Changes in Intracellular K^+ and Na^+ Ion Concentrations During Cell Growth and Differentiation

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ABSTRACT We compared intracellular K^+ and Na^+ ion concentrations during cell growth and differentiation of a mouse myeloid leukemia M1 cell line. Cells undergoing mitosis had higher K^+ concentrations than quiescent cells. Treatment with a K^+ channel blocker and furosemide enhanced cell growth and produced a slight increase in the intracellular K^+ concentration. Treatment with reagents that reduced the intracellular K^+ concentration stopped cell growth. Induction of differentiation in this cell line produced a decrease in the K^+ concentration, which always was accompanied by an increase in the Na^+ concentration. Treatment with ouabain, which decreased the intracellular K^+ concentration, did not, however, induce differentiation in the M1 cell line. The data suggest that cell growth and differentiation in the M1 cells are accompanied by changes in the intracellular K^+ and Na^+ concentrations but that the changes in the contents of these monovalent cations do not necessarily induce differentiation in this cell line.

Accumulated data suggest that one early cellular response to various growth factors is a change in the intracellular K^+ and Na^+ concentrations ($[K^+]_i$, $[Na^+]_i$) and that this change apparently triggers quiescent cells into beginning mitosis (2, 20, 21). Receptor-mediated endocytosis in macrophages (16, 17) and phagocytosis in fibroblasts (13–15) also are cases in which cation fluxes appear to be a signal from the membrane to the cytoplasmic components.

Recently, we isolated two actin-gelation factors, 38K- and 105K-dimer proteins, from myeloid leukemia cell line designated M1 (23, 12). The gelation activity of these proteins could be controlled by low concentrations of K^+ and Na^+ ions. Interestingly, the sensitivity of the 38K-dimer protein to monovalent cations decreased after M1 cells had been induced to differentiate into macrophages.

Actin-gelation has an important role in various cellular functions, including phagocytosis (22) and cell locomotion (6). These findings aroused our interest in the changes in intracellular monovalent cation concentrations before and after differentiation as well as during different phases of cell growth.

We here have compared $[K^+]_i$ and $[Na^+]_i$ in M1 line cells in different growth state as well as during differentiation. We confirmed that changes in monovalent cation concentrations function in the control of cell growth and differentiation.

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MATERIALS AND METHODS

Cell culture. Mouse myeloid leukemia M1 line cells were cultured in Eagle's medium (EM) that contained double concentrations of amino acids vitamins supplements of 5% horse serum (HS), 100 U/ml penicillin and 100 U/ml streptomycin. Cells were incubated in a humidified atmosphere of 10% CO₂ and 90% air at 37°C, the mass culture taking place in 600 ml or 3,000 ml glass bottles that were constantly agitated on a magnetic stirrer. Cell density supply of fresh medium. To induce differentiation in this cell line, we added 10–20% conditioned medium (CM) harvested from rat embryo fibroblasts for the last 3 days of culture. Differentiation into macrophages was assessed by the phagocytosis test (7, 8).

Preparation of the cell lysate and determination of the intracellular water space. M1 cells were harvested by centrifugation at 1,500 rpm for 5 min. The packed cells were divided; one part being used to determine the dry weight (D), the other to prepare the cell lysate after measuring the wet weight (W). To prepare the cell lysate, we resuspended the pellet in 8 volumes of 0.5% Triton X-100 solution then sonicated the suspension for 1 min.

The intracellular water space was determined by culturing the cells in ³H-water for 2 days, after which ¹⁴C-inulin was added. The cultured cells were harvested then resuspended in 1 ml 0.5% Triton X-100 solution and combined with 10 ml of Aquasol-2 (New England Nuclear). ³H and ¹⁴C were counted with scintillation counter (Packard, Model TRI-CABB 460) to determine the whole water volume (H) or the extracellular water (C) contained in the cell pellet. The intracellular space could be calculated by (W–D–C) or (H–C).

Measurement of monovalent cation concentration. The K⁺ and Na⁺ concentrations in the cell lysate and medium were measured with a highly selective liquid ion-exchange electrode equilibrated with a standard electrolyte solution (Photovolt Analyzer, Model PVA-4). The intracellular ion concentrations were expressed in millimoles per liter of cell water.

DNA synthesis. Cells were plated in a culture dish (3 cm dia.) at a density of 1–1.2 × 10⁶ cells per dish or in a 96-microwell plate at 7.5–10 × 10³ cells per well, then incubated in EM with the concentrations of HS reagents indicated in each experiment. After 24–72 h of incubation, [methyl-³H]thymidine (1 μCi/ml) (ICN: 2Ci/mmol) was added and cultures kept for 2 h (culture dish) or 6 h (microwell). Labeled cells were harvested on a membrane filter or with a microharvester, then they were combined with 2 ml of Univergel (Nakarai Chem. Co.) and counted with a liquid scintillation counter.

Reagents. Ouabain was obtained from Sigma Chemical Company, amiloride from Merck, and 4-aminopyridine from Nakarai Chemical Company. Furosemide was gift from Hoechst Japan Company, potassium canrenoate was a gift from Searle Drug Company.

RESULTS

Changes in [K⁺]_i and [Na⁺]_i during cell growth and differentiation. The [K⁺]_i value in quiescent M1 cells (HS-free culture) was 74.6 ± 6.5 mM. As the cells began exponential proliferation, [K⁺]_i increased rapidly to 139 ± 15.6 mM, this value being maintained as long as cell growth continued (Fig. 1). We also found a remarkable correlation between [³H]thymidine incorporation and [K⁺]_i in growing cells (Fig. 1).

When CM was added to the culture, [K⁺]_i gradually decreased, reaching 74.6 ± 25.9 mM after 3 days (Fig. 2). By that time, M1 cells had differentiated to macrophages, and 60% of the cells showed active phagocytosis. [Na⁺]_i gradually increased during this differentiation (Fig. 2).

Fig. 1. Relation of the intracellular K⁺ concentration to DNA synthesis. M1 cells were cultured with varied concentrations (0.2, 0.5, 1.0, 1.5, 2.5, 5.0%) of HS in the medium. After 2 days of incubation, [³H]thymidine (1 μ Ci/ml) was added. The cultures kept for 2 h. [K⁺]_i and [³H]thymidine incorporation were determined as described in MATERIALS AND METHODS. Samples (●) contained 300–400 mg of packed cells and were assayed in triplicate. [³H]thymidine incorporation is presented as a percent of the control (HS 5%) and is plotted against [K⁺]_i.

Effects of reagents on cell growth and on [K⁺]_i and [Na⁺]_i. The K⁺ concentration in M1 cells appears to depend on cell growth (Fig. 1). If so, any treatment that increases [K⁺]_i may enhance cell growth and any treatment that decrease [K⁺]_i may stop it.

Amiloride (75 μ M), ouabain (50 μ M) and potassium canrenoate (100 μ M) decreased both [K⁺]_i and [³H]thymidine incorporation in treated M1 cells (Fig. 3 and Table 1). Treatment with these three reagents did not, however, induce any sign of differentiation in the M1 cells (data not shown), evidence that changes in the ionic concentrations alone are not sufficient for differentiation.

The effects on both [K⁺]_i and cell growth were reversible when cells were transferred to normal medium (Fig. 3 and Table 1).

Effects of furosemide and 4-aminopyridine on cell growth. Furosemide, which did not decrease [K⁺]_i, also did not decrease cell growth, but it did enhance thymidine

Fig. 2. Intracellular K^+ and Na^+ concentrations during the differentiation of M1 cells. M1 cells were cultured in 10-cm dia. culture dishes. On day 0, 20% CM was added. $[K^+]_i$ and $[Na^+]_i$ were measured with an ion-selective electrode as described in MATERIALS AND METHODS. Data shown are the means $\pm$ S.E.M. Number of measurements; 35 for day 0, 6 for day 1, 5 for day 2 and 14 for day 3. Samples contained 300–400 mg of packed cells and were assayed in triplicate. $[K^+]_i$ —●—, $[Na^+]_i$ ---○---.

TABLE 1. INTRACELLULAR K^+ AND Na^+ CONCENTRATIONS IN M1 CELLS (5% HS MEDIUM)

Treatment	Number of experiments done	Ion concentration (mM)		
		$K^+ \pm SEM$	$Na^+ \pm SEM$	K^+/Na^+
None (5% HS alone)	35	139.9 ± 15.6	42.1 ± 9.8	3.30
Ouabain	4	65.7 ± 0.9	120.7 ± 5.4	0.54
Ouabain then*	3	133.7 ± 4.1	27.9 ± 4.3	4.79
Normal medium				
Amiloride	5	65.5 ± 2.2	85.1 ± 11.4	0.77
Amiloride then*	2	124.4 ± 2.4	35.9 ± 4.4	3.47
normal medium				
Canrenoate	3	80.5 ± 13.6	97.3 ± 11.2	0.83
Furosemide	3	147.5 ± 8.2	48.2 ± 7.2	3.06
CM (day 3)	14	74.6 ± 25.9	92.6 ± 17.5	0.81

M1 cells were grown for 2 days in culture medium containing 5% HS (control) and then with reagents; 50 μM ouabain, 75 μM amiloride, 100 μM potassium canrenoate, 30 μM furosemide, or in CM. For each measurement 300 to 400 mg of packed cells was used. *: cells that had been cultured for 2 days in the presence of ouabain (50 μM) or amiloride (75 μM) were transferred to normal medium. After 1 day, the ion concentrations were measured.

Fig. 3. Effects of reagents on DNA synthesis. Cells were cultured in the presence of various reagents; A: amiloride (75 μ M, ●), B: furosemide (60 μ M, ●), C: Ouabain (50 μ M, ●), D: potassium canrenoate (100 μ M, ▲, 50 μ M, ●) or normal medium ($\cdots\triangle\cdots$, or $\cdots\circ\cdots$). Cells that had been cultured for 1 or 2 days in the presence of a reagent were transferred to normal medium ($\downarrow$). Incorporation of [³H]thymidine was measured as described in MATERIALS AND METHODS.

incorporation (Fig. 3). To determine the optimal conditions for the furosemide effect on cell growth, M1 cells were incubated with various concentrations of HS and furosemide (Fig. 4). When the cells were incubated without HS, furosemide did not stimulate cell growth and [³H]thymidine incorporation; but, in the presence of 0.1–0.5% HS, furosemide (30–60 μ M) enhanced cell growth about 1.5–2 times the value for the control. A K-channel blocker, 4-aminopyridine, also enhanced [³H]thymidine incorporation under the same conditions that furosemide did (Fig. 5).

To confirm our contention that [K⁺]_i and cell growth are correlated in M1 cells, we investigated the intracellular cation concentrations. Both furosemide and 4-

Fig. 4. Effects on cell growth of furosemide combined with different concentrations of horse serum. Cells were plated in culture dishes (3 cm dia.) at a density of 5×10^5 cells/ml then cultured for 3 days with various concentrations of HS without furosemide. The cell number was counted with a hemocytometer, after which the cells were reseeded with various concentrations of furosemide leaving the serum concentrations, as shown, unchanged for each culture. Each dot represents the mean of 3 samples expressed as a percentage of the control (5% HS alone).

TABLE 2. INTRACELLULAR K^+ AND Na^+ CONCENTRATIONS IN M1 CELLS (LOW HS MEDIUM)

Treatment		Number of experiments done	Ion concentration (mM)		
HS	Reagents		$K^+ \pm SEM$	$Na^+ \pm SEM$	K^+/Na^+
0%	none	4	74.6 ± 6.5	118.0 ± 9.4	0.63
0.2%	none	3	71.3 ± 1.9	118.9 ± 2.7	0.60
0.2%	Furosemide	4	92.4 ± 5.9	114.4 ± 8.3	0.81
0.2%	4-aminopyridine	3	101.6 ± 15.3	41.2 ± 2.5	2.47

See the legend to Table 1.

Fig. 5. DNA synthesis in response to furosemide or 4-aminopyridine. M1 cells were plated in a 96-microwell plate at a density of 10,000 cells per well. Cells were incubated in EM without HS (0% HS), or with 0.2% or 5% in the presence of 30 μ M furosemide (F) or 100 μ M 4 aminopyridine (A). No treatment (C). Values are the means $\pm$ S.E.M. of 3 experiments.

aminopyridine produced [K^+]_i above the values for the control cultures (Table 2).

DISCUSSION

The intracellular K⁺ concentration in M1 cells during normal growth had the same value as reported for HL-60, a human promyelocytic leukemia cell line (4); whereas the [Na^+]_i was slightly higher than in HL-60 cells. This may be due to the following differences: 1) In our study, cell homogenates were made soluble in 0.5% Triton X-100 solution then sonicated, after which both free and protein bound Na⁺ ions were counted. 2) We used a glass electrode to assay the [Na^+]_i of the M1 cells. The selectivity of a sodium electrode ranges from 100 to 1,000 Na/K ratios, depending on the composition of the glass. As Pelleg and Levy (18) demonstrated, when a sample contains a large amount of K⁺, the Na⁺ concentration is overestimated if measured with a sodium electrode rather than a flame photometer.

In our previous report (23), we presented the K⁺ and Na⁺ contents in the total cell lysate, not the concentrations in the intracellular fluid as here. Furthermore, in that study, cell were washed in sucrose solution before harvest; therefore, some of the ion contents may have leaked from the cells.

The $[K^+]_i$ and $[Na^+]_i$ in the M1 cells change with cell growth (Fig. 1). A similar observation has been reported for the mitogenic response of 3T3 cells to growth factors (20). These findings suggest that an increase in $[K^+]_i$ above a certain concentration (85–90 mM) may be necessary for the stimulation of DNA synthesis and that a lower $[K^+]_i$ stops cell growth.

A number of reports have demonstrated correlations between the activity of Na-K ATPase and cell growth (11, 19, 24). In M1 cells, inhibition of Na-K ATPase by ouabain reversibly reduced $[^3H]$ thymidine incorporation (Fig. 3). Inhibition of Na/H antiporter by amiloride also reduced $[^3H]$ thymidine incorporation and $[K^+]_i$ in M1 cells. This may be a secondary effect because decrease in $[Na^+]_i$ inhibits Na-K ATPase activity.

In contrast, treatment with furosemide, $K^+/Na^+/Cl^-$ cotransporter inhibitor, enhanced $[^3H]$ thymidine incorporation and the replicative ability of M1 cells (Figs. 3, 4, 5) in low concentrations of serum. Furthermore, 4-aminopyridine, a K^+ -channel blocker, enhanced $[^3H]$ thymidine incorporation. Both reagents increased the amount of $[K^+]_i$ in M1 cells. Additional evidence of this action is provided by other studies (9, 10, 20), according to which cultured cells were unable to grow in a low K^+ medium, but which the addition of furosemide or bumetanide reduced the K^+ efflux and permitted growth of the treated cells. These findings suggest that a high K^+ concentration enhances the replicative ability of the cells.

Our recent observations (to be published) show that a K^+ , Na^+ -sensitive actin-gelation factor (a 38K-dimer protein) mainly is found free in the cytoplasm before the differentiation of M1 cells, and that it binds to cytoskeletal components after differentiation is induced. We conclude that the differentiation of M1 cells brings about a decrease of $[K^+]_i$ which leads to the assembly of cytoskeletal components with the 38K protein. This produces local gelation of the cytoplasm which, in turn, induces phagocytic activity in the differentiated cells.

Acknowledgements. We thank Dr. Y. Okada (Department of Physiology, Faculty of Medicine, Kyoto Univ.), Prof. E. Takahashi and Dr. T. Mato (Faculty of Agriculture, Kyoto Univ.) for their technical advice and comments, also Dr. H. Kitagawa (Hoechst Japan, Co.) and Mr. K. Takioka (Searle Drug Co.) for the gifts of furosemide and potassium canrenoate. This research was supported by a Grant from the Ministry of Education, Science and Culture of Japan.

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(Received for publication, June 4, 1986)