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[^{3}H]OUABAIN BINDING AND ^{86}RUBIDIUM UPTAKE DURING THE CELL GROWTH CYCLE IN L5178Y MURINE LYMPHOBLASTS

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SUMMARY

There was a parallel alteration in [^{3}H]ouabain binding and $^{86}Rb^+$ uptake during the cell growth cycle in L5178Y murine lymphoblasts. The initial rate of Rb^+ uptake and [^{3}H]ouabain binding was highest at the stationary phase of the cell growth cycle. The possible relationship between changes in cation transport and membrane properties to the cell growth cycle is discussed.

INTRODUCTION

The cell growth cycle of cells grown in culture (and perhaps in vivo) can be divided into four phases; the lag phase, the exponential phase, the stationary phase and the death phase. Because of hypotheses concerning programmed cell death, control of cell growth, cell-cell interaction, etc., it is very important to monitor enzyme molecule levels, especially of "pivotal" enzymes such as $(Na^+ + K^+)$-ATPase.

Evidence from several different laboratories indicates that changes in $(Na^+ + K^+)$-ATPase activities occur during the cell growth cycle and upon cell transformation. A several-fold increase in the activity of $(Na^+ + K^+)$-ATPase in the late interphase stage of virally transformed hamster fibroblasts (NIL-2HSV) has been demonstrated [1]. Also, two reports of increased $(Na^+ + K^+)$-ATPase activity and $^{86}Rb^+$ uptake in several transformed 3T3 lines have recently appeared [2, 3]. In the present report we demonstrate the fluctuations in $(Na^+ + K^+)$-ATPase activity as well as number of enzyme molecules as measured by initial rate of $^{86}Rb^-$ uptake and [^{3}H]ouabain binding during the cell growth cycle of L5178Y mouse lymphoblasts.

MATERIALS AND METHODS

L5178Y cells were grown and maintained as described previously [4, 5]. The assay of specific binding of [^{3}H]ouabain to L5178Y cells was similar to that described for the binding of ^{125}I-labeled nerve growth factor to isolated cell membranes [6, 7]. The binding assay used consisted of incubation of a cell suspension (10^6 cells/ml) at

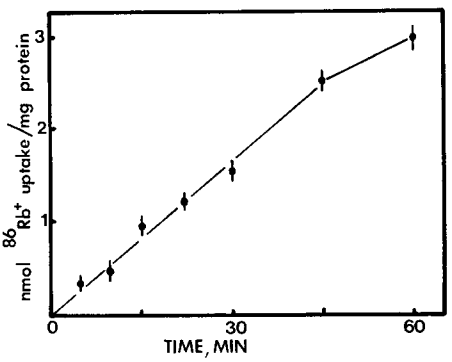

Fig. 1. The time course of ouabain-sensitive $^{86}Rb^+$ uptake in L5178Y cells during the mid-exponential phase.

24 °C for 60 min in 1 ml of Fischer's medium ($[K^+] = 4$ mM) (GIBCO, Grand Island, N.Y., USA) and 0.16 μM [^{3}H]ouabain (New England Nuclear; specific activity 12 Ci/mmol). After incubation, 3 ml of ice-cold 0.9 % NaCl were added to each tube and the mixture was filtered and washed over glass fiber filter papers (Reeve-Angel). Corrections were made for nonspecific accumulation of [^{3}H]ouabain in the cells by assaying parallel incubations in which excess unlabeled ouabain (0.25 mM) was added to the cell suspensions before adding the [^{3}H]ouabain. Specific binding was obtained by subtracting from the total bound radioactivity the amount that was not displaced by unlabeled ouabain.

The turnover of $(Na^+ + K^+)$-ATPase was estimated by the determination of initial rate of uptake of $^{86}Rb^+$ as $^{86}RbCl$ (New England Nuclear). $^{86}Rb^+$ was used as a marker for K^+ because of its longer half-life compared to $^{42}K^+$ and because $^{86}Rb^+$ has been shown to be taken up by a similar mechanism to that of K^+ in erythrocytes [8] and tissue cultured cells [9]. Assay tubes containing L5178Y cell suspensions (10^6 cells/ml) in 1 ml of Fischer's medium were incubated with 5 μCi of $^{86}RbCl$ (carrier free; specific activity 6.57 mCi/mg) at 37 °C for 30 min. Nonspecific $^{86}Rb^+$ uptake was obtained by parallel incubations in the presence of 1.25 mM ouabain. After incubation, 3 ml of ice-cold 0.9 % NaCl was added to each tube, and the mixture was filtered and washed over glass fiber filters, as described above. The radioactivity associated with the cells was measured using a Packard 5220 gamma counter. Protein was measured by the method of Lowry et al. [10]. Initial experiments showed that the $^{86}Rb^+$ uptake remains linear for at least 45 min in L5178Y cells during the mid-exponential phase (Fig. 1). Therefore, to determine the initial rate of $^{86}Rb^+$ uptake, the cells were incubated for 30 min in subsequent experiments.

RESULTS AND DISCUSSION

Alterations in $(Na^+ + K^+)$-ATPase activity during the cell growth cycle or cell transformation may be due to changes in the rate of ion translocation or number of transport macromolecules. Since ouabain is a specific inhibitor of $(Na^+ + K^+)$-ATPase and binds to cell surfaces externally [11, 12], [^{3}H]ouabain binding provides an estimate of number of $(Na^+ + K^+)$-ATPase molecules in a cell population. Fur-

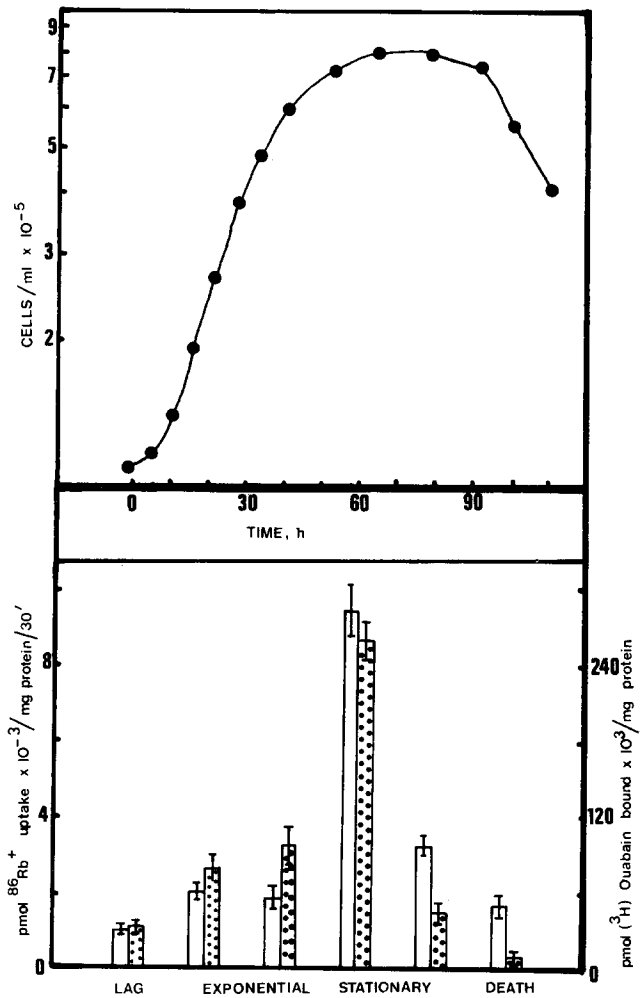

Fig. 2. The initial rate of $^{86}Rb^+$ uptake (□) and [^{3}H]ouabain binding (⊡) during the cell growth cycle in L5178Y murine lymphoblasts. The results shown are averages of two experiments, each run in triplicate.

thermore, the initial rate of $^{86}Rb^+$ uptake is an estimate of the rate of monovalent cation translocation.

The initial rate of $^{86}Rb^+$ uptake was lowest during the lag phase of cell growth of the L5178Y cells, doubled at early exponential, and remained fairly constant throughout the exponential phase. A dramatic increase in the initial velocity of $^{86}Rb^+$ uptake was observed as the cell growth of L5178Y entered the early stationary phase. This increase was about eight times that seen at the lag phase of growth curve (Fig. 2). The velocity of $^{86}Rb^+$ uptake rapidly declined at late stationary phase and continued to decrease as the cell growth curve entered the death phase. This increase may have been due to changes in cell volume and membrane area per cell during

TABLE I

RATE OF UPTAKE OF $^{86}Rb^+$ IN L5178Y CELLS

The results are averages of three determinations.

Cell growth cycle	Time after inoculation (h)	nmol of $^{86}Rb^+$ accumulated/30 min	
		per mg protein	per 10^6 cells
Lag phase	1	1.1	1.2
Early exponential	27	0.6	0.8
Mid-exponential	48	1.2	1.4
Early stationary	72	6.3	6.1
Stationary	97	3.8	4.2

the growth cycle. To test this possibility, $^{86}Rb^+$ uptake was determined per mg protein as well as per 10^6 cells. The results shown in Table I indicate that changes in the rate of $^{86}Rb^+$ uptake per mg protein were not due to changes in number of cells per mg protein during different phases of the cell growth cycle. $^{86}Rb^+$ uptake is increased in SV40-transformed 3T3 cells as compared to normal 3T3 cells [2]. This increase may be due to increase in phospholipid fluidity of the plasma membrane of the transformed cells [2, 12, 13]. However, almost identical alterations in [^{3}H]ouabain binding as the rate of $^{86}Rb^+$ uptake during cell growth of L5178Y mouse lymphoblasts (Fig. 2) suggest that changes in number of $(Na^+ + K^+)$-ATPase enzyme molecules may account for changes in rate of cation translocation. Therefore, membrane fluidity may not be the only factor responsible for fluctuations in cation movement during the growth curve of L5178Y.

The specificity of [^{3}H]ouabain binding to $(Na^+ + K^+)$-ATPase of mouse lymphoblastomas is not definitely established. There is evidence that [^{3}H]ouabain binds specifically to the external surface of erythrocytes [15, 16] and squid giant axons [17]. Although several workers [18–20] have used [^{3}H]ouabain binding to measure number of $(Na^+ + K^+)$-ATPase enzyme molecules in HeLa cells, Baker and Willis [21] have shown nonspecific uptake of [^{3}H]ouabain in the same cell line. Since we subtracted [^{3}H]ouabain uptake in the presence of excess unlabeled ouabain from total accumulation of [^{3}H]ouabain in mouse lymphoblastoma, the nonspecific uptake of the inhibitor was probably eliminated. This possibility is supported by the parallel changes in the rate of $^{86}Rb^+$ uptake and [^{3}H]ouabain binding during cell growth.

The mechanism of the remarkable increase in the rate of uptake of $^{86}Rb^+$ at the early stationary phase of cell growth is not clear. It may be due to synthesis of new $(Na^+ + K^+)$-ATPase macromolecules or exposure of "cryptic" transport sites or increased turnover of the enzyme system. Parallel changes in the rate of $^{86}Rb^+$ uptake and [^{3}H]ouabain binding would suggest an increase in the number of $(Na^+ + K^+)$-ATPase molecules at the stationary phase. Since [^{3}H]ouabain is known to bind to $(Na^+ + K^+)$-ATPase inactivated by phospholipase C [22, 23], a comparison of apparent K_m and V of initial rate of $^{86}Rb^+$ uptake during lag and early stationary phases may help to elucidate further the mechanisms for fluctuations in the movement of monovalent cations during the cell growth cycle. In any event, the

data presented here demonstrate that dramatic changes occur in $^{86}Rb^{+}$ uptake and [^{3}H]ouabain binding during the cell growth cycle of L5178Y cells. The implications of these changes remain to be elucidated, but it is tempting to question whether the alterations in cation movement during the cell growth cycle are casual or causal results of membrane properties as a function of temporal position in the cell growth cycle.

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