K. Y.

THE INHIBITION OF THE INDUCTION OF ORNITHINE DECARBOXYLASE BY CATIONS

Kuangyu Chen, John S. Heller, and E.S. Canellakis

Department of Pharmacology, Yale University School of Medicine

New Haven, Connecticut 06510

Received March 8,1976

SUMMARY: L1210 cells reinitiate growth after dilution with fresh medium; during this time there occurs a transient increase in ornithine decarboxylase (EC4.1.1.17) (ODC) activity. The addition of 10 to 20 mM Na^+, K^+ or Mg^{++} completely inhibits this induction of ODC activity with no effect on cell growth. These cations also inhibit the increase of ODC activity in neuroblastoma cells and in H-35 cells which is induced by prostaglandin E_1 plus 3-isobutyl-1-methylxanthine and by 15% fetal calf serum respectively. This inhibitory effect of low levels of cations on the induction of ODC activity in different cell lines suggests that the intracellular function of ODC, and of the products of the reaction it catalyzes (putrescine, spermidine and spermine), may be intimately involved with changes in cation pools.

We have previously shown that the intracellular ornithine decarboxylase activity of L1210 cells can be affected by agents which disrupt the microtubule-microfilament cytoskeleton (1). This phenomenon was considered as a model system for reactions which link the perturbation of the cell membrane with intracellular changes in ODC activity.

ODC is the rate-limiting enzyme in polyamine synthesis and its intracellular activity in eukaryotic cells can be induced by increases in the rate of growth (2,3). The polyamines are implicated in RNA polymerase activity (4), tRNA methylation (5), polypeptide chain elongation (6) and other cellular functions. It is this breadth of association of these polyamines that makes it difficult to assess their exact role in cell metabolism.

In most of the reactions mentioned above, there occurs an overlap between the function of the polyamines and other cations; specifically potassium and magnesium (3). Alterations in the intracellular osmotic pressure (7), in the membrane potential (7) as well as changes in the transport of non-electrolytes (8) and in macromolecular synthesis (9,10) have been shown to be affected by

alterations of K^+:Na^+ ratios in the interior and the exterior milieu of the cell. Furthermore it has been reported that lectin stimulated lymphocytes (11) and Ehrlich ascites cells (12), virus transformed fibroblasts (13) and quiescent cells stimulated by serum (14) show increased K^+ transport associated, in many cases, with changes in ODC activity (1,15,16). Studies in plant tissues have also emphasized that the plant polyamines and the physiological cations are interchangeable (3,17). Because of these associations we have attempted to relate, in the present work, modifications of cation concentrations in the growth medium of cells grown in culture to fluctuations of intracellular ODC activity.

MATERIALS AND METHODS

The following compounds were purchased: [1-^{14}C]-DL-ornithine, 10∿20 mCi/mM; aquasol; [^{3}H]thymidine, [^{3}H]uridine, and [^{3}H]leucine from New England Nuclear, Boston, Mass.; horse serum, fetal calf serum, Fischer's medium and minimal essential medium from Grand Island Biochem.; prostaglandin E_1 (PGE_1) from Dr. J. Pike, Upjohn Co.; 3-isobutyl-1-methylxantine (IBMX) from Aldrich Chem. Co. All other chemicals were of reagent grade and were obtained from Fischer Scientific Co., Springfield, N.J.

Cell Culture. L1210 cells were grown as previously described (18). The cells were grown to the late log phase (1.3 x 10^6 cells/ml) and then diluted with fresh Fischer's medium plus 10% horse serum to about 4 x 10^5 cells/ml for subsequent studies. Different amounts of cations were added to the fresh medium to observe their effects on the induction of ODC activity after cell dilution. H-35 cells were grown on 100 mm Falcon plastic plates in Eagle's minimal medium plus 15% fetal calf serum. At confluency, the plates were rinsed with saline, fresh medium minus serum was added and 24 hrs later ODC activity stimulated by the addition of fresh medium plus 15% fetal calf serum. The cells were harvested 2 hrs later and ODC activity assayed. Neuroblastoma cells were grown on 100 mm Falcon plastic plates in Dulbecco's modified Eagle's minimal medium with 10% fetal calf serum. At confluency, the plates were rinsed with Dulbecco's medium containing 25 mM HEPES pH 7.4 and no serum. IBMX and PGE_1 (0.5% in ethanol) were added to each plate containing the serum free medium at the final concentration of 0.5 mM and 10 mM respectively. The cells were harvested 4 hrs later and ODC activity assayed.

Assay of ODC Activity. Ornithine decarboxylase was determined by measuring the release of $^{14}CO_2$ from [1-^{14}C]-DL-ornithine as previously described (1). The specific activity of the enzyme is expressed as nmoles $^{14}CO_2$ released per mg protein per hour.

RESULTS AND DISCUSSION

1. Effect of cations on ODC activity of neuroblastoma and H-35 cells.

The basal ODC activity of confluent cultures of H-35 and neuroblastoma cells is very low, however the enzyme activity can be stimulated approximately

Table 1. Effect of cations on the induction of ODC activity of neuroblastoma cells and H-35 cells

Growth medium* $MgCl_2$ mM	Induction medium* $MgCl_2$ mM	Cellular ODC activity nmoles CO_2/mg/hr	Inhibition Per Cent
Neuroblastoma cells			
0	0	3.2	0
0	10	1.6	50
0	20	0.86	73
10	10	1.65	48
10	0	2.80	12
20	20	1.61	50
20	0	3.65	stimulation (14%)
H-35 cells			
0	0	0.36	0
0	10	0.030	92
0	20	0.001	99
10	10	0.073	80
10	0	0.40	stimulation (11%)
20	20	0.030	92
20	0	0.43	stimulation (20%)

* The composition of growth medium and induction medium were described in Materials and Methods.

200-fold within 2 hrs by the addition of medium plus serum, to serum-depleted H-35 cells or within 4 hrs by the addition of PGE_1 and IBMX to neuroblastoma cells. The addition of 10-20 mM $MgCl_2$ to the inducing media inhibits completely the rise in ODC activity in H-35 cells; the rise in ODC activity in neuroblastoma cells is inhibited by more than 50% (Table 1). In addition, this table shows that regardless of whether the cells were grown in the presence or ab-

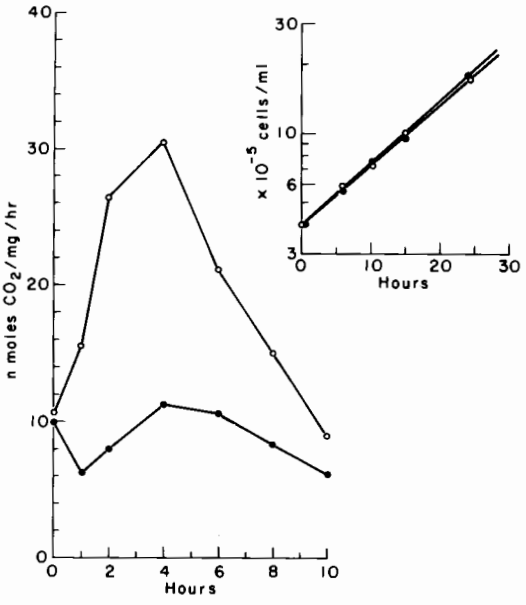

Fig. 1. Effect of 15 mM KCl on the induction of ODC activity of L1210 cells. L1210 cells at late log phase were diluted with fresh Fischer's medium plus 10% horse serum and ODC activity was assayed at designated time periods. -o-, control; -●-, 15 mM KCl was added to the fresh medium before dilution. Inset is the growth curve.

sence of added Mg^{++}, the presence of Mg^{++} in the induction medium prevents the induction of ODC. The converse also obtains, because when stationary cells are diluted in the absence of Mg^{++}, ODC activity is always induced.

2. Inhibition of the induction of ODC activity in L1210 cells by cations.

Dilution of L1210 cells at late log phase or at stationary phase into fresh medium results in a stimulation of ODC activity reaching a maximum approximately 2-4 hrs after dilution. Fig. 1 shows that if the fresh medium, at the time of dilution also contains 15 mM KCl, the increase of ODC activity is completely inhibited with no undue effects on the subsequent growth of the cells. A similar result is obtained if the dilution is done in medium with 15 mM NaCl or with 15 mM $MgCl_2$ (these concentrations of Na^+, K^+ and Mg^{++} have little effect on ODC activity when added to an in vitro assay). This involvement of the cations on the regulation of the induction of ODC activity is

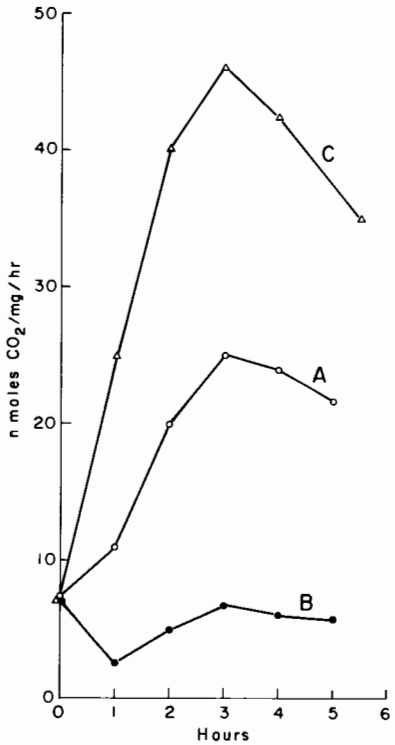

Fig. 2. Effect of 15 mM $MgCl_2$ on the induction of ODC activity of L1210 cells. L1210 cells were grown in normal medium to the late log phase and were diluted with fresh Fischer's medium plus 10% horse serum in the presence (-●-) or in the absence (-o-) of added 15 mM $MgCl_2$. -Δ-: L1210 cells were grown in Fischer's medium with added 15 mM $MgCl_2$ for 2 generations and were then diluted into the normal fresh Fischer's medium.

further emphasized by the converse experiment. Fig. 2 shows that when cells which have been grown in the presence of 20 mM $MgCl_2$ up to the stationary phase, are diluted into normal fresh medium (i.e., lower $MgCl_2$), there occurs an almost two-fold greater increase in ODC activity than the increase in ODC activity usually obtained. This indicates that whereas the high Mg^{++} concentration inhibits the induction of ODC activity a lowering of Mg^{++} concentration enhances the induction of ODC activity.

3. The adaptation of L1210 cells to a high Mg^{++} medium.

L1210 cells grown in the presence of 20 mM $MgCl_2$ over several generations

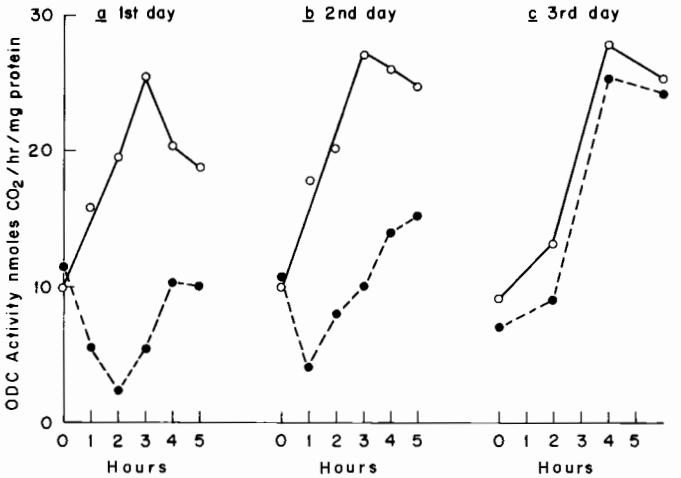

Fig. 3. Adaptation of L1210 cells in the presence of 20 mM $MgCl_2$. L1210 cells grew in Fischer's medium and were diluted every 24 hrs (roughly 2 generations). a, L1210 cells at 1.2 x 10^6 cells/ml were diluted in the absence (-o-) or in the presence (-●-) of 20 mM $MgCl_2$ to 3 x 10^5 cells/ml. b, control cells were diluted with normal medium; the Mg-treated cells were diluted with 20 mM Mg^{++} containing medium. c, control cells were diluted with normal medium and Mg-treated cells were diluted with 20 mM Mg^{++} containing medium.

adapt slowly to this higher Mg^{++} level until they reach the stage where they again show a normal induction of ODC activity even when diluted at stationary phase with high Mg^{++} media (Fig. 3). The figure shows that the induction of ODC activity in L1210 cells in Fischer's medium is inhibited by 20 mM Mg^{++} (Fig. 3a); this result corresponds to that obtained with K^+ (Fig. 1). However if the L1210 cells are grown in a high Mg^{++} medium for 2 generations and then diluted at stationary phase with a high Mg^{++} medium, a low but definite induction of ODC activity is observed (Fig. 3b). In addition, if the L1210 cells have been grown for more than 4 generations in a high Mg^{++} medium, then dilution at stationary phase with a high Mg^{++} medium results in an induction of ODC activity which is almost equivalent to that of the control (Fig. 3c). These results emphasize that the cell adapts to the new environment with a normal induction of ODC activity in the presence of what was an inhibitory Mg^{++} concentration; furthermore it appears that the phenomena we are observing

are central to the function of the cell, but that the particular conditions that are required to elicit them will vary depending upon the prior conditioning of the cell.

4. The effect of cations on macromolecular synthesis in L1210 cells.

Although the three cations inhibit the induction of ODC activity at 10-20 mM, they have no adverse effect on cell growth. We also found that they do not inhibit the incorporation of thymidine into DNA and leucine into protein. However, a small percentage (less than 10%) stimulation of uridine incorporation in the presence of exogenous cations is consistently observed (data not shown). The results clearly indicate that KCl and $MgCl_2$ as well as NaCl, when added in concentrations which have no apparent effect on the growth rate of the cells or on macromolecular synthesis, can abolish the characteristic normal induction of ODC which occurs after the reinitiation of growth in L1210 cells and after the stimulation of growth of neuroblastoma and H-35 cells.

This study pertains strictly to the inhibition of the induction of ODC activity by cations and should be differentiated from the inhibition of the basal intracellular activity of ODC. Changes in the latter have been shown by Munro et al. (19) to occur at high changes of osmolality of the surrounding medium; for instance, an increase by 50 mM NaCl inhibits the basal ODC activity by less than 60%. This effect should be contrasted with the almost complete inhibition of induction of ODC activity noted in the present experiments with 10 mM NaCl

The inhibition of the induction of ODC activity does not appear to be due to osmotic changes but due to cation specific effects. The osmolality of Fischer's medium is approximately 325 mOsm/Kg (20). Adding 10 to 20 mM KCl increases the osmolality by approximately 7% to 15% and causes a complete inhibition of the induction of ODC activity. However, we find that an increase of the osmolality by the addition of 20 to 40 mM sucrose, inhibits the induction of ODC activity only by 30% to 50%. Consequently, the change in osmolality is not sufficient in itself to have a profound effect on the inhibition of the induction of ODC activity. Furthermore, the marginal inhibition of ODC activity that we have

observed, when the osmolality of the medium is changed by the addition of sucrose, may well be a reflection of changes in intracellular concentrations of those cations which are the proximal effectors of the inhibition of the induction of ODC.

In additional experiments we find that the same effect is produced by the chloride and the sulfate anions of a given cation. In contrast, we find that some changes seem to be cation specific. For instance, the induction of ODC activity in H-35 cells is less sensitive to changes in Na^+ than to changes in K^+; in contrast, the induction of ODC activity in L1210 cells is equally sensitive to changes in Na^+ and K^+.

Our current working hypothesis is that the inhibition of the induction of ODC activity in response to membrane perturbations (1) and the inhibition of the induction of ODC activity observed with small changes of cation pools may be causally related; it is possible that fluctuations of cation pools may act as a linkage between the microtubule-microfilament cytoskeleton and ODC.

Acknowledgment: The authors wish to thank Ms. S.M. Tu for her excellent technical assistance. This work was supported by American Cancer Society Grant BC75 and USPHS Grant CA-04823 and a USPHS Research Career Award to E.S.C. No. K06-GM-03070.

REFERENCES

1. Chen, K.Y., Heller, J. and Canellakis, E.S. (1976) Biochem. Biophys. Res. Comm. 68, 401-408.
2. Russell, D.H. in Polyamines in Normal and Neoplastic Growth (1973), ed. by Russell, D.H., pp. 1-13, Raven Press, New York.
3. Cohen, S.S. Introduction to the Polyamines (1971), Prentice-Hall, N.J.
4. Herbst, E.J., Byus, C.V. and Nuss, D.L. in Polyamines in Normal and Neoplastic Growth (1973), ed. by Russell, D.H., pp. 71-90, Raven Press, New York.
5. Hacker, B. in Polyamines in Normal and Neoplastic Growth (1973), ed. by Russell, D.H., pp. 55-70, Raven Press, New York.
6. Igarashi, K., Hara, K., Watanabe, Y., Hirose, S. and Takeda, Y. (1975) Biochem. Biophys. Res. Comm. 64, 897-904.
7. Sheppard, J.R. (1972) Nature New Biol. 236, 14-16.
8. Schwartz, A., Lindenmayer, G.F. and Allen, J.C. (1972) in Current Topics in Membranes and Transport, eds. Bronner, F. and Kleinzeller, A., p. 1, Academic Press, New York.
9. Lubin, M. (1967) Nature 213, 451-453.
10. Appel, S.A., Autilo, L., Festoff, B.N. and Escueta, A.V. (1969) J. Biol. Chem. 244, 3166-3172.

11. Wright, P., Quastel, M.R. and Kaplan, J.G. (1973) Proc. Seventh Leukocyte Conference, pp. 87-104.
12. Quastel, M.R. and Kaplan, J.G. (1971) Exp. Cell Res. 63, 230-233.
13. Kimelberg, H.K. and Mayhew, E. (1975) J. Biol. Chem. 250, 100-104.
14. Rozengurt, E. and Heppel, L.A. (1975) Proc. Nat. Acad. Sci. (U.S.A.) 72, 4492-4495.
15. Watanabe, T., Kishimoto, T., Miyake, T., Nishijawa, Y., Inoue, H., Takeda, Y. and Yamamura, Y. (1975) J. Immunol. 115, 1185-1190.
16. Hogan, B., Shields, R. and Curtis, D. (1974) Cell 2, 229-233.
17. Sinclair, C. (1967) Nature 213, 214-215.
18. Tsai, C.M., Chen, K.Y. and Canellakis, E.S. (1975) Biochim. Biophys. Acta 401, 196-212.
19. Munro, G.F., Miller, R.A., Bell, C.A. and Verderber, E.L. (1975) Biochim. Biophys. Acta 411, 263-281.
20. Waymouth, C.(1970) In Vitro 6, 109-127.