Influence of medium amino acids on the ouabain sensitive and insensitive $^{86}Rb^+$-fluxes in HeLa cells

A. Schaefer and K.-H. Munter[1]

Abteilung Molekularbiologie, Universität Hamburg, Grindelallee 117 IV, D-2000 Hamburg 13 (Federal Republic of Germany),
10 June 1985

Summary. Components of the $^{86}Rb^+$-influx in HeLa cells were investigated in Joklik minimal essential medium, or in Earle's balanced salt solution with and without medium amino acids. The presence of amino acids led to the stimulation of the ouabain sensitive $^{86}Rb^+$-uptake and inhibition of the diuretic-sensitive and residual $^{86}Rb^+$-fluxes. These results show that the presence of amino acids is an important regulator of the K^+/Rb^+-fluxes under normal conditions in growth medium.
Key words. HeLa cells; Na^+, K^+-pump; Na^+, K^+, $2Cl^-$-cotransport; amino acids; monensin.

The intracellular Na^+-level is an important determinant of the Na^+, K^+-pump activity[2–4]. Since under normal conditions the cellular sodium concentration is low, the Na^+, K^+-pump is operating below its capacity and its activity depends on the rate of sodium influx[5,6]. Parallel changes in the sodium influx and the Na^+, K^+-pump activity measured as ouabain sensitive $^{86}Rb^+$-influx are well-documented in several cell-culture systems[7–12]. Na^+, K^+-ATPase dependent cation pumping has been shown to respond to sodium coupled transport of solutes such as alanine, taurocholate, glycine or alpha-aminoisobutyric acid[4,13]. Several amino acids in tissue-culture medium enter the cell by a Na^+-coupled transport[14]. Therefore, we were interested in the question of how the presence of amino acids in growth medium contributes to the regulation of the activity of the Na^+, K^+-pump in HeLa cells. Three components of the $^{86}Rb^+$-influx ($^{86}Rb^+$ is a K^+ analogue in HeLa cells: see Ikehara et al.[15]) were investigated: the ouabain sensitive $^{86}Rb^+$-influx, reflecting the Na^+, K^+-pump activity, the diuretic-inhibitable Na^+, K^+, 2 Cl^--cotransport system[16,17] and the residual $^{86}Rb^+$-influx in the presence of both ouabain and piretanide. This latter $^{86}Rb^+$-influx may be an indicator of changes in the passive permeability of the plasma membrane towards monovalent cations[18].

Materials and methods. Logarithmically growing HeLa S3 cells were harvested by centrifugation and washed once in the corresponding incubation medium (see below). Cells (3×10^6, 500 µl) were incubated with about 0.3 µCi $^{86}Rb^+$-chloride (New England Nuclear Corp., 1.94 mCi/mg) in Joklik minimal essential medium (MEM) and in Earle's balanced salt solution (EBSS) wihtout or with the amino acids present in Joklik MEM (105 mg/l L-arginine-HCl, 29.6 mg/l L-cystine-Na, 294 mg/l L-glutamine, 41.9 mg/l L-histidine-HCl, 52 mg/l L-isoleucine, 52 mg/l L-leucine, 72.5 mg/l L-lysine-HCl, 15 mg/l L-methionine-HCl, 32 mg/l phenylalanine, 48 mg/l L-threonine, 10 mg/l L-tryptophan, 37.8 mg/l L-tyrosine, 46 mg/l L-valine, purchased from Seromed, München). The ouabain sensitive, diuretic sensitive and residual $^{86}Rb^+$-influxes were measured by using the inhibitors ouabain (1 mM, Serva) and piretanide (1 mM, Hoechst) in the incubation mixtures. If not otherwise stated, incubations were carried out for 8 min at 37 °C and stopped by centrifugation of the cell suspension through a dibutylphthalate-dinonylphthalate (Fluka) 3:1 oil mixture. $^{86}Rb^+$-uptake was measured and calculated as described previously[12]. Zero point values were substracted in order to correct the results for non-specific binding of radioactivity by the cell pellet. The cellular protein content was determined by the method of Lowry et al.[19].

Results. Figure 1 shows the uptake of $^{86}Rb^+$ in HeLa cells during the first 10 min of incubation in Joklik MEM. $^{86}Rb^+$-uptake was strongly inhibited by ouabain, which indicated that the major part of $^{86}Rb^+$ was taken up via the Na^+, K^+-pump under these conditions. Inhibition of the $^{86}Rb^+$-uptake by the Na^+, K^+, $2Cl^-$-cotransport-inhibitor piretanide[17] could be observed only when the $^{86}Rb^+$-uptake had already been reduced by the presence of ouabain.

In further experiments, components of the $^{86}Rb^+$-uptake were studied in EBSS with and without Joklik MEM amino acids (fig. 2). The effect of amino acids on the $^{86}Rb^+$-fluxes was compared with the effect of the sodium ionophore monensin. Monensin is known to stimulate the Na^+, K^+-pump activity by increasing the sodium influx and thus the supply of sodium to the pump[8,9]. Replacement of Joklik MEM with EBSS caused marked alteration in the $^{86}Rb^+$-fluxes. The contribution of the ouabain sensitive $^{86}Rb^+$-uptake became smaller and there was an increase in the ouabain insensitive $^{86}Rb^+$-influx. Addition of amino acids to EBSS at the same concentrations as in Joklik MEM led to enhancement of the ouabain-sensitive $^{86}Rb^+$-uptake and inhibition of the ouabain insensitive $^{86}Rb^+$-influx. Within the ouabain insensitive $^{86}Rb^+$-influx, both the piretanide inhibitable and the residual $^{86}Rb^+$-influxes were significantly reduced.

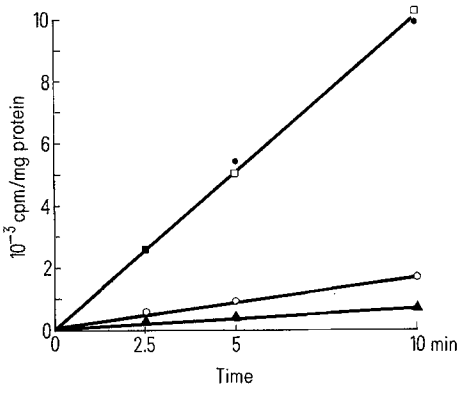

Figure 1. Time course of the initial $^{86}Rb^+$-uptake of HeLa cells incubated in Joklik MEM. Symbols: control (●), 1 mM piretanide (□), 1 mM ouabain (○), 1 mM ouabain + 1 mM piretanide (▲).

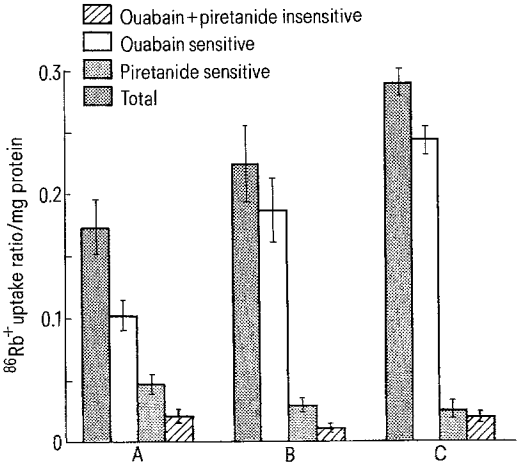

Figure 2. Components of the $^{86}Rb^+$-influx of HeLa cells measured in EBSS. *A* control, *B* in the presence of Joklik MEM amino acids, *C* in the presence of 2 µM monensin. The concentration of the inhibitors used was 1 mM. Piretanide sensitive $^{86}Rb^+$-influx was estimated as part of the ouabain insensitive uptake. Uptake ratios were calculated as activity in cell pellet/total radioactivity in 200 µl samples[12]. Results show the means of four experiments ±SE.

Experiments with 2 μM monensin yielded comparable results regarding the stimulation of the ouabain sensitive $^{86}Rb^+$-uptake and inhibition of the piretanide sensitive $^{86}Rb^+$-influx in the presence of ouabain. However, the residual $^{86}Rb^+$-influx in the presence of ouabain and piretanide was not affected by monensin.

In order to investigate whether or not the effect of the presence of amino acids on the $^{86}Rb^+$-fluxes could be brought about by the addition of only one amino acid, we studied the effects of alanine, methionine and of the model amino acid alpha-amino-isobutyric acid (AIB) on the $^{86}Rb^+$-fluxes in EBSS (table). These amino acids are taken up mostly by Na^+-cotransport[14]. Amino acids were added at a concentration of 2 mM. These results show, that the stimulation of the ouabain sensitive $^{86}Rb^+$-uptake and reduction of the piretanide inhibitable part of the ouabain insensitive $^{86}Rb^+$-uptake could be brought about by one of these amino acids, whereas reduction in the residual $^{86}Rb^+$-influx in the presence of ouabain and piretanide was not observable. This situation did not change when, in the case of alanine, a higher concentration (4 mM) was investigated.

Discussion. The results of this study present further evidence that Na^+-coupled amino acid transport is an important regulator of the Na^+, K^+-pump. The cation pumping activity may therefore respond to physiological changes in which the amino acid uptake is altered. On the other hand, the presence of the amino acids in the cell-culture medium affects also the ouabain insensitive components of the $^{86}Rb^+$-uptake. Inhibition of the $^{86}Rb^+$-influx via the Na^+, K^+, 2 Cl^--cotransport system by amino acids may be related to an increased sodium influx, since the sodium ionophore monensin had a similar effect. However, a change in cell volume due to the accumulation of amino acids may also contribute to the inhibition of the diuretic sensitive $^{86}Rb^+$-influx[17,20]. The residual $^{86}Rb^+$-uptake in the presence of ouabain and piretanide was reduced only when all the amino acids of Joklik MEM were added. This effect cannot be related to the sodium influx, since it was not observed after treatment by monensin or the investigated amino acids. The mechanism by which the presence of amino acids inhibits this $^{86}Rb^+$-influx is unclear. The reduction of the residual $^{86}Rb^+$-uptake may indicate a decreased permeability of the plasma membrane under these conditions.

Effect of amino acids on the $^{86}Rb^+$-influx in HeLa cells

Components of $^{86}Rb^+$-influx	$^{86}Rb^+$-uptake in the presence of amino acid (% of control)		
	Alanine	Methionine	AIB
Ouabain sensitive	165.4	145.0	168.2
Ouabain insensitive, piretanide inhibitable	65.8	58.7	63.4
Ouabain and piretanide insensitive	109.2	91.3	117.5

$^{86}Rb^+$-uptake was measured in EBSS with and without amino acid (2 mM). The results are expressed as percentages of the corresponding control values without amino acid. The concentration of the inhibitor used was 1 mM.

In summary, all components of the $^{86}Rb^+$-influx studied were shown to be affected by the presence of amino acids in the cell-culture medium. Thus the marked alteration in the $^{86}Rb^+$-influx due to the absence of amino acids in the incubation medium indicates that data on ion transport mechanisms obtained with cells in buffer solutions must be interpreted cautiously since these data may not reflect ionic fluxes of cells grown under normal culture conditions.

1 Acknowledgments. We thank Dr G. Steinheider for his help in the preparation of this manuscript. This work was supported by the Deutsche Forschungsgemeinschaft.

2 Post, R.L., Regulatory Functions of the Biological Membranes, p. 163. Elsevier, Amsterdam 1968.
3 Pietrzyk, C., Geck, P., and Heinz, E., Biochim. biophys. Acta *513* (1978) 89.
4 Van Dyke, R.W., and Scharschmidt, B.F., J. biol. Chem. *258* (1983) 12912.
5 Yamamoto, S., Akera, T., and Brody, T.M., Biochim. biophys. Acta *555* (1979) 270.
6 Akera, T., Yamamoto, S., Temma, K., Kim, D.-H., and Brody, T.M., Biochim. Biophys. Acta *640* (1981) 779.
7 Smith, J.B., and Rozengurt, E., Proc. natn. Acad. Sci. USA *75* (1978) 5560.
8 Mendoza, S.A., Wigglesworth, N.M., Pohjanpelto, P., and Rozengurt, E., J. Cell Physiol. *103* (1980) 17.
9 Rozengurt, E., Gelehrter, T.D., Legg, A., and Pettican, P., Cell *23* (1981) 789.
10 Moolenaar, W.H., Mummery, C.L., Van der Saag, P.T., and de Laat, S., Cell *23* (1981) 789.
11 Pouyssegur, J., Chambard, J.C., Franchi, A., Paris, S., and Van Obberghen-Schilling, E., Proc. natn. Acad. Sci. USA *79* (1982) 3935.
12 Schaefer, A., Munter, K.-H., Geck, P., and Koch, G., J. Cell Physiol. *119* (1984) 335.
13 Racker, E., Johnson, J.H., and Blackwell, M.T., J. biol. Chem. *258* (1983) 3702.
14 Shotwell, M.A., Kilberg, M.S., and Oxender, D.V., Biochim. biophys. Acta *737* (1983) 267.
15 Ikehara, T., Sakai, T., Miyamoto, H., and Kaniike, K., Jap. J. Physiol. *32* (1982) 13.
16 Geck, P., Pietrzyk, C., Burckhardt, B.-C., and Heinz, E., Biochim. biophys. Acta *600* (1980) 432.
17 Geck, P., Heinz, E., and Pfeiffer, B., Ototoxic Side Effect of Diuretics. Eds R. Klinke, W. Lahn, H. Querfurth and J. Scholtholt. Scand. Audiol., suppl. 14 (1981) 25.
18 Schaefer, A., Geck, P., Zibirre, R., Kühne, J., and Koch, G., Virology *136* (1984) 457.
19 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
20 Ueberschar, S., and Bakker-Grunwald, T., Biochim. biophys. Acta *731* (1983) 243.

0014-4754/86/040436-02$1.50 + 0.20/0

Cytokinesis in onion roots: inhibition by vanadate and caffeine

P. Navas, A. Hidalgo and G. Garcia-Herdugo

Departamento de Morfología y Fisiología, Facultad de Ciencias, Universidad de Córdoba, E-14071 Córdoba (Spain), 4 March 1985

Summary. The effect of vanadate ions on plant cytokinesis has been studied in *Allium cepa* root meristematic cells. Vanadate induces binucleate cells by inhibiting cell plate formation. Moreover, vanadate and caffeine have additive effects in the induction of binucleate cells.

Key words. Allium cepa; cytokinesis inhibitor; root meristem; vanadate; caffeine.