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CONTROL BY TREATMENT WITH LITHIUM CHLORIDE OF ORNITHINE DECARBOXYLASE IN EHRLICH ASCITES TUMOR CELLS

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Abstract—The activity of ornithine decarboxylase (ODC) was increased in Ehrlich ascites tumor cells by a change of the medium. This increase in the activity was inhibited by the addition of LiCl to the medium. Na^+ and Mg^{2+} did not affect the enzyme activity. The inhibition of the enzyme activity with LiCl was not reversed by the addition of inositol or dibutyryl cyclic AMP. Total RNA was isolated from cells treated with LiCl and the relative abundance of the ODC mRNA was measured by Northern blot analysis. These levels in cells treated with LiCl were comparable to those in control cells. In the treated cells, the biological half-life of ODC was 14 min, which was the same as for the control cells. The inhibition by LiCl of ODC activity was not due to the nonspecific toxicity of LiCl. These results suggest that treatment of Ehrlich ascites tumor cells with LiCl suppressed ODC induction during translation, not during transcription or after translation.

Cell development is a complex sequential process consisting of various intra- and intercellular events involving specific structural and functional alterations in the cell. Cellular polyamine levels and their corresponding biosynthetic enzymes such as ornithine decarboxylase (ODC) and *S*-adenysylmethionine decarboxylase increase during the early phase of cell growth and development. ODC is the first and key enzyme in polyamine biosynthesis in mammalian cells, and rapidly responds to hormones, growth factors and other stimuli [1, 2]. Thus, ODC is important in the control of cell growth and differentiation in many types of cells.

Lithium has strong effects on human behavior and also on early embryonic development. Lithium is the main drug now used to treat manic-depression. Despite its therapeutic success, little is known about the pharmacological mechanism by which lithium acts. Recently, it has been reported that lithium affects various signal transduction pathways. One of the proposed mechanisms could involve inhibition of *myo*-inositol phosphate phosphatase [3], resulting in the inhibition of phosphatidylinositol turnover. It is also proposed that lithium inhibits components of various neurotransmitter signaling pathways, such as those for the formation of cyclic AMP [4] or cyclic GMP [5]. On the other hand, lithium stimulates the membrane-bound phospholipase C of PC12 cells exposed to nerve growth factor both *in vitro* and *in vivo* [6]. Here. we found that lithium inhibited ODC induction in Ehrlich ascites tumor cells and that the inhibition was due to post-transcriptional, and not post-translational, regulation.

MATERIALS AND METHODS

Ehrlich ascites tumor cells were cultured in a humidified atmosphere of 5% CO_2 in air at 37° for 2–4 days in Eagle's minimum essential medium containing 10% fetal calf serum, and then washed and suspended at the cell concentration of 1×10^6/mL in fresh medium [7].

ODC activity was assayed as described previously [8]. In brief, cells were washed with phosphate-buffered saline and suspended in 0.15 mL of 50 mM Tris–HCl buffer (pH 7.5) containing 250 μM pyridoxal phosphate, 0.1 mM EDTA and 2.5 mM dithiothreitol. Then the cells were disrupted by being frozen and thawed three times, and crude extracts were prepared for the enzyme assay by centrifugation at 30,000 *g* for 20 min. ODC activity was measured by estimation of the amounts of putrescine formed from L-[5-^{14}C]ornithine as described previously [9]. The protein concentration was measured by a dye-binding assay [10].

For isolation of RNA, cells were washed with Eagle's minimum essential medium twice and collected by centrifugation at 400 *g* for 5 min. The cells were lysed by homogenization in 4 M guanidinium isothiocyanate and the total RNA was collected by ultracentrifugation through a block gradient of 5.7 M CsCl by the method of Chirgwin *et al.* [11]. The RNA was further purified by sequential ethanol precipitation. The amount of RNA was measured by the absorbance at 260 nm.

RNA hybridization was done as described elsewhere [12]. Total RNA was denatured by being heated in 6% formaldehyde and 50% formation at 60° for 15 min before gel electrophoresis. Samples were fractionated by electrophoresis on 1% agarose gels containing 2.2 M formaldehyde and transferred to a nylon membrane (Hybond-N, Amersham). The membranes were prehybridized at 42° for 20 hr in 10% dextran sulfate, 5 × SSPE (standard saline phosphate EDTA), 5 × Denhardt's solution and

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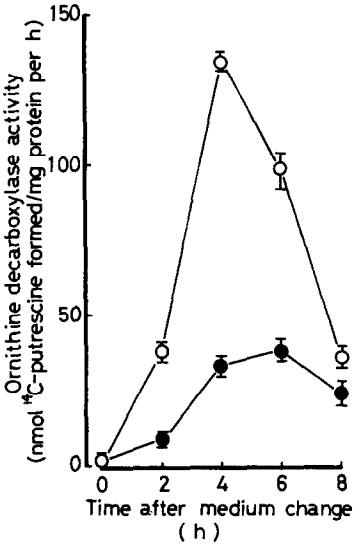


Fig. 1. Effect of LiCl on ornithine decarboxylase activity in Ehrlich ascites tumor cells stimulated with serum. After 4 days of culture the medium was changed. Then the cells were cultured with or without 10 mM LiCl for up to 8 hr. Key: (○) control; (●) LiCl.

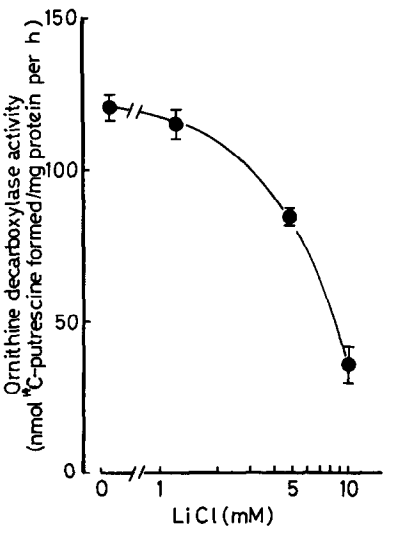


Fig. 2. Effect of LiCl on ornithine decarboxylase activity in Ehrlich ascites tumor cells. After a change to fresh medium, the cells were cultured with various concentrations of LiCl and harvested 4 hr later.

50% formamide, and then hybridized in the same solution for 38 hr with ODC cDNA or actin cDNA labeled with [^{32}P]dCTP with use of a multiprime labeling system (Amersham). Plasmid sp64 containing ODC cDNA was kindly supplied by Dr C. Kahana (Weizman Institute of Science) [13]. To normalize the amounts of RNA applied to the gel, the relative amounts of ODC and β-actin mRNA were calculated by densitometric scanning of the autoradiogram films (LKB Ultroscan XL laser densitometer).

RESULTS

After culture of Ehrlich ascites tumor cells for 2–4 days in Eagle's minimum essential medium containing 10% fetal calf serum, the cells were cultured in fresh medium at 37°. The ODC activity changed with time after the change of medium, increasing rapidly and with a peak at 4 hr. This increase was transient, declining to the level of the control at 8 hr. The activation of ODC is affected by cycloheximide but is unaffected by actinomycin D [12]. The addition of LiCl significantly suppressed ODC activity enhanced after a change to fresh medium (Fig. 1). The inhibition of ODC activity with LiCl was dose-dependent (Fig. 2).

To rule out the possibility that LiCl acts directly on the enzyme to suppress its activity, ODC activity was assayed *in vitro* in the presence of LiCl at various concentrations up to 10 mM. The enzyme was inhibited little if at all at the concentrations of LiCl used (Table 1).

To see if the effects of Li were the result of changes in ionic strength and osmolarity, sodium chloride or magnesium chloride was used as the control. Na^+ or Mg^{2+} had no effect on ODC activity in cells (Table 2).

It has been reported that LiCl inhibits *myo*-inositol phosphate phosphatase [3] and adenylate cyclase [4], so we examined the effects of inositol or dibutyryl cyclic AMP on ODC activity in cells treated with LiCl. The inhibition of the enzyme activity with LiCl was not reversed with the addition of inositol or dibutyryl cyclic AMP significantly (Table 3). Cells differ in their ability to take up and accumulate inositol [14]. To check the possibility that the lack of effect of inositol is due to inositol not being taken up during the experiment, we treated cells with 5 mM inositol for 1 day and then incubated the cells with LiCl for 4 hr. In inositol-treated cells, LiCl suppressed the ODC activity the same as in control cells (Table 4).

The inhibition observed when LiCl was added to the culture medium at 2 hr after a change to fresh medium was similar to that in the cells treated with LiCl immediately after the change in medium (Table 5). When LiCl was added to the culture at 3 hr and the cells were harvested 1 hr later, the drug caused 30% inhibition of the enzyme induction. These results suggest that LiCl affects the late stage of enzyme induction.

To find whether the inhibition of ODC activation was due to changes in the level of ODC mRNA, total RNA was isolated from the tumor cells treated with or without LiCl and the relative abundance of the mRNA for ODC was measured by Northern blot analysis. The ODC mRNA level in the cells treated with LiCl was comparable to that in control cells (Fig. 3). These results suggest that the LiCl treatment of cells suppressed ODC induction post-transcriptionally.

To examine the rate of turnover of ODC, cells that were cultured for 4 hr after a change to fresh medium were treated with cycloheximide and the decreases in their ODC activity were monitored. In

Table 1. Effect of LiCl on ODC activity *in vitro*

Concentration of LiCl (mM)	ODC activity (nmol [^{14}C]putrescine formed/mg protein/hr)
0	113.1 ± 2.1
0.625	115.0 ± 2.9
1.25	114.0 ± 0.7
2.5	116.0 ± 1.0
5.0	116.0 ± 2.2
10.0	116.0 ± 0.7

ODC activity was assayed *in vitro* in the presence of various concentrations of LiCl. The results are the means ± SE at three assays.

Table 2. Effects of LiCl, NaCl and $MgCl_2$ on ODC activity

Treatment	ODC activity (nmol [^{14}C]putrescine formed/mg protein/hr)
None, 0 hr	2.7 ± 0.1
None, 4 hr	91.2 ± 8.6
LiCl, 4 hr	31.9 ± 2.3
NaCl, 4 hr	97.2 ± 7.5
$MgCl_2$, 4 hr	84.7 ± 5.0

Cells were treated with LiCl (10 mM), NaCl (10 mM) or $MgCl_2$ (10 mM) for 4 hr after a change to fresh medium. The results are the means ± SE of three experiments.

Table 3. Effect of inositol and dibutyryl cyclic AMP (db-cAMP) on ODC activity in cells treated with LiCl

Treatment	ODC activity (nmol [^{14}C]putrescine formed/mg protein/hr)
Expt. 1	
None	131.0 ± 13.0
LiCl	46.0 ± 1.2
LiCl + inositol, 2.5 mM	46.4 ± 3.1*
LiCl + inositol, 5 mM	44.5 ± 1.3*
LiCl + inositol, 10 mM	40.1 ± 9.2*
Expt. 2	
None	103.0 ± 8.9
LiCl	31.2 ± 4.1
LiCl + db-cAMP, 2.5 mM	31.6 ± 4.2*
LiCl + db-cAMP, 5 mM	39.2 ± 5.1*
LiCl + db-cAMP, 10 mM	38.1 ± 3.9*

After a change to the medium, cells were cultured for 4 hr with LiCl (10 mM), inositol or db-cAMP. The results are the means ± SE of three experients.

* Not significant by the Student's *t*-test compared with the activity in LiCl-treated cells.

the cells treated with LiCl, the biological half-life of ODC was 14 min, the same time as for control cells (Fig. 4). These results suggest that treatment with LiCl of Ehrlich ascites tumor cells suppressed ODC induction during translation, not later.

Treatment of the cells for 4 hr with 10 mM LiCl followed by removal of LiCl by washing did not inhibit an increase in ODC caused by a change to fresh medium in the next 2 hr. The activity of ODC in medium from which LiCl had been removed was estimated to be about the same as control cells cultured for 4 hr after a change to fresh medium (Fig. 5). These results show that the effect of LiCl was fully reversible.

To examine whether LiCl interferes with the induction of ODC by other stimuli, we studied the effect of LiCl on the activity induced with 12-*O*-tetradecanoylphorbol 13-acetate. The stimulation with the phorbol was suppressed completely with LiCl (Table 6).

Table 4. Effect of treatment with inositol on ODC activity

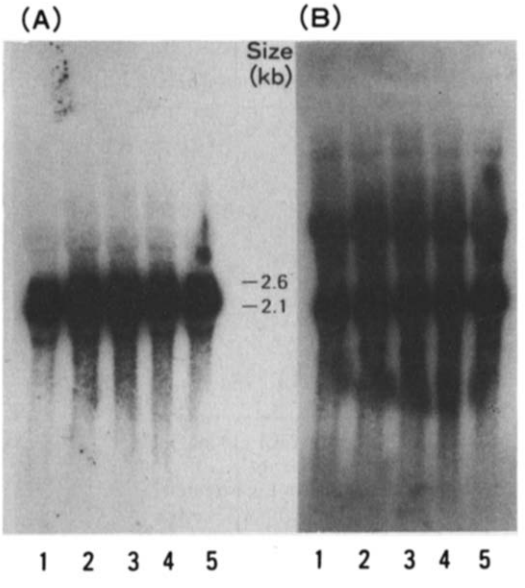
First treatment	Second treatment	ODC activity (nmol [^{14}C]putrescine formed/ mg protein/hr)
None	None	59.0 ± 9.0
None	LiCl	7.3 ± 1.3
Inositol	None	54.6 ± 2.7
Inositol	LiCl + inositol	7.7 ± 4.9

Cells were treated with inositol (5 mM) for 24 hr. The cells were moved to fresh medium and treated with LiCl (10 mM) for 4 hr. The results are the means ± SE of three experiments.

Table 5. Effect of delayed addition of LiCl on ODC activity

Treatment	ODC activity (nmol [^{14}C]putrescine formed/mg protein/hr)
None	128.6 ± 5.1
LiCl at 0 hr	44.1 ± 8.3
LiCl at 1 hr	32.6 ± 2.1
LiCl at 2 hr	40.7 ± 6.2
LiCl at 3 hr	89.0 ± 7.9
LiCl at 4 hr	133.9 ± 7.7

After a change to fresh medium, cells were cultured for 4 hr. LiCl was added at 0, 1, 2, 3 or 4 hr. The results are the means ± SE of three experiments.



(C)

Lane	Densitometric units ODC	Actin	ODC/Actin
1	1.08	0.67	1.61
2	1.23	0.77	1.60
3	1.30	0.79	1.65
4	1.28	0.78	1.64
5	1.31	0.81	1.62

Fig. 3. Effect of LiCl on ornithine decarboxylase mRNA (A) and actin mRNA (B) in Ehrlich ascites tumor cells. After a change to fresh medium, cells were cultured with or without 10 mM LiCl. Total RNA from 7×10^6 cells was isolated and electrophoresed in a formaldehyde–agarose gel (10 μg per lane). Following transfer to a nylon membrane, the sample was hybridized to a mouse ornithine decarboxylase probe and detected by autoradiography. The membrane was rehybridized to a mouse β-actin probe. To normalize the amounts of RNA applied on the gel, the relative amounts of ornithine and β-actin mRNAs were found by densitometric scanning of the autoradiogram films (C). Lane 1, cells after change to fresh medium. Lane 2, cells cultured without LiCl for 2 hr. Lane 3, cells cultured without LiCl for 4 hr. Lane 4, cells cultured with LiCl for 2 hr. Lane 5, cells cultured with LiCl for 4 hr.

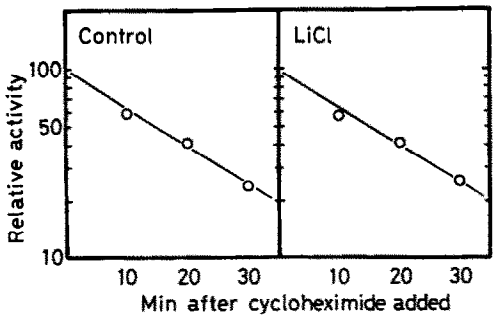


Fig. 4. Decline in ornithine decarboxylase activity after treatment of Ehrlich ascites tumor cells by cycloheximide. After a change to fresh medium the cells were cultured with or without LiCl. Then the cells were treated with cycloheximide (20 μg/mL).

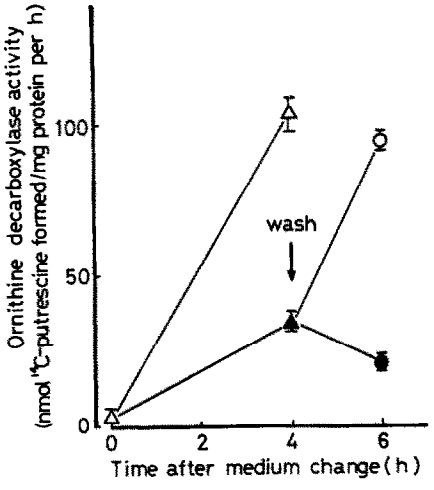


Fig. 5. Reversibility of inhibition by LiCl. Cells were treated for 4 hr with (▲) or without (△) LiCl. After the cells were washed, they were treated for 2 hr with (●) or without (○) LiCl in fresh medium. The results are the means ± SE of three experiments.

To ascertain whether the inhibition by LiCl of ODC activity is selective, the effect of LiCl on the activity of spermidine/spermine N^1-acetyltransferase, an inducible enzyme that has a rapid turnover, was studied. The enzyme activity increased two-fold 4 hr after the addition of LiCl (data not shown). These results suggest that the inhibition by LiCl of ODC activity enhanced after a change to fresh medium is not due to the nonspecific toxicity of LiCl.

DISCUSSION

The results of this study showed that treatment of Ehrlich ascites tumor cells with LiCl suppresses ODC induction dose-dependently, but did not affect the biological half-life of the enzyme activity. Such treatment did not change the level of ODC mRNA. These results confirm previous findings about the inhibition of ODC activity with LiCl treatment [15, 16]. The observation that LiCl treatment suppresses ODC induction during translation is new. The results also show that the suppression of the increase in ODC activity is to be fully reversible and not to be the result of non-specific cellular toxicity of LiCl.

There are many reports that the regulation of ODC occurs primarily at the level of translation in a variety of cells. Kahana and Nathans [17] have shown that neither the amounts of ODC mRNA in the cell nor the half-life of ODC protein is affected when cells overproducing ODC are exposed to polyamines. We have also shown that hyperthermic treatment of Ehrlich ascites tumor cells suppressed ODC induction during translation [12].

The mechanism of inhibition during translation with LiCl treatment is not understood. Lithium inhibits inositol 1-phosphate degradation; it consequently results in the accumulation of inosotol 1-phosphate and its precursor, and in the depletion of intracellular inositol [3]. Lithium also inhibits adenylate cyclase in a number of cell types. Scott *et al.* [18] have reported that mitogenic ligands to human lymphocytes activate ODC within minutes, and that this rapid activation is unaffected by cycloheximide. They also found that this rapid increase in ODC activity is inhibited with 1 mM LiCl present for 30 min in an inositol-free medium. This inhibition restored the inducibility of ODC within 10 min with the addition of 1 mM inositol [16]. It is unlikely that these effects of LiCl caused the inhibition of ODC induction in Ehrlich ascites tumor

Table 6. Effect of LiCl on ODC activity in Ehrlich ascites tumor cells treated with TPA

Treatment	ODC activity (nmol [^{14}C]putrescine formed/mg protein/hr)
None, 0 hr	6.3 ± 0.9
None, 4 hr	5.7 ± 1.0
LiCl, 4 hr	0.2 ± 0.1
TPA, 4 hr	10.6 ± 1.4
TPA + LiCl, 4 hr	0.7 ± 0.1

After a change to fresh medium, cells were cultured for 16 hr to avoid the effect of renewal of the culture medium. Then the cells were cultured with or without 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (50 ng/mL) and LiCl (10 mM). The results are the means ± SE of three experiments.

cells, because the addition of *myo*-inositol or dibutyryl cyclic AMP did not restore the ODC induction.

Whether inhibition of ODC by lithium contributes to its therapeutic efficacy in affective disorders is not known. Verapamil, which is used to treat manic-depression illness, also inhibits the ODC activity induced by parathyroid hormone and forskolin in an osteoblast cell line [19]. Several investigations have found circadian or diurnal rhythm in the levels of ODC activity in the liver and small intestine. There is evidence that the circadian system is altered in many individuals suffering from manic-depressive illness. Lithium is particularly effective in those patients who have the fastest circadian rhythm [20]. These observations suggest that the acting of lithium on ODC activity might be part of the mechanism of its effects in manic-depressive illness.

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