High-Temperature Inactivation of Passive Potassium Transport in Electrically Non-Excitable Cells

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Mouse Cell Cultures, Passive Potassium Efflux, Temperature Dependence

High-temperature inactivation of passive potassium transport is demonstrated for mouse cell cultures and discussed in analogy to recent results on model systems from phospholipid/cholesterol doted with channel-forming antibiotics.

Introduction

Following early suggestions [1 – 4] of vital membrane functions being regulated by cooperative transitions of the membrane, very detailed studies of sugar transport in bacteria [5 – 11] and active alkali-ion transport in mammalian cell membranes [12 – 16] have established a close correlation between these transport functions and the phase equilibria of outer cell membranes. In essentially all these and other cases [17] studied, transport or enzymatic functions of the membrane were optimally active (with fairly low activation energy of typically about 60 kJ mol^{-1}) in the high-temperature (or "fluid") phase, but were functioning with relatively high activation energy (of typically about 120 kJ mol^{-1}) and/or were largely inactivated in the low-temperature ("solid" or "gel") phase. Freeze-etch electron microscopic data indicated a preferential location of membrane proteins in the "fluid" domains of the membrane [18, 19]. These results would appear consistent with the generalization that transport and catalytic membrane proteins function optimally and partition preferentially in the fluid domains of a heterogeneous membrane.

Recent evidence, however, suggests that the sodium channels in nerve membranes are functional only with their surrounding lipids being in the "gel" phase [20].

In the following, we wish to present data on passive potassium transport in the plasma membrane of permanent mouse cell cultures showing a tripartite temperature characteristic: a high-temperature branch with relatively large activation energy, a low-temperature branch with much lower activation energy, and a decrease at intermediate temperatures from the low-to the high-temperature branch. This characteristic of high-temperature inactivation is consistent with passive potassium transport molecules (pores or carriers) in the plasma membrane of these cells at a given temperature functioning more effectively in "solid" than in "fluid" regions of the membrane.

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Materials and Methods

Swiss 3T3 cells and SV40-3T3 cells (line 101) were grown as described earlier [21]. In order to minimally perturb the physiological state of the cells growing on the culture substrate, potassium transport was measured with ion-specific electrodes, recording potassium ionic activity directly above the cells during incubation with low-potassium (0.1 mM) modified Ringer solution. Low external potassium concentration in the incubation medium served two purposes: i) to provide for an electrochemical gradient of potassium across the cell membrane leading to net K^+-efflux, ii) to practically eliminate active potassium influx. Inactivation of active K^+-influx at external K^+-concentration of about 0.1 mM has previously been demonstrated for the same cell types [22]. Controls with 1 – 3 mM ouabain in the incubation solution under the experimental conditions of the present study yielded the same results as experiments without ouabain. Thus, K^+effluxes in the present experiment may be regarded as purely passive.

For measurement, the cell sheet was rinsed twice with 5 ml of thermostated modified Ringer solution (MRS) at pH 7.4, containing 144 mM NaCl, 0.1 mM KCl, 1 mM $CaCl_2$, and 10 mM triethanol amine: HCl, and then incubated with 10 ml MRS at the desired temperature in a thermostated aluminium block, the whole setup being mounted on a gyratory moving table and agitated with about 15 cycles per

minute. Potassium ion activity was recorded continuously from the incubation solution using an ion-specific potassium electrode (model IS 561 K, Philips Elektronik, Hamburg) and double junction reference electrode (model 90 – 92, Orion Research, Cambridge, Mass.). Potassium efflux φ_K in mol $cm^{-2}\,s^{-1}$ was evaluated from the initial slope dE/dt of the experimental recording of electrical potential difference E between potassium and reference electrodes versus time t using the relation

$$\varphi_K = \frac{2.303\,V\,K_{e0}}{SNA}\left(\frac{dE}{dt}\right)_{t=0},$$

where $V = 10$ ml is the incubation volume, S the response characteristic of the electrode assembly given in mV/log (K_{e1}/K_{e0}) and calibrated with 10 ml MRS containing $K_{e0} = 0.1$ mM and $K_{e1} = 1$ mM before and after each recording, using the same conditions. Cell number N per plate was determined by electrical counting (model ZF, Coulter Electronics, Krefeld) after release of the cells from the plate in Ca-free MRS with 0.25% trypsin (Flow Laboratories, Bonn), this procedure following each experiment. Cell surface areas A were taken from an independent parallel study [23] and are reproduced in Table II. Temperatures are accurate within ± 0.5 °C as determined by Pt 100 resistance thermometer.

Results and Discussion

Passive K^+-effluxes are plotted in Fig. 1 on a logarithmic scale *versus* reciprocal temperature. These data are taken at a cell density of 3T3 cells of $3 \times 10^4\,cm^{-2}$, which corresponds to a cell monolayer near saturation density, and at a cell density of SV40-3T3 cells of $5 \times 10^4\,cm^{-2}$, which approximates monolayer covering of the cell plate. Evidently, temperature characteristics of passive K^+-efflux for both cell lines shown in Fig. 1 differ markedly from those observed for active K^+-influx for the same cell types [16], for (Na + K)-activated ATPase [12 – 15], or for sugar transport into bacterial cells [5 – 11]. The phenomenon of high-temperature inactivation of K^+-efflux, as exhibited by the temperature characteristic of 3T3 cells at 3×10^4 cells/cm^2, is even more pronounced at lower cell densities and leads to lower K^+-effluxes at 37 °C than at 25 °C. We have investigated K^+-effluxes φ_K at 25 °C and 37 °C for different cellular growth densities D of both cell lines. Within experimental error these data may well be described by double logarithmic linear regression analysis (see Table I) using:

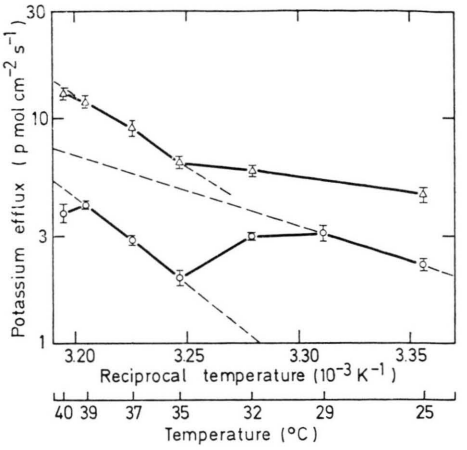

Fig. 1. Dependence on reciprocal temperature of passive potassium efflux from mouse cell cultures. 3T3 cells and SV40-3T3 cells were measured at densities of $3 \times 10^4\,cm^{-2}$ and $5 \times 10^4\,cm^{-2}$, respectively. Experimental points are averages from three independent measurements of K^+-effluxes using different petri plates. Error bars indicate standard error of the mean. 3T3 cells (○); SV40-3T3 cells (△); broken lines indicate procedure for evaluation of activation energies given in Table II (see text).

$$\varphi_K = \varphi_1\left(\frac{D}{D_1}\right)^b \qquad (1)$$

where $D_1 = 1 \times 10^4\,cm^{-2}$, and φ_1 and b are coefficients adjusted by regression analysis [24]. In Table I are given minimum and maximum cell densities, D_{min} and D_{max}, respectively, number n of independent experiments, correlation coefficient r, and the coefficients b and $\ln \varphi_1$ with their standard deviations s_b and $s_{\ln \varphi_1}$, respectively. Furthermore, we have computed the average K^+-efflux $\varphi_{0.3}$ at a cell density of $0.3 \times 10^4\,cm^{-2}$ and given in Table I as the logarithm and its standard deviation. From these data it is evident that for both cell lines at $D \leqq 10^4\,cm^{-2}$ the K^+-efflux at 25 °C is larger than at 37 °C, implying clearly the phenomenon of high-temperature inactivation. Interestingly, both normal and transformed cells behave very similar in their temperature dependence at small cell densities: the ratio of K^+-efflux at 25 °C to that at 37 °C is 1.80 ± 0.01 at $0.3 \times 10^4\,cm^{-2}$ and 1.34 ± 0.02 at $10^4\,cm^{-2}$ for both cell lines. However, at larger cell densities, temperature characteristics of normal and transformed 3T3 cells differ as is exhibited in Fig. 1

Table I. Results on K^+-effluxes of 3T3 and SV40-3T3 cells at different growth densities and parameters of double logarithmic linear regression analysis according to $\varphi_K = \varphi_1 (D/D_1)^b$ with $D_1 = 10^4\ cm^{-2}$, and b, φ_1 and $\varphi_{0.3}$ ($= \varphi_K$ at $3 \times 10^3\ cm^{-2}$) obtained by regression analysis; φ_K, φ_1 and $\varphi_{0.3}$ being given in pmol $cm^{-2}\ s^{-1}$.

Cell type	T [°C]	D_{min} [$10^4\ cm^{-2}$]	D_{max} [$10^4\ cm^{-2}$]	n	$-r$	$-(b \pm s_b)$	$\ln \varphi_1 \pm s_{\ln \varphi_1}$	$\ln \varphi_{0.3} \pm s_{\ln \varphi_{0.3}}$
3T3	25	0.2	7.6	29	0.968	0.913 ± 0.046	2.47 ± 0.07	3.57 ± 0.12
	37	0.4	3.6	15	0.975	0.663 ± 0.042	2.18 ± 0.04	2.98 ± 0.07
SV40-3T3	25	0.2	9.1	16	0.943	0.854 ± 0.033	3.33 ± 0.04	4.355 ± 0.075
	37	0.3	1.3	12	0.959	0.614 ± 0.057	3.025 ± 0.035	3.76 ± 0.06

and as was confirmed at still higher densities of SV40-3T3 cells, yielding $\varphi_K(25\ °C = 4.69 \pm 0.88(4)$ pmol $cm^{-2}\ s^{-1}$ and $\varphi_K(37\ °C = 11.44 \pm 0.61(3)$ pmol $cm^{-2}\ s^{-1}$ at $D = 13.2 \times 10^4\ cm^{-2}$ (data $\pm$ s. e. m., number of experiments in parenthesis).

Phenomenologically, the tripartite temperature characteristic for 3T3 cells may be described by:

$$\varphi_K = (1 - \nu) A_0 \exp(-E_0/RT) + \nu A_1 \exp(-E_1/RT) \quad (2)$$

where $0 \leqq \nu \leqq 1$ approaches 0 or 1 at low or high temperatures, respectively, as indicated by broken lines in Fig. 1, and are given in Table II. For a more detailed interpretation we observe that passive K^+-permeability of these cells at 25 °C [25] and at 37 °C [Adam, Ernst and Seher, submitted] follows closely K^+-efflux in its dependence on cell density. Thus, we may interpret the high-temperature inactivation of K^+-efflux shown by our data as reflecting a virtually parallel high-temperature inactivation of passive K^+-permeability.

Table II. Growth parameters and parameters describing asymptotic temperature characteristics of K^+-efflux for 3T3 and SV40-3T3 cells.

Cell line	3T3	SV40-3T3
Cell growth density [cm^{-2}]	3×10^4	5×10^4
Cell surface area $\pm$ s. e. m. * [$10^{-5}\ cm^2$]	4.53 ± 0.14 (43)	1.68 ± 0.08 (9)
E_0 [kJ mol^{-1}]	62	—
A_0 [pmol $cm^{-2}\ s^{-1}$]	1.51×10^{11}	—
E_1 [kJ mol^{-1}]	150	125
A_1 [pmol $cm^{-2}\ s^{-1}$]	4.62×10^{25}	8.73×10^{21}

* From ref. 23, (number of independent measurements in parentheses).

Interestingly, investigation of phase properties by measuring the partitioning coefficient f of TEMPO spin label into 3T3 and SV40-3T3 cells grown in calf serum revealed inflection points at 32, 18 and 12 °C, which could be shown to shift after changing the lipid composition of the cells [26]. The uppermost of these inflection points corresponds well with the temperature region of permeability changes described above. Furthermore, in close correlation to the results taken from Table I, of almost parallel temperature changes of normal and transformed cells at *low* cell densities, the above authors using fairly low cell densities do not find differences in temperature dependence of spectroscopic signals from spin labels in normal and transformed cells [26]. Thus, the temperature characteristics reported in the present study might well reflect changes of the lateral phase equilibria of the lipid protein matrix of the plasma membrane. This suggestion is supported by results of studies of passive K^+-efflux from *Acholeplasma laidlawii* or liposomes from egg lecithin in the presence of cholesterol and certain polyene antibiotics such as amphotericin B, nystatin or etruscomycin which demonstrate also the phenomenon of high-temperature inactivation [27]. These antibiotics have been shown to aggregate with cholesterol [28, 29] and might well serve as models for transport molecules in native biological membranes. Thus, high-temperature inactivation of passive K^+-permeation in cells, as reported here, might be reduced mechanistically to the following possibilities (which are not mutually exclusive): i) complexes of K^+-transport molecules and cholesterol from stable pores at low temperatures and dissociate at rising temperatures [27], ii) K^+-transport molecules are more effective and/or reside preferentially in "quasicrystalline" regions of a laterally heterogeneous plasma membrane.

Then, the above relation immediately suggests a mechanistic interpretation of the data in terms of

fractions $\nu = N_1/N$ and $(1-\nu) = N_0/N$ of N potassium transport molecules (pores or carriers) residing in the fluid (cholesterol-deficient) and in the solid (cholesterol-rich) regions, respectively, of the membrane. Here, the sum $N = N_0 + N_1$ of transport molecules in the "solid" and "fluid" phases is implied to be independent of temperature. The dependence of ν on temperature does not necessarily represent the temperature dependence of the membrane transition as preferential partitioning of the transport molecules into the "fluid" or the "solid" domains would lower or raise, respectively, the temperature region of K^+-permeability change relative to that of the underlying membrane transition.

The temperature characteristics of SV40-3T3 cells at higher cell densities according to the data given in Fig. 1 and reported above, are clearly different from those of 3T3 cells, even at a comparable monolayer coverage of the plate as intended in the experiments given in Fig. 1.

For a more detailed discussion of the temperature characteristic, in particular below 35 °C, the data on SV40-3T3 cells are not sufficient and, therefore, activation energies are not derived for the low-temperature branch in this case.

It is of considerable interest that our recent investigation on K^+-transport of isolated rat liver cells [30] demonstrated high activation energies of 125 to 200 kJ mol^{-1} at high temperatures and low activation energies of 15 to 45 kJ mol^{-1} at low temperatures, which is very similar to the results presented here. Clearly, work on other cell types and other ionic species (*e. g.* Na^+, Cl^-) is needed in order to check the intriguing possibility suggested from previous work [20, 30] and from the present data: that passive ion transport through cell membranes generally functions more effectively in "solid" than in "fluid" domains of cell membranes.

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