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Energetic cooperation via ion-permeable junctions in mixed animal cell cultures

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Low ouabain concentration (1×10^{-6} M) is shown to decrease intracellular K^+ (K^+_{in}) and to increase intracellular Na^+ (Na^+_{in}) in human fibroblast cell cultures. The same ouabain concentration was without effect upon K^+_{in} ad Na^+_{in} in rodent cultures such as BHK-21, mouse fibroblasts and rat glyoma C6 cells. K^+_{in} and Na^+_{in} in the mixed cultures of human and BHK-21 fibroblasts or human and mouse fibroblasts were found to be resistant to 1×10^{-4} M ouabain whereas that of the mixtures of human and rat glyoma C6 cells proved to be ouabain-sensitive. The gap-junction-mediated dye transfer was revealed between human and BHK-21 cells. Such an effect was very small in the human–C6 cell mixed culture. It is concluded that cells with active ion pumps can support the maintenance of K^+ and Na^+ gradients in cells with inactive pumps, provided that effective ion transport via gap junctions takes place.

Intercellular junction; Intracellular ion content; Cell culture; Ouabain; Energy transfer

1. INTRODUCTION

The ionic pumps of animal plasma membranes are known to maintain electrochemical gradients of K^+ and Na^+ ions, the latter being used to support the uptake of many ions and metabolites [1–4]. This fact has stimulated interest in the study of the functional role of the electrical and ionic coupling between animal cells through junctions permeable for a number of the low molecular compounds [5–8].

Cells of animals of different species are able to form heterotypic permeable junctions in a mixed culture (for review, see [9]). Combined cultivation of animal cells with different sensitivities to ouabain in the presence of ouabain doses specifically inhibiting ion pumps in one cell type is known to normalize such cells with respect to a number of parameters, including genetics [10], viability [11] and protein synthesis [12,13]. It was postulated that such cell co-operation is due to ion fluxes through permeable junctions.

A mathematical model developed in this group [14] suggests that in a cell population consisting of cells with (a) active and (b) completely inactive ion pumps, the ion gradients can be maintained not only in (a) but also in (b) cells provided that two types of cells form permeable junctions [7,8]. To check this prediction, we have studied mixed cultures of cells that differed considerably in their sensitivities to the Na^+/K^+-ATPase inhibitor ouabain.

We have already published some estimates of morphological and electrophysiological characteristics for a permeable junction-connected mixed animal cell culture [7], and for permeable junction-connected cells from the fungal hyphae tops of *Neurospora crassa* [15].

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2. MATERIALS AND METHODS

Cultures of human and mouse primary embryonic fibroblasts, hamster fibroblasts of the BHK-21 line (obtained from a collection of cultures of the Institute of Virology, Acad. Sci. USSR) and rat glyoma C6 cells (obtained from a collection of cultures of the Institute of Cytology, Acad. Sci. USSR) were maintained in Eagle's medium supplemented with 10% fetal calf serum and 50 mg/ml gentamycin as described in [7,8]. To produce mixed cultures of desirable densities and to estimate the proportion of each cell type in the final mixture we have used some specifical procedures described in detail in [8].

To measure the intracellular ion content, cells were incubated with or without 1 μM ouabain for 2 h in the usual culture medium. The medium was then removed, the culture rapidly washed three times with cold (4°C) Tris-HCl buffer, pH 7.2, and ions were extracted with 5% trichloroacetic acid. Ion concentrations in the extract were determined by flame-emission photometry using a Perkin-Elmer AA spectrophotometer (model AA 306) as described in [16,17]. Protein concentration was determined according to Lowry [18]. Every figure in the tables represents a mean value of triplicate measurements, the values agreeing within 1–5%.

Formation of gap junctions in heterotypic cell contacts was shown by using intracellular glass microelectrodes with a tip diameter of less than 0.3 μm to inject fluorescent dyes, gap junction markers, including sodium fluorescein (mol. wt. 330, Sigma) or Lucifer yellow (mol. wt. 660, Sigma) as described in [9]. The efficiency of gap junc-

Table I

Effects of 1 μM ouabain on the potassium and sodium content of the human and rodent cells in pure cultures

Cell cultures	Total density of the cell culture, × 10^4 of cells/cm²	Without ouabain (mmol of ion/g protein)			With ouabain (mmol of ion/g protein)		
		K^+_{in}	Na^+_{in}	K^+_{in}/Na^+_{in}	K^+_{in}	Na^+_{in}	K^+_{in}/Na^+_{in}
Human fibroblasts:							
(a)	3.5	0.76	0.14	5.3	0.24	0.58	0.4
(b)	4.0	0.76	0.17	4.3	0.27	0.63	0.4
(c)	8.0	0.65	0.14	4.8	0.15	0.96	0.2
Mouse fibroblasts	12.0	0.72	0.12	6.1	0.68	0.11	6.3
Hamster fibroblasts, BHK-21	44.0	0.90	0.10	9.0	0.93	0.10	9.0
Rat glyoma C6 cells	11.0	0.73	0.12	6.1	0.71	0.11	6.3

Table II

Effects of 1 μM ouabain on the potassium and sodium content in mixed cultures of the human and rodent cells

Cell cultures	Total density of the cell culture, × 10^4 of cells/cm²	Human-to-rodent cell ratio		Degree of cell-to-cell coupling, (%)	Without ouabain (mmol of ion/g protein)			With ouabain (mmol of ion/g protein)		
		by cell numbers	by protein content		K^+_{in}	Na^+_{in}	K^+_{in}/Na^+_{in}	K^+_{in}	Na^+_{in}	K^+_{in}/Na^+_{in}
Human fibroblasts (b), mouse fibroblasts	9.0	1:3	1:15	100*	0.63	0.12	5.2	0.61	0.12	5.0
Human fibroblasts (c), BHK-21	44.0	1:5	1:2	83	0.90	0.14	6.4	0.94	0.15	6.4
Human fibroblasts (a), glyoma C6	14.5	1:3	1:1	30	0.71	0.10	7.4	0.45	0.27	1.7

* The data from ref. [9]

tions ('degree of the cell-to-cell coupling') was estimated by the percentage of dye-loaded cells transferring the label into neighbouring cells during 2 min after the 1 min injection.

3. RESULTS

As was to be expected, Na^+_{in} and K^+_{in} of the rodent cells resistant to 1 μM ouabain did not change after 2 h incubation with this dose of the inhibitor while in human fibroblasts, a 2- to 4-fold decrease in K^+_{in} and a 3- to 6-fold increase in Na^+_{in} occurred (Table I).

A study of the distribution of a fluorescent dye between the different cell types in mixed cultures revealed permeable junctions between human fibroblasts and rodent fibroblasts to be more effective than between human fibroblasts and rat glyoma C6 cells (see ref. [8] for details).

When human and rodent fibroblasts were cultured together, ouabain failed to decrease the K^+_{in}/Na^+_{in} ratio (Table II). In mixed cultures of human fibroblasts and rat glyoma C6 cells, ouabain was found to decrease this ratio (Table II).

Thus, the above experiments showed that mixed cell cultures, containing a considerable number of cells with completely inactivated ion pumps, can maintain the K^+_{in}/Na^+_{in} ratio close to normal, provided that effective permeable junctions are formed between the ouabain-sensitive and ouabain-resistant cells.

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