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The effect of cyclic AMP on Na^+ and K^+ transport systems in mouse macrophages

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Summary. Exogenous cyclic AMP (cAMP) inhibits the Na^+, K^+-cotransport system and stimulates the Na^+, K^+-pump and Na^+, Ca^{2+} exchange in mouse macrophages. These effects are enhanced by inhibition of phosphodiesterase with methylisobutylxanthine (MIX). MIX alone showed little or no effect. A similar response was observed after stimulation of endogenous production of cAMP by isoproterenol.
Key words. Na^+; K^+ transport; macrophages; cyclic AMP; mouse.

It is a classical view that any disturbance of ion metabolism may be corrected by hormonal modulation of gastrointestinal ion absorption and/or renal ion excretion. However, a more complex mechanism was recently suggested by the observation that ion transport may also be submitted to hormonal homeostasis in non-epithelial cells. For instance, in avians, catecholamines may correct the hypokalemia resulting from flight by the stimulation of K^+ entry into red cells. This particular mechanism involves the adenylate cyclase-dependent stimulation of a bumetanide-sensitive Na^+, K^+-cotransport system[1,2]. In mammals, catecholamines may correct the hyperkalemia resulting from muscular exercise by enhancing K^+ gain into striated muscle cells. This mechanism involves the stimulation of a ouabain-sensitive Na^+, K^+-pump by cyclic AMP (cAMP)[3]. Thus, ion transport in non-epithelial cells may be modulated by hormones through second messengers such as cAMP.

Ion transport systems are well characterized at the molecular level in human red cell membranes[4]. On the other hand human erythrocytes, like many other cells, have a cAMP translocating system[5,6]. This regulatory mechanism maintains a cAMP erythrocyte content about 100 times lower than the extracellular cAMP concentration[5]. Furthermore, it catalyzes the rapid incorporation of a small but non-negligible amount of exogenous cAMP[5]. This allowed us to study the effect of internal cAMP on ion transport in intact erythrocytes. We thus observed that exogenous cAMP is able to inhibit the Na^+, K^+-cotransport system in these cells[5]. Unfortunately, the study of cAMP-ion transport interactions is hampered by the fact that human erythrocytes do not have a beta-adrenergic stimulated adenylate cyclase. We thus decided to investigate these interactions further in other non-epithelial cells.

A transport study at the a molecular level requires a large amount of cells, and they must be in suspension under natural conditions. It appeared to us that both conditions were fulfilled by mouse macrophages elicited in the peritoneal cavity by injection of thioglycollate.

2.5–3 ml of sterile thioglycollate medium were injected into the peritoneal cavity of female mice 5–8 weeks old, of the 57 BL/5 ($H\text{-}2^b$) and DBA/2 ($H\text{-}2^d$) inbred strains. 3 to 5 days later, the cells were collected by washing the peritoneal cavity with Hank's balanced salt solution. An average of 10^7 cells were obtained per mouse. More than 80% of these cells presented the morphological aspect of macrophages. A pool of peritoneal cells from 5–15 mice was used in each experiment.

Na^+ and K^+ contents, measured in cells washed once with $MgCl_2$ 110 mM, varied between 15 and 24 mmoles/l·cells and between 50 and 80 mmoles/l·cells, respectively.

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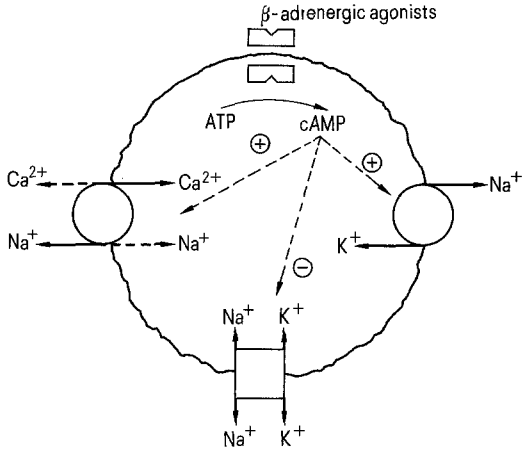
Transport system	Control	Isoproterenol 10^{-5} M	cAMP 2 mM	cAMP+MIX 2 mM 0.5 mM
Na^+,K^+-pump				
Na^+ efflux	6.5 ± 4.6	12.1 ± 4.2	17.2 ± 5.8	24.6 ± 6.9
Na^+,K^+-co-transport				
Na^+ efflux	5.1 ± 2.3	0	1.3 ± 0.7	2.7 ± 1.8
K^+ efflux	11.2 ± 3.6	0	9.8 ± 4.6	7.1 ± 0.7
Na^+,Ca^{2+} exchange				
Na^+ efflux	3.7 ± 2.1	10.8 ± 2.9	5.2 ± 3.5	9.5 ± 2.4

Values are given as mean ± SD of 4–7 experiments. Fluxes are expressed as mmol/(l·cells·h).

Na^+ and K^+ fluxes were measured in fresh macrophages incubated in Mg^{2+}, sucrose medium (see Garay et al.[4,9] for previously published methods for human red cells) containing (mM): 1) *K^+ medium:* 2 KCl, 2) *ouabain medium:* 1 ouabain, 3) *bumetanide medium:* 1 ouabain + 0.02 bumetanide and *Ca^{2+} medium:* 1 ouabain + 0.02 bumetanide + 1 $CaCl_2$. The difference in Na^+ efflux between K^+ medium and ouabain medium is a measure of Na^+, K^+ pump activity. The flux difference between ouabain medium and bumetanide medium is a measure of outward Na^+, K^+-cotransport. The difference in Na^+ efflux between Ca^{2+} medium and bumetanide medium is a measure of Na^+, Ca^{2+} exchange. In control experiments we observed that all these fluxes deviate from initial rate conditions after 2–10 min incubation. In all the experiments reported in this paper, we measured the initial rate of Na^+ and K^+ efflux. In 12 different experiments, the ouabain-sensitive Na^+ efflux varied between 4 and 10 mmoles $(l \cdot cells \cdot h)^{-1}$. Bumetanide-sensitive Na^+ efflux varied between 2.5 and 7.6 mmoles $(l \cdot cells \cdot h)^{-1}$ and bumetanide sensitive K^+ efflux lied between 7 and 15 mmoles $(l \cdot cells \cdot h)^{-1} \cdot Ca^{2+}$-stimulated, Na^+ efflux varied from 2 to 9 mmoles $(l \cdot cells \cdot h)^{-1}$. Most of the total Na^+ efflux was resistant to ouabain and bumetanide (20 ± 8 mmoles $(l \cdot cells \cdot h)^{-1}$, mean $\pm$ SD).

The table shows that the addition of 2 mM exogenous cAMP to the incubation medium is associated with: 1) a strong stimulation of ouabain-sensitive Na^+ efflux, 2) a significant decrease in bumetanide-sensitive Na^+ efflux and to a lesser extent in bumetanide-sensitive K^+ efflux and 3) enhanced Ca^{2+}-stimulated Na^+ efflux. The effects of exogenous cAMP were potentiated by inhibition of phosphodiesterase with 0.5 mM methylisobutylxanthine (MIX). MIX alone showed little or no effect (not presented in the table). In addition, isoproterenol, a beta-adrenergic agonist, is able 1) to completely block bumetanide-sensitive Na^+ and K^+ efflux, 2) to stimulate ouabain-sensitive Na^+ efflux and 3) to significantly increase Ca^{2+}-stimulated Na^+ efflux.

Transmembrane Na^+ and K^+ movements appear to play an important role for many vital functions of macrophages[7,8]. Inspection of data presented in the table clearly shows that ion fluxes catalyzed by different transport systems are quantitatively and in some cases qualitatively different from those classically recognized in human red cells. First, Na^+ efflux through the cotransport system is of similar magnitude to that catalyzed by the Na^+, K^+-pump. Secondly, the Na^+ to K^+ stoichiometry of outward cotransport is about 1:2 (a ratio which is close to one in human red cells, see Garay et al.[9]). Third, in contrast to the human red cell, external Ca^{2+} stimulates a ouabain- and bumetanide-resistant Na^+ efflux. The results presented here indicate that ion transport systems in macrophages may be modulated by catecholamines and other agents acting on adenylate cyclase (fig.). This may be important for the rapid recovery of a normal cell Na^+ and Ca^{2+} content after macrophage activation.



Cyclic AMP-dependent regulation of cell cation content in macrophages.

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Natural killer-like activity in human cultured lymphoid cells propagated in the presence of interleukin-2: acquired resistance to prostaglandin E_2- or dexamethasone-mediated suppression

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Summary. The cytotoxic activity of human peripheral blood lymphocytes against the natural killer-sensitive target K562 was suppressed both by prostaglandin E_2 and dexamethasone. On the other hand, cultured lymphoid cells propagated in the presence of interleukin-2 showed strong cytotoxic reactivity against K562 targets, and were resistant to prostaglandin E_2- or dexamethasone-mediated suppression.

Key words. Lymphoid cells, human; cytotoxic activity; Killer-sensitive target; interleukin-2; prostaglandin E_2; dexamathasone.

Natural killer (NK) cells, present in the peripheral blood lymphocytes (PBL) of normal donors, lyse a variety of tumor and virus-infected cells without prior antigenic stimulation[1,2]. PBL cultured in medium alone rapidly lose NK activity[3]. However, recent studies have shown that in vitro incubation of lymphocytes with interleukin-2 (IL-2), a soluble mediator which is produced by activated T cells, could lead to the propagation of cultured lymphoid cells (CLC-IL-2) with substantial cytotoxic