Volume 262, number 1, 69–71 FEBS 08218 March 1990

Energy transfer via cell-to-cell junctions

Ouabain-resistant cells maintain a membrane potential in ouabain-sensitive cells

T.V. Potapova, K.B. Aslanidi* and L.Ju. Boitzova

A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 119899 and **Institute of Biological Physics, Acad. Sci. USSR, Pushchino, Moscow Region 142292, USSR*

Received 16 January 1990

Animal cell cooperation has been studied in a mixed cell culture. Membrane potentials of human embryonic cells and hamster BHK-21 cells were recorded by intracellular microelectrodes. The Na^+/K^+-ATPase inhibitor ouabain (1×10^{-6} M; 2 h) caused strong depolarisation of the human cells in pure culture. The same treatment reduced only slightly the membrane potential in the hamster cells in pure or mixed cultures, as well as in the human cells in mixed culture. The above data can be explained by effective ion fluxes through heterotypic gap junctions in mixed culture. Thus, in the presence of ouabain the Na^+/K^+-ATPase of hamster cells creates transmembrane differences of the electrochemical potential of ions not only in the hamster cells, but in the human cells as well.

Membrane potential; Intercellular junction; Ouabain; Energy transmission

1. INTRODUCTION

The energy required for the accumulation of a wide range of solutes in living cells is provided by the action of primary ion pumps, which create transmembrane differences of the electrochemical potential of ions (for reviews, see [1,2]). It is well known that effective ion transfer ('electric coupling') through the permeable cell-to-cell junctions exists in the majority of the multicellular systems: organs, tissues, multicellular microorganisms (for reviews, see [3,4]). The possibility of energy distribution between adjacent cells was substantiated for various multicellular systems via ionic fluxes that pass through permeable cell-to-cell junctions [2,3,5–8].

Previously, we have revealed with the aid of intracellular microelectrodes that the apical cells of *Neurospora crassa* hyphae electrically coupled with other hyphae compartments maintain high membrane potential levels due to the intercellular ion fluxes which are comparable with the ion fluxes generated by the primary ionic pump [9]. Thus, the membrane potential in apical cell with inactive H^+-ATPase (see [10,11]) is supported by the trunk cells where the ATPase is active.

The purpose of this study was to test the ability of animal cells possessing active ion pumps to maintain the membrane potential in the adjacent cells in which the pumps are inactivated.

Correspondence address: T.V. Potapova, A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 119899, USSR

2. MATERIALS AND METHODS

Experiments were carried out on pure and mixed cultures of hamster cells (BHK-21) and primary human fibroblasts (embryonic, from 4th up to 17th passages). A mixed suspension of two cell types (approximately 1×10^5 cells per ml) was passed in flasks containing cover glasses, and grown in the Eagle medium with addition of glutamate and 10% calf serum for 2–3 days by 37°C. The medium was renewed 3–5 h before the experiment.

Electrophysiological measurements were performed with glass microelectrodes (1 M potassium citrate; 50–70 MΩ). Measurements were made in a flow of the cultivation medium by 37°C. Microelectrode insertion was considered to be successful if the cell depolarization in 1–2 min after was negligible. Every other measurement was performed at a distance not less than 5 cells from the previous one. After membrane potential measuring in 10–15 cells of every type (20–30 min in pure cultures and 40–50 min in mixed ones), the cover glass was placed in a control flask or in a flask with 1×10^{-6} M ouabain for 2 h. Then the next set of measurements was performed. About 600 cells have been tested. In the experiment performed by Dr Mittelman, the existence of gap junctions in heterotypic cell culture was shown. For this purpose the distribution of Lucifer yellow between cells in a balanced salt medium by room temperature was studied as it was described previously [3].

3. RESULTS AND DISCUSSION

In this study, we have used the mathematical model developed by Aslanidi and Panfilov to describe ion-osmotic homeostasis in the single animal cell [12]. This model includes a detailed description of ionic transport, when the Na^+/K^+-ATPase hydrolyzes ATP and supports formation of the membrane potential and ion concentration gradients through the plasma membrane. The formation of gap junctions between the two cells (one of them with completely inactive Na^+/K^+-ATPase and the other cell with the active

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

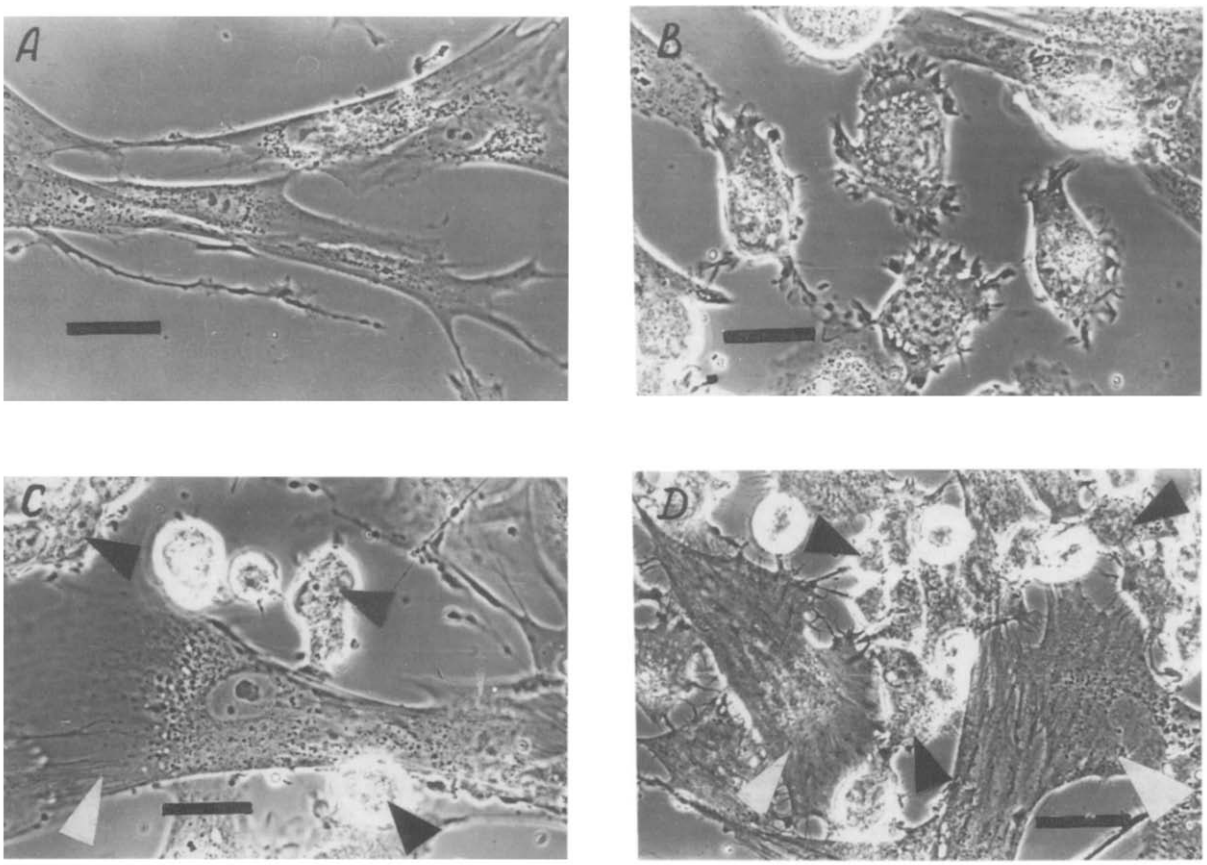

Fig.1. Comparison of the ouabain effect on human fibroblasts in pure and mixed cell cultures. Phase-contrast micrographs of human fibroblasts (white arrowheads) in pure (A,B) or in mixed culture (C,D) with hamster cells (black arrowheads). A,C, incubation without ouabain. B,D, 2 h incubation with 1×10^{-6} M ouabain. Bar, 10 μm.

pump) corresponds, within the framework of the model, to some increase of the plasma membrane permeability in the active cell. A twofold permeability increase leads to a membrane potential decrease in the active cell by 1 mV only. At the same time, the membrane potential in the inactive cell, which is completely abolished in absence of gap junctions, was predicted to be near normal level due to the effective electric coupling via gap junctions with the active cell.

Two types of the animal cells were investigated, i.e. (i) ouabain-sensitive human fibroblasts and (ii) a hamster cell culture which was found to be resistant to low ouabain concentrations. The cultivated cells were rather polymorphous in our experiments. However, as a rule, among hamster cells we could not find cells stretching along the axis. As to the human cells, no small round ones were found (fig.1A,C). After 2 h of incubation with 1×10^{-6} M ouabain, the morphology of human fibroblasts changes considerably: some of them have lost the axis polarization (fig.1B). Under the same conditions, human fibroblasts joined to BHK-21 cells remained spread and elongated in spite of the presence of ouabain (fig.1D).

The results of the electrophysiological measurements are presented in table 1. The average membrane potential values of human fibroblasts and hamster cells in pure cultures were about −40 and −50 mV, respectively. Upon contact with hamster cells, the human fibroblasts were hyperpolarized to a level typical for hamster cells. As it was shown by Hyde et al. [13], gap junction formation between the fibroblasts and myoblasts from the cardiac tissue of 2–7 days old rats in the mixed culture caused hyperpolarization of the fibroblasts from −20 to −60 mV. This effect is similar to that observed in our experiments.

Ouabain treatment came to almost complete depolarization of the human cells in pure culture (table 1 and fig.2). At the same time, the membrane potential in hamster cells was lowered by ouabain only slightly.

Table 1

Effects of ouabain (1×10^{-6} M, 2 h) on the membrane potentials (mV, mean ± SE) of human and hamster cells in pure and mixed cultures

Cell type	Pure cultures		Mixed cultures	
	Control	Ouabain	Control	Ouabain
Hamster cells	−51 ± 1.8	−46 ± 1.8	−47 ± 1.7	−42 ± 1.7
Human cells	−38 ± 1.3	−6 ± 0.6	−47 ± 1.6	−37 ± 1.8

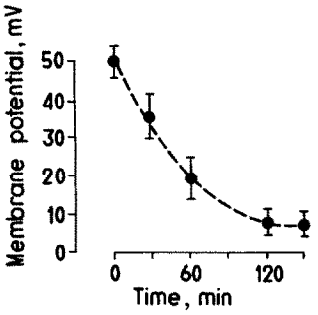

Fig.2. The 1×10^{-6} M ouabain-induced membrane potential changes in human fibroblasts.

The membrane potential values of the human cells joined to hamster cells in the mixed culture were found to be rather high in spite of the ouabain treatment (see table 1).

Thus, the human cells with the inactive Na^+/K^+-ATPases coupled by the gap junctions with the hamster cells maintain high membrane potential level, most probably due to intercellular ionic fluxes which serve as the mechanism of transferring energy from the hamster cells to the human cells. It should be mentioned in this context that cultivation of ouabain-sensitive cells with resistant ones in the presence of ouabain is shown to restore division [14], protein synthesis [15,16] and viability [17] of the sensitive cells.

Different examples of spatial separation of primary and secondary plasma membrane ion transport mechanisms are known [18–22]. According to suggestions of Skulachev [23], Mitchell [24], and Crane [25], the energy coupling of the spatially segregated primary and secondary transport mechanisms can be carried out by means of lateral ion currents. The local electric currents were really demonstrated for some animal and plant cells, but their functional significance remained virtually obscure [1,10,11,18,19,21]. We have once more obtained evidence (see also [6,9]) that ionic interactions through permeable intercellular junctions might be involved in the energy redistribution between the adjacent cells. These observations might be useful for the study of the energy budget of the tissue cells.

Acknowledgements: We would like to thank Professor V.P. Skulachev, Professor L.M. Chailakhyan and Dr D.B. Zorov for useful advice and Mrs A.L. Kulakova for technical assistance.

REFERENCES

[1] Harold, F.M. (1986) The Vital Force: A Study of Bioenergetics, Freeman, New York.
[2] Skulachev, V.P. (1988) Membrane Bioenergetics, Springer, Berlin.
[3] Berkinblit, M.B., Boitzova, L.Ju., Bozshkova, V.P., Mittelman, L.A., Potapova, T.V., Chailakhyan, L.M. and Sharovskaya, Ju.Ju. (1981) High-Permeable Contact Membranes, Nauka, Moscow (in Russian).
[4] Satir, B.H., ed. (1988) in: Gap Junctions. Modern Cell Biology 7, Liss, New York.
[5] Skulachev, V.P. (1980) Biochim. Biophys. Acta 604, 297–320.
[6] Chailakhyan, L.M., Glagolev, A.K., Glagoleva, T.N., Murvanidze, G.V., Potapova, T.V. and Skulachev, V.P. (1982) Biochim. Biophys. Acta 679, 60–67.
[7] Aslanidi, K.B., Potapova, T.V. and Chailakhyan, L.M. (1988) Dokl. Akad. Nauk SSSR 299, 992–996.
[8] Potapova, T.V., Aslanidi, K.B. and Chailakhyan, L.M. (1988) Biol. Membr. 5, 613–627.
[9] Potapova, T.V., Aslanidi, K.B., Belozerskaya, T.A. and Levina, N.N. (1988) FEBS Lett. 241, 173–176.
[10] Harold, F.M., Schrauts, W.J., Harold, R.L. and Caldwell, J.C. (1985) Microbiol. Sci. 2, 363–366.
[11] Takeuchi, Y., Schmidt, J., Caldwell, J.H. and Harold, F.M. (1988) J. Membr. Biol. 101, 33–41.
[12] Aslanidi, K.B. and Panfilov, A.N. (1986) Mathem. Biosci. 79, 45–54.
[13] Hyde, A., Blondel, B. and Matter, A. (1969) Progr. Brain Res. 31, 283–311.
[14] Corsaro, C.M. and Migeon, R.R. (1977) Nature 268, 737–739.
[15] Ledbetter, M.L.S. and Lubin, M. (1979) J. Cell Biol. 80, 150–165.
[16] Ledbetter, M.L.S., Young, G.J. and Wright, E.R. (1986) Am. J. Physiol. 250, C306–C313.
[17] Potapova, T.V. and Fetisova, E.K. (1981) Tsitologia 23, 38–47.
[18] Jaffe, L.E., Robinson, K.R. and Nuccitelli, R. (1974) Ann. NY Acad. Sci. 238, 372–389.
[19] Stern, C.D. and McKennzie, D.D. (1983) J. Embryol. Exp. Morphol. 77, 73–98.
[20] Ahlmers, W. and Stirling, C. (1984) J. Membr. Biol. 77, 169–186.
[21] DeLoof, A. (1985) Compar. Biochem. Physiol. 80A, 453–459.
[22] Rossier, B.C., Geering, K. and Krauhenbuhl, J.P. (1987) Trends Biol. Sci. 12, 483–487.
[23] Skulachev, V.P. (1971) Curr. Top. Bioenerg. 4, 127–190.
[24] Mitchell, P. (1973) J. Bioenerg. 4, 63–91.
[25] Crane, R. (1977) Rev. Physiol. Biomed. Pharmacol. 78, 99–159.