Regulation of the Na^+-K^+ pump activity and estimation of the reserve capacity in intact rat peritoneal mast cells

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Evidence is provided that regulation of the Na^+-K^+ pump activity in rat peritoneal mast cells occurs mainly through stimulation of the pump from inside the plasma membrane by sodium. It is demonstrated that there is a large reserve capacity for the exchange of intracellular sodium with extracellular potassium in these cells. The maximal pump activity was estimated to be 3230 pmol/10^6cells per min and K_m for extracellular potassium was 1.5 mM.

Mast cell; Na^+-K^+ pump; Na^+, K^+-ATPase; Sodium; Potassium

1. INTRODUCTION

Previously, the Na^+-K^+ pump in the plasma membrane of rat peritoneal mast cell has been characterized, and evidence was provided that extracellular calcium influences the activity of the pump [1,2]. Ouabain has been shown to enhance the secretion of histamine from rat mast cells, but only if the cells were incubated in a calcium-free medium [3–8]. The receptor for ouabain is the Na^+-K^+ pump (for review, see [9]). In the present investigation we have, therefore, studied the ionic regulation of the pump activity in intact mast cells incubated in a calcium-free medium and estimated the reserve capacity of the pump activity of the cell.

2. MATERIALS AND METHODS

Male Sprague-Dawley rats, 320–410 g, were used for the experiments. The rats were killed by decapitation under light ether anaesthesia. Peritoneal mast cells were isolated by differential centrifugation of mixed peritoneal cells in a selfgenerating gradient of Percoll as described previously [1]. Cell isolation was performed at 4°C, and then the mast cells were suspended in a calcium-free Krebs-Ringer solution. A Coulter counter (Model 134, Analys Instrument AB, Sweden) was used to count the number of cells. The purity of the mast cell suspensions was determined by inspection of smears stained with Toluidine blue. The mast cells constituted mean 97.8% (94.5%–99.0%) of the cell population. Mast cell suspensions pooled from 4–5 rats were divided into samples with the same cell density in a final volume of 0.4 ml. The samples were preincubated at 37°C for various time periods as indicated in the legends to the figures. For determination of cellular potassium-uptake ($K(^{86}Rb)$-uptake), 100 μl of a solution containing, in addition to potassium, trace amounts of ^{86}Rb was added to the samples. The radioactive concentration during the incubation was mean 2.2 μCi/ml (0.4–4.5 μCi/ml). The incubation with the $K(^{86}Rb)$-solution lasted for 2–10 min and was terminated by the addition of 9.5 ml ice-chilled Krebs-Ringer solution. The cells were washed twice by centrifugation at 600 × g for 15 min at 41°C followed by the addition of 0.5 ml of an aqueous solution of NaOH (47.6 mM) to the samples. The whole content of each sample was then transferred to a scintillation vial and mixed with Ecoscint. Cellular $K(^{86}Rb)$-uptake was measured in a Mark III Liquid Scintillation Spectrometer (Nuclear Chicago) using the preset window for ^{32}P. The specific activity of potassium in the extracellular medium was used to calculate the cellular uptake of $K(^{86}Rb)$. The statistical analysis was performed by use of the Mann-Whitney U-test. $P < 0.05$ was considered statistically significant.

The calcium-free Krebs-Ringer solution had the following composition (mM): NaCl 136.8, KCl 4.75, $MgCl_2$ 1.2, Tris-HCl 12.5. When the concentration of potassium was varied (Fig. 4), the concentration of sodium was varied accordingly in order to maintain isotonicity. All solutions contained bovine serum albumin, 1 mg/ml, and glucose, 1 mg/ml. The pH was 7.4 (room temperature).

Bovine serum albumin was supplied by Sigma Chemical Company (St. Louis, USA), glucose by E. Merck (Darmstadt, FRG), Percoll by Pharmacia Fine Chemicals (Sweden), Ecoscint by BN Plastics (Helsinge, Denmark) and ^{86}Rb by Amersham (Buckinghamshire, UK). Ouabain (Mecobenzon, Denmark) and all other chemicals were of analytical grade.

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3. RESULTS

The cellular uptake of $K(^{86}Rb)$ was dependent on the time period of preincubation of the cells at 37°C. In addition, it was influenced by exposure of the cells to potassium prior to measurement of the uptake (Fig. 1). While the initial levels of $K(^{86}Rb)$-uptake were the same ($P > 0.1$), the uptake after preincubation of the cells for 5 min or more were significantly different ($P < 0.01$). Preincubation of the cells with potassium caused a rapid fall in the uptake until a stable level of $K(^{86}Rb)$-uptake was attained after 45 min. There was a time-dependent fall with a low rate of decrease in the uptake by preincubation of the cells in the absence of potassium. The steady-state level of $K(^{86}Rb)$-uptake in presence of potassium was about 600 pmol/10^6 cells per min, and in the absence of potassium it was about 1250 pmol/10^6 cells per min following a preincubation period of 90 min.

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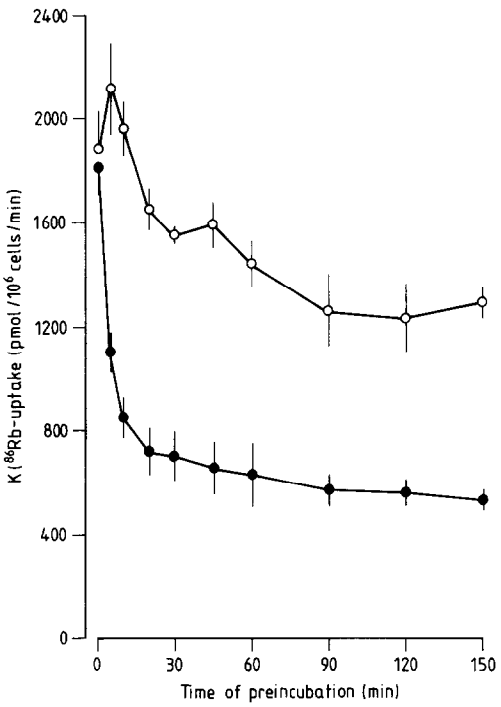

Fig. 1. Effect of time of preincubation in presence and absence of potassium 4.75 mM on cellular K(^{86}Rb)-uptake. The cells were isolated in a potassium-free medium at 4°C followed by preincubation at 37°C for the time indicated on the abscissa scale in presence (•) or absence (○) of potassium 4.75 mM. The incubation with K(^{86}Rb) lasted for 5 min. The ordinate scale shows the cellular K(^{86}Rb)-uptake in pmol/10^6 cells per min. Results are mean ± SE from 5 experiments.

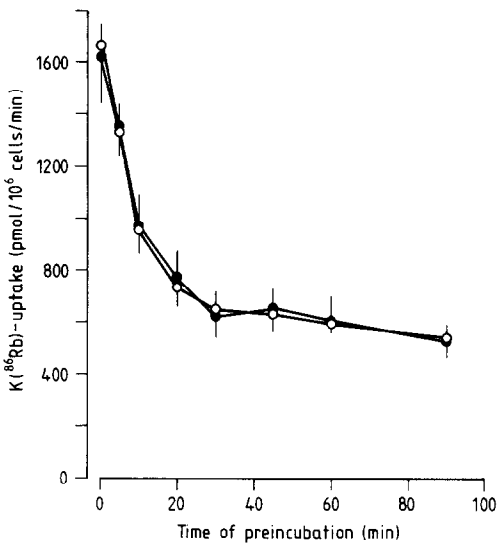

Fig. 2. Effect of cell isolation in presence and absence of potassium 4.75 mM on cellular K(^{86}Rb)-uptake. Cells were isolated in absence (○) or presence (•) of potassium 4.75 mM at 4°C. This was followed by preincubation at 37°C for the time indicated on the abscissa scale in the presence of potassium 4.75 mM. The incubation with K(^{86}Rb) lasted for 5 min. The ordinate scale shows the cellular K(^{86}Rb)-uptake in pmol/10^6 cells per min. Mean ± SE from five experiments are shown.

The presence of potassium during cell isolation had no effect on the rate of decrease in cellular K(^{86}Rb)-uptake that occurred after preincubation at 37°C in presence of potassium if the isolation procedure was performed at 4°C (Fig. 2). However, if cell isolation was performed at room temperature there was a significant difference in K(^{86}Rb)-uptake until the cells have been preincubated for more than 5 min at 37°C with potassium (Fig. 3) ($P < 0.05$ or less). The uptake of K(^{86}Rb) was almost immediately at steady-state level when the isolation at room temperature occurred in presence of potassium. It may be observed that the steady-state levels of K(^{86}Rb)-uptake seemed to be less stable after cell isolation at room temperature than when cells were isolated at 4°C.

The dose-response relationship between the extracellular potassium concentration and the activity of the Na^+-K^+ pump in sodium loaded cells is shown in Fig. 4a. There was a large increase in the pump activity when the concentration of potassium was increased in the interval from 0.5–2.86 mM, while in the range of 2.86–15 mM there was only a small increase in the ouabain-sensitive uptake. Estimation of the values for the maximal uptake velocity (V_{max}) and K_m was performed by transformation of the data into an Eadie-Hofstee plot (Fig. 4b). K_m was estimated to 1.5 mM and V_{max} to 3230 pmol/10^6 cells per min. The ouabain-resistant K(^{86}Rb)-uptake was proportional to the extracellular concentration of potassium and varied from 10 pmol/10^6 cells per min in presence of potassium 0.5 mM–420 pmol/10^6 cells per min in presence of potassium 15 mM.

4. DISCUSSION

The time-dependent decreases in the rate of the cellular K(^{86}Rb)-uptake observed during preincubation of the cells at 37°C (Figs 1, 2 and 3) are likely to represent changes in the activity of the Na^+-K^+ pump, since, in the mast cells, the rate ouabain-resistant K(^{86}Rb)-uptake is not dependent on time of preincubation (unpublished control experiments). Exposure of the cells to low temperature and incubation of the cells in potassium-free media are both procedures that inhibit the Na^+-K^+ pump activity. Both of these procedures are likely to increase the intracellular concentration of sodium, and this may explain the high initial level of potassium uptake due to stimulation of the Na^+-K^+ pump by sodium from inside the plasma membrane.

The populations of Na^+-K^+ pump units in various tissues are considered to have a large reserve capacity [10,11]. This is also the case concerning the mast cells, since the calculated V_{max} value for the ouabain-sensitive K(^{86}Rb)-uptake (3230 pmol/10^6 cells per min) is very large compared to the value observed when mast cells were incubated in the presence of concentrations of

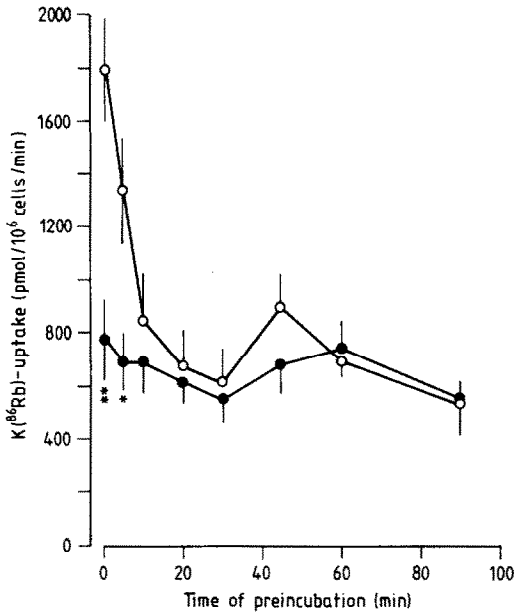

Fig. 3. Effect of cell isolation at room temperature (21°C) on cellular K(^{86}Rb)-uptake. The cells were isolated in absence (○) and presence (●) of potassium 4.75 mM at room temperature. Following the isolation procedure the cells were preincubated at 37°C in presence of potassium 4.75 mM for the time indicated on the abscissa scale. The incubation with K(^{86}Rb) lasted for 5 min. Ordinate scale shows the cellular K(^{86}Rb)-uptake in pmol/10^6 cells per min. Results are mean ± SE from five (○) or six (●) experiments. * $P < 0.05$; ** $P < 0.01$.

calcium and potassium being physiologically relevant (50–100 pmol/10^6 cells per min [8,9]).

The reason for the observation of a reserve capacity is the submaximal stimulation of the Na^+-K^+ pump by the normally low intracellular concentration of sodium. The K_m values reported for the activation of the pump mechanism by sodium in intact cells were 18–22 mM [11–14]. The K_m value for sodium activation of an isolated Na^+,K^+-ATPase preparation incorporated into lipid vesicles was 15 mM [15]. The intracellular concentrations of sodium are reported to be somewhat lower, 3.6–12 mM [11,13,16]. Consequently, when intact cells are incubated under conditions considered to be physiological, the activity of the Na^+-K^+ pump is determined mainly by the intracellular concentration of sodium. Any alterations in the sodium influx would immediately change the activity of the pump. This has been demonstrated by Akera et al. [17], who showed a sodium-dependent increase in the ouabain-sensitive ^{86}Rb uptake into guinea pig left atrial preparations when these were stimulated electrically. In addition, Yamamoto et al. [18] observed an increased ouabain-sensitive ^{86}Rb uptake in guinea pig atrial preparations following electrical stimulation as well as after treatment of the tissue with the sodium ionophore monensin. The effect of monensin is in accordance with our finding of an increased ouabain-sensitive K(^{86}Rb)-uptake into mast cells treated with monensin [9]. An increased concentration of sodium in rat hepatocytes caused an increased ouabain-sensitive ^{86}Rb uptake [13], and similarly, as a result of an increased sodium concentration in guinea pig auricles, an increased ouabain-sensitive sodium efflux was observed [12]. When rat skeletal myotubes were treated with tetrodotoxin, which is known to decrease sodium influx across the plasma membrane, the ouabain-sensitive ^{86}Rb influx was diminished [19]. These observations support the view that intracellular sodium plays a key role in the regulation of the Na^+-K^+ pump activity in most cells including the mast cells.

When the mast cells were preincubated in the absence of potassium a time-dependent decrease in the K(^{86}Rb)-uptake was observed (Fig. 1). The reason for this decrease is unclear. However, only small concentrations of extracellular potassium are necessary for ac-

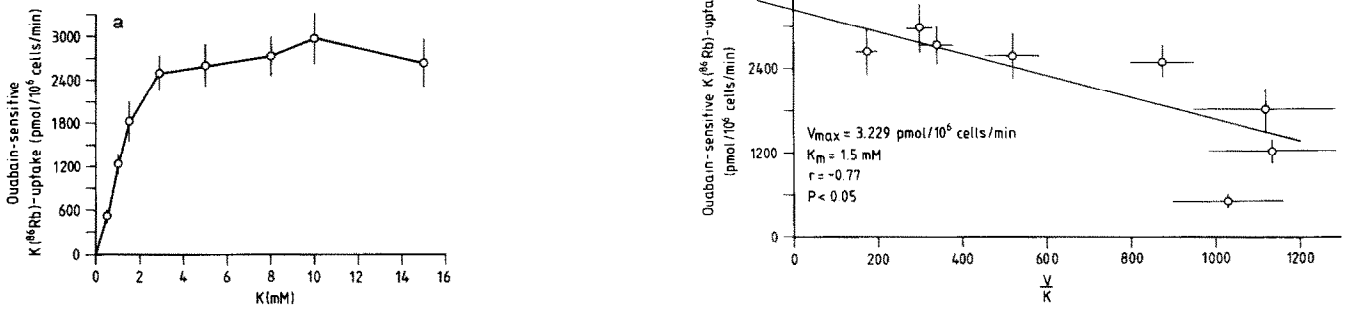

Fig. 4. (a) Effect of increasing concentrations of potassium on the ouabain-sensitive cellular K(^{86}Rb)-uptake. The cells were preincubated for 5 min in a calcium- and potassium-free medium in the absence or presence of ouabain 1 mM. The preincubation was followed by incubation with K(^{86}Rb) in concentrations as indicated on the abscissa scale. The incubation lasted for 2 min in the absence and for 10 min in the presence of ouabain. The ordinate scale shows the ouabain-sensitive K(^{86}Rb)-uptake in pmol/10^6 cells per min. The ouabain-sensitive uptake is the difference between the uptake in samples incubated with or without ouabain. Mean ± SE from four experiments are shown. (b) The figure shows the data from Fig. 4a transformed into an Eadie-Hofstee plot. The abscissa scale shows the velocity of the K(^{86}Rb)-uptake divided by the concentration of potassium ((pmol/10^6 cells/min) mM^{-1}). The ordinate scale shows cellular K(^{86}Rb)-uptake in pmol/10^6 cells per min. Linear interpolation was performed by the method of least squares. Maximal velocity (V_{max}), which is the intercept with the ordinate, and the K_m value, which is the slope of the line, are indicated as is the correlation coefficient. Mean values and SE from four experiments are shown.

tivation of the pump [12]. Thus, the possibility exists that potassium leakage from the cells during the preincubation period increases the extracellular concentration of potassium to a level sufficient for activation of the pump mechanism.

The K_m for potassium activation was estimated to be 1.5 mM. Therefore, the potassium binding sites are likely to be almost fully saturated at the concentration of potassium (4.75 mM) used in these experiments. It follows that even in a calcium-free medium, the Na^+-K^+ pump of the mast cells has a large reserve capacity, and the activity of the pump is mainly determined by the intracellular concentration of sodium.

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REFERENCES

[1] Knudsen, T. and Johansen, T. (1989) Br. J. Pharmacol. 96, 773–778.
[2] Knudsen, T. and Johansen, T. (1989) Br. J. Pharmacol. 98, 1119–1126.
[3] Frossard, N., Amellal, M. and Landry, Y. (1983) Biochem. Pharmac. 32, 3259–3262.
[4] Amellal, M., Binck, M., Frossard, N., Ilien, B. and Landry, Y. (1984) Br. J. Pharmacol. 82, 423–430.
[5] Amellal, M., Bronner, C. and Landry, Y. (1985) Br. J. Pharmacol. 85, 819–826.
[6] Binck, M., Frossard, N. and Landry, Y. (1985) Agents and Actions 16, 118–121.
[7] Fewtrell, C.M.S. and Gomperts, B.D. (1977) Nature 265, 635–636.
[8] Johansen, T., Knudsen, T. and Bertelsen, H. (1990) FEBS Lett. 262, 228–230.
[9] Hansen, O. (1984) Pharmacol. Rev. 36, 143–163.
[10] Akera, T. and Brody, T.M. (1982) Annu. Rev. Physiol. 44, 375–388.
[11] Akera, T. and Brody, T.M. (1985) Trends Pharmacol. Sci. 6, 156–159.
[12] Glitsch, H.C., Pusch, H. and Venetz, K. (1976) Pflügers Arch. 365, 29–36.
[13] Van Dyke, R.W. and Scharschmidt, B.F. (1983) J. Biol. Chem. 258, 12912–12919.
[14] Post, R.L., Merrit, C.R., Kinsolving, C.R. and Albright, C.D. (1960) J. Biol. Chem. 235, 1796–1802.
[15] Apell, H.-J. and Marcus, M.M. (1986) Biochim. Biophys. Acta 862, 254–264.
[16] Thomas, R.C. (1972) J. Physiol. 220, 55–71.
[17] Akera, T., Yamamoto, S., Temma, K., Kim, D.-H. and Brody, T.M. (1981) Biochim. Biophys. Acta 640, 779–790.
[18] Yamamoto, S., Akera, T. and Brody, M. (1979) Biochim. Biophys. Acta 555, 270–284.
[19] Brodie, C. and Sampson, S.R. (1989) J. Cell. Physiol. 140, 131–138.