, while all hormones activate potassium channels within the same cell, the sensitivity to PTX discloses differences in cellular transduction.

The hyperpolarizing effect of epinephrine involves obviously two mechanisms. A PTX-insensitive mechanism allows for a transient hyperpolarization, while a mechanism involving apparently PTX-sensitive G-proteins accounts for the sustained hyperpolarization (fig.1, table 1). Both mechanisms are mediated by α-receptors, since epinephrine-induced hyperpolarization is abolished in the presence of the unselective α-channel blocker pentolamine [7]. Selective stimulation of β-receptors by isoproterenol leads to a shallow depolarization and an increase of the chloride selectivity of the cell membrane, an effect mimicked and most likely mediated by cAMP [13]. Previous studies reported both PTX-dependent and -independent effects of epinephrine: no effect

of PTX has been shown on epinephrine binding in hepatocytes [14] and MDCK cells [15], on epinephrine-stimulated phosphorylase in hepatocytes [14] or on phosphoinositide hydrolysis and prostaglandin E_2 formation in MDCK cells [15]. On the other hand, PTX blocked norepinephrine inhibition of calcium channels in dorsal root ganglion neurones [16], epinephrine inhibition of adenylate cyclase [17] and epinephrine stimulated phosphoinositide turnover [18].

The seeming insensitivity of bradykinin- and ATP-induced hyperpolarization to PTX (fig.1, table 1) documents that the hormones are capable of stimulating potassium conductance by mechanisms independent of PTX-sensitive G-proteins. In parallel to our observations PTX has no effect on bradykinin-stimulated formation of inositol phosphates in neuroblastoma × glioma hybrid NG108-15 cells [19], 3T3 fibroblasts [20], A431 carcinoma cells [21] and NG115-401L neuronal cells [22] as well as bradykinin-induced increase of intracellular calcium concentration in neuroblastoma × glioma hybrid NG108-15 cells [19] and NG115-401L neuronal cells [22]. Furthermore, no effect of PTX has been shown on bradykinin-stimulated prostacyclin formation in endothelial cells [23] and bradykinin-stimulated prostaglandin E_2 formation in 3T3 fibroblasts [20]. On the other hand, PTX blocked bradykinin-stimulated arachidonic acid release, bradykinin-stimulated calcium uptake and bradykinin inhibition of adenylate cyclase in 3T3 fibroblasts [24]. In addition, PTX reduced bradykinin-stimulated

inositol-1,4,5-triphosphate formation in neuroblastoma × dorsal root ganglion sensory (F-11) cells [25] and bradykinin-induced changes in cell membrane potential of NG108-15 neuroblastoma × glioma hybrid cells [26]. In the latter cells bradykinin stimulation of GTPase has been reported to be inhibited by [26] or not sensitive [27] to PTX. To our knowledge, no experiments have been published on PTX sensitivity of extracellular ATP effects.

The complete abolishment of acetylcholine- and serotonin-induced hyperpolarization indicates that cellular transmission of potassium channel activation by these hormones depends at some step on PTX-sensitive G-proteins (fig.1, table 1). These observations parallel previous reports on PTX-sensitive effects of these hormones on increase of smooth muscle intracellular calcium concentration [28], activation of chloride channels in *Xenopus* oocytes [29], activation of cardiac potassium channels [30–34] as well as inactivation of calcium currents in neurones [35] and heart [36].

In conclusion, in the same MDCK cells, both PTX-sensitive and -insensitive intracellular mechanisms operate in parallel to activate potassium channels.

REFERENCES

[1] Findlay, I. (1984) J. Physiol. 350, 179–195.
[2] Trautmann, A. and Marty, A. (1984) Proc. Natl. Acad. Sci. USA 81, 611–615.
[3] Petersen, O.H. and Findlay, I. (1987) Physiol. Rev. 67, 1054–1116.
[4] Putney, J.W., jr (1983) Cell Calcium 4, 439–449.
[5] Welsh, M.J. (1987) Physiol. Rev. 67, 1143–1184.
[6] Simmons, N.L., Brown, C.D.A. and Rugg, E.L. (1984) Fed. Proc. 43, 2225–2229.
[7] Paulmichl, M., Defregger, M. and Lang, F. (1986) Pflügers Arch. 406, 367–371.
[8] Lang, F., Klotz, L. and Paulmichl, M. (1988) Biochim. Biophys. Acta, in press.
[9] Paulmichl, M., Friedrich, F. and Lang, F. (1987) Pflügers Arch. 408, 408–413.
[10] Paulmichl, M., Friedrich, F., Wöll, E., Weiss, H. and Lang, F. (1988) Pflügers Arch. 411, 394–400.
[11] Jungwirth, A., Paulmichl, M. and Lang, F. (1988) Pflügers Arch. 411, R78, 145.
[12] Katada, T. and Ui, M. (1982) Proc. Natl. Acad. Sci. USA 79, 3129–3133.
[13] Lang, F., Defregger, M. and Paulmichl, M. (1988) Pflügers Arch. 407, 158–162.
[14] Lynch, C.J., Prpic, V., Blackmore, P.F. and Exton, J.H. (1986) Mol. Pharmacol. 29, 196–203.
[15] Terman, B.I., Slivka, S.R., Hughes, R.J. and Insel, P.A. (1987) Mol. Pharmacol. 31, 12–20.
[16] Holz, G.G., iv, Rane, S.G. and Dunlap, K. (1986) Nature 319, 670–672.
[17] Nomura, Y., Kawata, K., Kitamura, Y. and Watanabe, H. (1987) Eur. J. Pharmacol. 134, 123–129.
[18] Moreno, F.J., Mills, I., Garcia-Sainz, J.A. and Fain, J.N. (1983) J. Biol. Chem. 258, 10938–10943.
[19] Osugi, T., Imaizumi, T., Mizushima, A., Uchida, S. and Yoshida, H. (1987) Eur. J. Pharmacol. 137, 207–218.
[20] Burch, R.M. and Axelrod, J. (1987) Proc. Natl. Acad. Sci. USA 84, 6374–6378.
[21] Tilly, B.C., Van Paridon, P.A., Verlaan, I., Wirtz, K.W.A., De Laat, S.W. and Moolenaar, W.H. (1987) Biochem. J. 244, 129–135.
[22] Jackson, T.R., Patterson, S.I., Wong, Y.H. and Hanley, M.R. (1987) Biochem. Biophys. Res. Commun. 148, 412–416.
[23] Clark, M.A., Conway, T.M., Bennett, C.F., Crooke, S.T. and Stadel, J.M. (1986) Proc. Natl. Acad. Sci. USA 83, 7320–7324.
[24] Murayama, T. and Ui, M. (1985) J. Biol. Chem. 260, 7226–7233.
[25] Francel, P.C., Miller, R.J. and Dawson, G. (1987) J. Neurochem. 48, 1632–1639.
[26] Higashida, H., Streaty, R.A., Klee, W. and Nirenberg, M. (1986) Proc. Natl. Acad. Sci. USA 83, 942–946.
[27] Grandt, R., Greiner, C., Zubin, P. and Jakobs, K.H. (1986) FEBS Lett. 196, 279–283.
[28] Bruns, C. and Marmé, D. (1987) FEBS Lett. 212, 40–44.
[29] Nomura, Y., Kaneko, S., Kato, K., Yamagishi, S. and Sugiyama, H. (1987) Mol. Brain Res. 2, 113–123.
[30] Sasaki, K. and Sato, M. (1987) Nature 325, 259–262.
[31] Pfaffinger, P.J., Martin, J.M., Hunter, D.D., Nathanson, N.M. and Hille, B. (1985) Nature 317, 536–538.
[32] Kurachi, Y., Nakajima, T. and Sugimoto, T. (1986) Pflügers Arch. 407, 264–274.
[33] Logothetis, D.E., Kurachi, Y., Galper, J., Neer, E.J. and Clapham, D.E. (1987) Nature 325, 321–326.
[34] Yatani, A., Codina, J., Brown, A.M. and Birnbaumer, L. (1987) Science 235, 207–211.
[35] Wanke, E., Ferroni, A., Malgaroli, A., Ambrosini, A., Pozzan, T. and Meldolesi, J. (1987) Proc. Natl. Acad. Sci. USA 84, 4313–4317.
[36] Hescheler, J., Kameyama, M. and Trautwein, W. (1986) Pflügers Arch. 407, 182–189.