Pflügers Arch (1991) 419:418–420
0031676891001962

Pflügers Archiv
European Journal of Physiology

Short communication

Alkaline stress transforms Madin-Darby canine kidney cells

Hans Oberleithner, Hans-Jürgen Westphale, and Birgit Gaßner

Department of Physiology, University of Würzburg, Röntgenring 9, W-8700 Würzburg, Federal Republic of Germany

Received June 17, 1991/Accepted July 3, 1991

ABSTRACT.
Similar to growth factors aldosterone stimulates Na^+/H^+ exchange in renal target cells leading to cytoplasmic alkalinization. An alkaline intracellular pH reduces the H^+ bonds between repressor proteins and DNA leading to the destabilization of the nuclear chromatin. We observed that sustained alkaline stress "per se" can lead to malignant transformation of Madin-Darby canine kidney (MDCK) cells. Cells grown for two weeks in alkaline culture medium (pH 7.8) developed multiple "foci" composed of spindle-shaped pleomorphic cells lacking contact inhibition and exhibiting poor adhesion to the culture support, typical characteristics of dedifferentiated tumor cells. "Focus" cells were cloned and grown in standard medium (pH 7.4). Cells maintained their abnormal growth pattern, indicating stable pH-induced genetic transformation. Cells were fused with polyethylene glycol to giant cells and impaled with microelectrodes. In contrast to non-transformed giant MDCK cells the plasma membrane potential showed spontaneous oscillations that could be virtually abolished by the omission of extracellular Ca^{2+} or by the addition of the K^+ channel blocker Ba^{2+}. We conclude that sustained alkaline stress can induce malignant transformation in MDCK cells indicated by an abnormal growth pattern and by membrane potential oscillations most likely due to Ca^{2+} activated K^+ channels in the plasma membrane.

Key words: MDCK cells, intracellular pH, cell transformation, renal carcinoma, membrane potential oscillations

INTRODUCTION

In Madin-Darby canine kidney (MDCK) cells aldosterone activates the Na^+/H^+ exchanger, resulting in an increase of cytoplasmic pH by 0.1 - 0.3 units in absence and presence of bicarbonate. This alkalinization triggers a cascade of cellular events including ion transport, cell differentiation and cell growth (6). In order to imitate aldosterone action in cultured cells we incubated MDCK cells in standard medium rendered alkaline by the addition of NaOH. We increased medium pH from 7.4 to 7.8, an alkaline pH tolerated by the MDCK cells in long-term experiments. Our initial goal was to change the phenotypical character of the cell by such extreme experimental conditions and to force cells to overexpress those plasma transport systems that allow them to survive systemic alkalosis. In addition we expected to gain better access to transport systems usually expressed by MDCK cells (but less pronounced) in response to aldosterone.

To our surprise and rather unexpected, MDCK cells developed the typical characteristics of tumor cells after about two weeks of continuous alkaline stress. Here, we report on the procedure of pH-induced transformation and give some first information on the altered plasma membrane properties of these cells.

METHODS

$2 \cdot 10^6$ MDCK cells obtained at passage 53 from the American Type Culture Collection were seeded in coated plastic culture dishes (growth area = 75 cm^2; Nunc, Wiesbaden, FRG) in 10 ml Minimu Essential Medium with Earle's salts, nonessential amino-acids an L-glutaminic acid (MEM medium, Biochrom KG, Berlin, FRG The MEM medium was supplemented with 10% fetal calf seru (Biochrom KG) and 26 mM $NaHCO_3$. This solution was equilibra ted with 5% CO_2, 95% room air and then titrated at 37° C to pH 7. by adding 16 ml of 1M NaOH to 1000 ml medium. Cells we grown for two weeks in this alkaline medium. Over this period c time MDCK cells were split 3 times. After about 10 days in alkali ne medium several so-called "foci" could be detected in the dish indicating cell transformation. By means of a pasteure pipette "foc cells were removed and transferred to another culture dish at steril conditions. These "foci" MDCK (MDCK-F) cells were grown furt her on in standard medium (pH 7.4). So far we have repeated thes experiments two times following the same procedure. In both serie the cells were successfully transformed.

For intracellular potential measurements MDCK-F cells were fuse to giant cells according to techniques published previously (3). Gi ant cells were seeded on glass cover slips, mounted on the stage c an inverted microscope and superfused with Ringer solution com posed of (mM) 130 NaCl, 5.4 KCl, 1.2 $CaCl_2$, 0.8 $MgCl_2$, 10 HE PES, 5 glucose, pH 7.4. Microelectrodes were applied to measu the plasma membrane potential while the cells were exposed to va rious superfusion solutions.

RESULTS

MDCK cells grown in standard culture medium (pH 7.4) form ho mogeneous monolayers on the bottom of a plastic culture dish. I figure 1A, B two petri dishes are shown, one with cells maintaine in pH 7.4 medium (fig. 1A), the other one grown in pH 7.8 medi um (fig. 1B). The cells of both dishes originated from the sam batch of cells (passage number 53) and were both split simulta neously 3 times over the last two weeks. The Giemsa staining iden tifies non-transformed cells forming islets with clear borders (fi 1B). In between multiple "foci" can be detected identifiable by the reticulum-like growth pattern. The "foci" have a multilayered cen tre (fig. 1C) from which cells grow out into the periphery. Due t the poor adhesion to the culture support a major portion of cel originating from a "focus" are found suspended in the culture me dium while a minor portion sticks to the dish. We transferred a fe cells from a "focus" to another dish and thus could grow a colony c transformed MDCK cells. These so-called MDCK-F cells (F = fo cus) were furtheron grown in standard medium (pH 7.4). They di not redifferentiate to their original growth pattern but maintaine their malignant features. Fig. 1D shows a homogeneous preparatio of MDCK-F cells forming multiple foci. The cells lack contact in hibition, grow over each other, are pleomorphic and never form a organized epithelial monolayer (fig. 1E). The MDCK-F cells hav been split already 10 times over the past 10 weeks. They still main tain their typical growth pattern. The cells were fused with polye hylene glycol to giant cells (fig. 1F). In contrast to the nor transformed parent cells that are usually round-shaped with cle

Offprint requests to: H. Oberleithner

cell borders (3), MDCK-F cells form many dendrite-like protrusions. The intracellular potential measured with microelectrodes in Ringer solution turned out to be instable (fig. 2A). In contrast to non-transformed giant MDCK cells the plasma membrane potential shows spontaneous hyperpolarizing spikes. Fig. 2B shows that superfusion with Ca^{2+}-free, EDTA (1mM) containing Ringer solution virtually abolishes the oscillations of the membrane potential. Treatment with 10 mM Ba^{2+}, a K^+ channel blocker, leads to a clear decrease in the spike amplitude and to a small reduction in frequency of the spontaneous oscillations (fig. 2C).

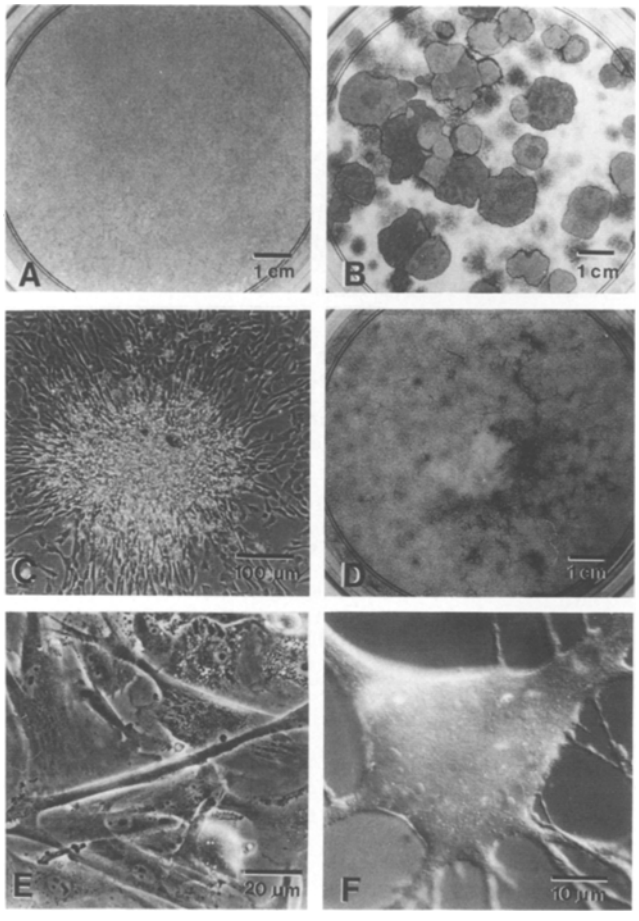

Figure 1
A: Confluent monolayer of MDCK cells maintained in standard culture medium (pH 7.4). The monolayer was stained with 4% Giemsa after ethanol fixation.
B: MDCK cells (same cell batch, same passage number and splitting procedures as in A) after 2 weeks in alkaline medium (pH 7.8). Please note the non-transformed cells forming solid islets and the transformed cells forming typical "foci". Giemsa stain as in A.
C: "Focus" formed by transformed MDCK cells.
D: Cloned "focus" cells (MDCK-F cells) maintained in standard medium for one week. Please note the multiple focus formation. Giemsa stain as in A.
E: Growth pattern of MDCK-F cells. Pleomorphic cells grow irregular in multilayers and lack contact inhibition. The phase contrast micrograph was taken at the outer border of a "focus".
F: Fused giant MDCK-F cell. Please, note several cell nuclei in the cell periphery and long protrusions that cannot be observed in the non-transformed parents cells.

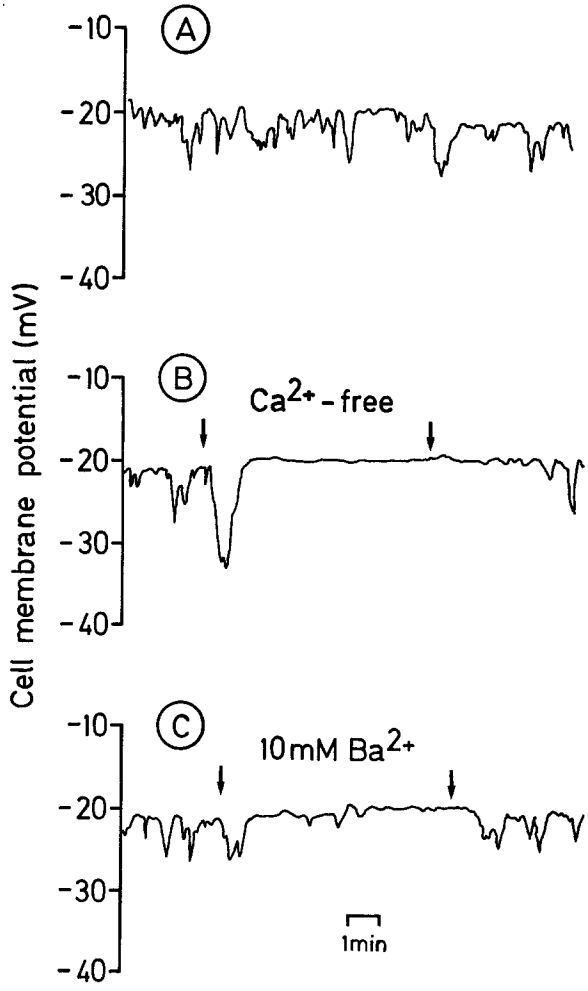

Figure 2
A: Spontaneous plasma membrane potential oscillations in MDCK-F cells.
B: Removal of extracellular Ca^{2+} (plus addition of 1mM EGTA) suppresses the membrane potential oscillations of MDCK-F cells completely.
C: Addition of Ba^{2+} diminishes the frequency and the amplitude of the membrane potential oscillations of MDCK-F cells.

DISCUSSION

A variety of mitogenic agents including serum, specific growth factors, tumor promoting phorbol esters and steroids have been found to activate the Na^+/H^+ exchanger, resulting in an increase of cytoplasmic pH by about 0.1 to 0.3 units. The magnitude of intracellular alkalinization depends on the resting level of intracellular pH which is determined also by the activity of the HCO_3^- -transport systems that protect the cell from severe alkalosis (2,7). By increasing HCO_3^- -concentraction to 65 mM (pH 7.8, 5 % CO_2) we applied an alkaline stress to the MDCK cells so that intracellular pH could not be down-regulated by the HCO_3^- -extrusion mechanisms available in these cells (6). With pH-sensitive microlectrodes we measured in (non-transformed) giant MDCK cells exposed to 65 mM HCO_3 (5% CO_2 pH 7.8) an intracellular pH of 7.60, a value about 0.35 pH-units above the value of intracellular pH measured in standard medium, pH_0 = 7.4 (unpublished observation in our laboratory).

It is known that cell nuclei swell at alkaline pH indicating that condensed inactive chromatin changes into dispersed chromatin which is active in RNA synthesis (5). Pronounced and sustained intracellular alkalinization (this is definitely different to the transient pH change induced by mitogens) could lead to the reduction of H^+ bonds between repressor proteins and DNA leading to the destabilization of the nuclear chromatin. By mechanisms yet unknown this may have led to the malignant transformation. In animals, neoplastically transformed cells lead to the formation of tumors; in cell

culture, this may result in the formation of discrete "foci" (10). We cloned these cells and could establish a pH-induced, transformed MDCK-F cell line that did not regain its original growth pattern in standard culture medium.

MDCK cells resemble intercalated cells of the renal collecting duct epithelium (6, 8) from which two types of renal cell carcinomas originate, the oncocytoma and the chromophobic renal cell carcinoma (11). So far, we have not yet classified the MDCK-F cells. At least the spindle-shaped pleomorphic morphology, the missing of tight junctions and the lack of contact inhibition suggest a high degree of dedifferentiation.

In giant MDCK-F cells the plasma membrane potential oscillates in contrast to the non-transformed parents cells. This phenomenon is most likely due to oscillations in the intracellular concentration of calcium. Lang and collaborators could recently demonstrate similar cell membrane potential oscillations in NIH-3T3 fibroblasts expressing the ras oncogene (4). In this elegant study they could show that bradykinin leads to a transient increase of intracellular calcium followed by plasma membrane potential oscillations. The hyperpolarizing spikes are most likely caused by the rhythmic stimulation of Ca^{2+} activated K^+ channels. Indeed, removal of extracellular Ca^{2+} or addition of the K^+ channel blocker Ba^{2+} can stabilize the membrane potential. An instable plasma membrane Ca^{2+} channel or a spontaneous release of calcium from intracellular stores are two putative trigger mechanisms for the membrane potential oscillations (1).

In conclusion, sustained alkaline stress leads to the transformation of MDCK cells which can be identified by their abnormal growth pattern and their oscillating membrane potential. This opens at least two interesting questions: Firstly, is an alkaline intracellular pH mediating tumor formation and might have inhibitors of Na^+/H^+ exchange (as amiloride) potential use as anticancer agents (9) and, secondly, are membrane potential oscillations a prerequisite for maintaining malignancy so that stabilizing the membrane potential could be of therapeutic use in the treatment of cancer?

Acknowledgements: We thank our colleagues Drs. S. Silbernagl, W. Steigner and L. Wojnowski for many helpful discussions during the course of the experiments. We are grateful to Mrs. B. Schuricht and M. Schulze for preparing the figures and Mrs. S. Kopp for secretarial assistance. This project was supported by Deutsche Forschungsgemeinschaft: SFB 176, A6.

REFERENCES

1. Berridge MJ (1988) Inositol trisphosphate-induced membrane potential oscillations in xenopus oocytes. J Physiol 403: 589 - 599

2. Biermann AJ, Cragoe EJ, de Laat SW, Moolenaar WH (1988) Bicarbonate determines cytoplasmic pH and suppresses mitogen-induced alkalinization in fibroblastic cells. J Biol Chem 263, 30: 15253 - 15256

3. Kersting U, Joha H, Steigner W, Gaßner B, Gstraunthaler G, Pfaller W, Oberleithner H (1988) Fusion of cultured dog kidney (MDCK) cells. I. Technique, fate of plasma membrane and cell nuclei. J Membr Biol 111: 37 - 48

4. Lang F, Friedrich F, Kahn E, Wöll E, Hammerer M, Waldegger S, Maly K, Grunicke H (1991) Bradykinin induced oscillations of cell membrane potential in cells expressing the Ha-ras oncogene. J Biol Chem 266, 8: 4938 - 4942

5. Leake RE, Trench ME, Barry JM (1972) Effect of cations on the condensation of hen erythrocyte nuclei and its relation to gene activation. Exp Cell Res 71: 17 - 26

6. Oberleithner H (1991) Acute aldosterone action in renal target cells. Cell Physiol Biochem 1: 2 - 12

7. Oberleithner H, Kersting U, Silbernagl S, Steigner W, Vogel U (1989) Fusion of cultured dog kidney (MDCK) cells: II. Relationship between cell pH and K^+ conductance in response to aldosterone. J Membr Biol 111: 49 - 56

8. Pfaller W, Gstraunthaler G, Kersting U, Oberleithner H (1989) Carbonic anhydrase activity in MDCK cells: Evidence for intercalated cell properties. Renal Physiol Biochem 12: 328 - 337

9. Rotin D, Steek-Norweed D, Grinstein S, Tannock I (1989) Requirement of Na^+/H^+ exchange for tumor growth. Canc res 49: 205 - 211

10. Rubin AL, Adam Y, Rubin H (1990) Relation of spontaneous transformation in cell culture to adaptive growth and clonal heterogeneity. Proc Natl Acad Sci USA, 87: 482 - 486

11. Störkel S, Steart PV, Drenckhahn D, Thoenes W (1989) The human chromophobe cell renal carcinoma: its probable relation to intercalated cells of the collecting duct. Virchows Archiv B Cell Pathol 56: 237 - 245