STUDIES ON THE REGULATION OF ORNITHINE DECARBOXYLASE ACTIVITY BY THE MICROTUBULES: THE EFFECT OF COLCHICINE AND VINBLASTINE

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

Department of Pharmacology, Yale University

New Haven, Connecticut 06510

Received November 3,1975

SUMMARY: Colchicine and vinblastine in micromolar concentrations inhibit the activity of ornithine decarboxylase (E.C.4.1.1.17) (ODC), of mouse leukemia L1210 cells, which has been stimulated by dilution of the cells with fresh medium and serum. The colchicine analogues, lumicolchicine and colchiceine, which do not affect microtubular strcuture, do not inhibit ODC activity even at 10^{-4}M. However, it appears that disruption of the microtubular structure is not in itself enough to inhibit ODC activity but that one or more additional temperature dependent steps are involved. We propose that the microtubule system is one of a series of components which regulates ODC activity.

Based on the study of the modulation of lymphocyte receptor mobility by ConA, dimeric succinyl ConA and anti-mitotic drugs, Edelman and his coworkers (1,2) proposed that the membrane receptor-cytoplasmic interactions are mediated by an assembly of colchicine-binding proteins, most likely the microtubules. According to this hypothesis, the disruption of the microtubule system should in general abolish the information mediated by the cell surface. Recent work on the spectrin and glycophorin of erythrocyte ghosts (3), the mosaic separation of phagocytic sites and transport sites (4) and the inhibition by colchicine of the mitogenic stimulation of lymphocytes by ConA (5) also support this hypothesis.

Ornithine decarboxylase, the rate-limiting enzyme in the polyamine biosynthetic pathway, exhibits high levels of activity in tissues or cells with high rates of growth (6). ODC activity is increased dramatically when cultured cells are transferred from the resting to the growing state by the addition of fresh medium and serum (7). It can also be induced in vivo by various reagents that affect cell growth, such as cyclic AMP (8), dexamethasone (9) and puromycin (10).

In previous experiments, we have shown that antibodies to L1210 membrane proteins in the absence of complement will inhibit the growth of cells; presumably by cell surface interactions (11,12). Furthermore, preliminary unpublished experiments from this laboratory indicated that this inhibition of growth was associated with a decrease in ODC activity of L1210 cells. Consequently, it seemed reasonable to speculate that these various responses of ODC could be associated with perturbation of the plasma membranes.

In testing a variety of microtubule disrupting agents we found that they inhibit ODC activity which has been stimulated by dilution of the L1210 cells with fresh medium plus serum. In contrast, structural analogs which are known not to affect the microtubule system, such as lumicolchicine and colchiceine had no effect on ODC activity.

MATERIALS AND METHODS

Chemicals. Colchicine, pyridoxal phosphate were purchased from Sigma Chemical Company, St. Louis, Mo. DL-[1-^{14}C]ornithine (sp. act. 7.5 mCi/mmole) and aquasol were products of New England Nuclear; DL ornithine monohydrochloride was obtained from Nutritional Biochem. Corp. Vinblastine was obtained from Eli Lilly Co. and cytochalasin B was from Aldrich. NCS tissue solubilizer was purchased from Amersham/Searle Corp. Lumicolchicine and colchiceine were kindly given by Dr. G.T. Shiau, Department of Pharmacology, Yale University.

Cell culture. L1210 cells were grown as described previously (13). The cells were grown to the late log phase (1.5 x 10^6/ml) and then diluted with fresh Fisher's medium plus 10% horse serum to about 4 x 10^5/ml for all subsequent studies.

Assay of ODC activity. Approximately 6 x 10^6 cells were harvested by centrifugation and washed twice with PBS at 4°C. The cells were resuspended in 0.4 ml 50 mM Tris (pH 7.2 at 37°C) containing 0.1 mM EDTA, 0.05 mM pyridoxal phosphate, 10 mM dithiothreitol and disrupted by one cycle of freezing and thawing. Enzymatic activity was assayed according to Fong et al. (14) with the following modification. Cell homogenate 0.1 ml and 10 µl ornithine (0.05 µmole, 7.55 µCi/µmole) were put into a 17x100 mm plastic tube with a well containing 0.1 ml NCS-toluene (1:1 V/V) suspended from the cap. The reaction was stopped with 0.2 ml of 10% TCA after 60 minutes at 37°C and the tube was incubated for another 90 minutes to insure complete absorption of the $^{14}CO_2$. The radioactivity was determined by placing the well into 10 ml of aquasol and assayed in a Packard Tri-carb liquid scintillation spectrometer.

RESULTS AND DISCUSSION

1. Time course of stimulation of ODC activity (Fig. 1).

Following dilution of stationary L1210 cells, an increase in ODC activity

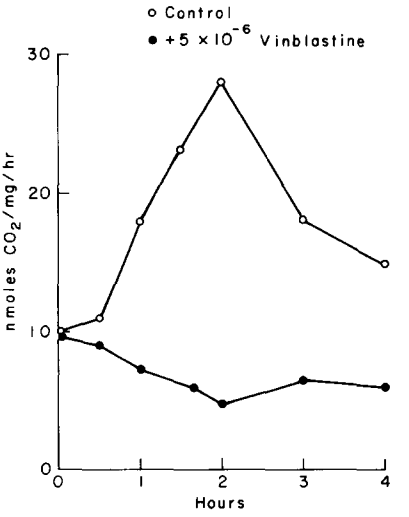

Fig. 1. Time course of the effect of vinblastine on the stimulation of ODC activity. Stationary L1210 cells were diluted with Fisher's medium plus 10% horse serum in the absence (o) or in the presence (●) of $5x10^{-6}$M vinblastine. Cells were collected at the designated times and ODC activity was measured as described in the text.

occurs which reaches a maximum after 2-6 hours. The exact time at which the peak activity is attained depends on the cell concentration at the stationary phase prior to dilution (K.Y. Chen, unpublished observations). In Fig. 1, the peak activity in the control sample is attained 2 hours after dilution of the cells. 10^{-6}M vinblastine added at time zero completely inhibits this stimulation of ODC activity. Colchicine has a similar effect.

2. Dose-response curve for colchicine, vinblastine, colchiceine and lumicolchicine on the inhibition of ODC activity (Fig. 2).

In this experiment enzyme activity was assayed 1 hour after stimulation as described in Fig. 1. Drugs at the appropriate concentrations were added at the time of dilution. Vinblastine produces a 50% inhibition of ODC activity at 8×10^{-8}M whereas colchicine caused a 50% inhibition at 7×10^{-7}M. This is in agreement with the greater effectiveness of vinblastine in disrupting the microtubular system (15). Lumicolchicine, an isomer of colchicine which has no effect on the microtubular system (16), does not inhibit ODC activity even at

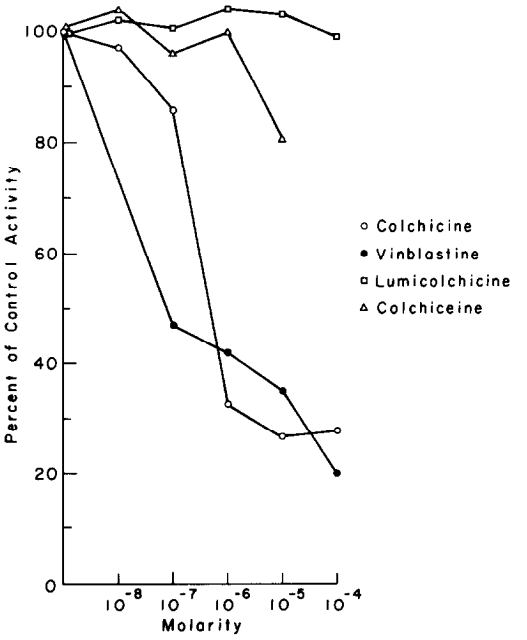

Fig. 2. Dose-response curve for colchicine (o), vinblastine (•), colchiceine (Δ) and lumicolchicine (□) on the inhibition of ODC activity of L1210 cells. Cells were diluted and various drugs were added simultaneously at different concentrations. ODC activity was assayed 1 hour after dilution.

5 x 10^{-4}M. Colchiceine, an analogue of colchicine which acts much less effectively as an anti-mitotic drug (17), inhibits ODC activity only 20% at 10^{-5}M. At these or higher concentrations, none of the compounds affect ODC activity when added to the enzyme assay, in vitro.

3. The effect of cytochalasin B and ouabain on the stimulation of ODC activity of L1210 cells (Fig. 3).

Since it has been proposed that the microtubular system functions cooperatively with the microfilament system as the cytoskeleton (2) cytochalasin B, a microfilament specific reagent, was tested. We found that it inhibited ODC activity by 50% at 7 x 10^{-6}M. We also tested ouabain, an inhibitor of $(Na^+ + K^+)$-ATPase, and found only 20% inhibition of ODC activity at 10^{-5}M. Neither of these compounds affected ODC activity when added directly to the enzyme assay.

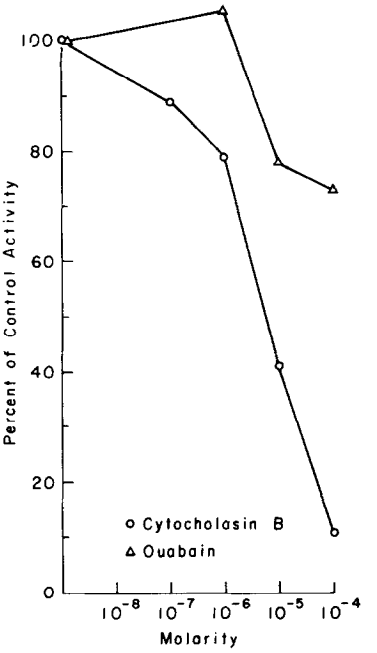

Fig. 3. Dose-response curve for cytochalasin B (o) and ouabain (Δ) on the inhibition of ODC activity of L1210 cells. Cells were diluted and cytochalasin B or ouabain was added simultaneously at different concentrations. ODC activity was assayed 1 hour after dilution.

4. Temperature effect on ODC activity of L1210 cells.

Various investigations have shown that the cytoplasmic microtubule system is sensitive to low temperature and that it is disrupted after storage of cells at 0° C for 15 minutes (18,19). We took advantage of this observation in order to ask the question whether the disruption of the microtubules is in itself enough for the inhibition of ODC activity. For this experiment the cellular ODC activity had been stimulated for 1 hr by dilution of L1210 cells, and then portions of cells were incubated at 0°C for 60 minutes both in the presence and in the absence of 10^{-6} M colchicine. We found that low temperature treatment of the cells in the presence or absence of colchicine did not alter the already increased ODC activity. The ODC activity of control cells was 754 cpm/10^5 cells/hr while the cold treated cells had a value of 740 cpm/10^5 cells/hr whether colchicine was present or not. This indicates that the disruption of the microtubule system is not enough in itself to inhibit ODC activity and

that there must exist temperature dependent steps between the disruption of the microtubules and the inhibition of ODC activity.

Colchicine and the vinca alkaloids are known as anti-mitotic drugs (20). These drugs also inhibit nucleoside transport in mammalian cells but do not inhibit the uptake of 2-deoxyglucose or α-amino isobutyric acid (21). They inhibit many secretory processes which generally are considered to be microtubule system related (22,23).

Although these drugs may affect many biological processes, the only known reaction of these drugs with the biological system on the molecular level is their specific binding with tubulin (24). A microtubule dependent biological process can be defined by the following criteria (25). 1) The concentration range where anti-mitotic drugs inhibit this process should be around 10^{-6}M or lower. 2) Colchicine analogues which are not anti-mitotic such as lumicolchicine should not inhibit this process. 3) The colchicine inhibition is temperature dependent, no inhibition should be osbserved at 0°C. A good example of applying these criteria and differentiating microtubule dependent processes from others is given by Mizel and Wilson (21) who showed that the effect of colchicine on nucleoside transport is not a process which depends on the integrity of the microtubule system.

Based on the above criteria our data strongly suggest that the regulation of ODC activity of L1210 cells is related to the integrity of the cytoplasmic microtubules. Cytochalasin B, which is thought to affect the microfilament system, inhibits ODC activity indicating that the microfilament system may also be involved in the mediation of membrane receptor-cytoplasmic interactions (2).

Recently it has been reported that colchicine, vinblastine and cytochalasin B at 10^{-4}M affect some plasma membrane enzymes activities, e.g. $(Na^+ + K^+)$-ATPase, Mg^{2+}-ATPase, adenylcyclase, etc. (26). It seems unlikely that the inhibition of ODC activity by colchicine is primarily due to the inhibition of these membrane enzymes because colchicine inhibits ODC activity at much lower concentrations. Furthermore, ouabain which inhibits $(Na^+ + K^+)$-ATPase does not significantly inhibit ODC activity even at 10^{-4}M.

Our low temperature experiment shows that the disruption of the microtubule system itself does not inhibit ODC activity, therefore, we have assumed that additional temperature sensitive steps are involved between the disruption of the microtubule system and the observed decrease in ODC activity.

Although at this stage we do not know exactly how the cell transfers information from its surface receptors to the microtubule system and then to the cytoplasm or nucleus, the discovery that ODC activity is affected by microtubule disrupting agents provides a model system which connects the perturbation of the membrane with a cytoplastic enzymatic marker. These results also acquire particular interest because of the interaction which has been demonstrated to occur between spermine and tubulin (27,28).

Acknowledgment: The authors wish to thank Ms. S. M. Tu for her excellent technical assistance. We are also grateful to Dr. G. T. Shiau for the helpful discussions. 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. KO6-GM-03070.

REFERENCES

1. Edelman, G.M., Yahara, I. and Wang, J.L. (1973) Proc. Nat. Acad. Sci. U.S.A. 70, 1442-1446.
2. Yahara, I. and Edelman, G.M. (1975) Exp. Cell Res. 91, 125-142.
3. Nicolson, G.U. and Painter, R.G. (1973) J. Cell Biol. 59, 395-406.
4. Ukena, T.E. and Berlin, R.D. (1972) J. Exp. Med. 136, 1-7.
5. Wang, J.L., Gunter, G.R. and Edelman, G.M. (1975) J. Cell Biol. 66, 128-144.
6. Russell, D.H. in Polyamines in Normal and Neoplastic Growth (1973), ed. Russell, D.H., pp. 1-13, Raven Press, New York.
7. Hogan, B., Shields, R. and Curtis, D. (1974) Cell 2, 229-233.
8. Richman, R.A., Underwood, L.E., Van Wyk, J.J. and Voina, S.J. (1971) Proc. Soc. Exp. Biol. Med. 138, 880-884.
9. Theoharides, T.C. and Canellakis, Z.N. (1975) Nature 255, 733-734.
10. Beck, W.T., Bellantone, R.A. and Canellakis, E.S. (1973) Nature 241, 275-277.
11. Chen, K.Y., Tsai, C.M. and Canellakis, E.S. (1975) Cancer Res. 35, 2403-2412.
12. Huang, C.C., Tsai, C.M. and Canellakis, E.S. (1974) Biochim. Biophys. Acta 332, 59-68.
13. Tsai, C.M., Chen, K.Y. and Canellakis, E.S. (1975) Biochim. Biophys. Acta 401, 196-212.
14. Fong, W.F., Heller, J. and Canellakis, E.S. (1975) Submitted to Biochim. Biophys. Acta.

15. Wilson, L., Anderson, K. and Creswell, K. (1974) J. Cell Biol. 63, 373a.
16. Wilson, L. and Friedkin, M. (1967) Biochem. 6, 3126-3135.
17. Shiau, G.T., personal communication.
18. Behnke, O. and Forer, A. (1967) J. Cell Sci. 2, 169-192.
19. Tilney, L.G., Hiramoto, Y. and Marsland, D. (1966) J. Cell Biol. 29, 77-95.
20. Creasey, W.A. in Handbuch der experimentallen Pharmakologie (1975) ed. Sartorelli, A.C., Johns, D.G. 38, 670-694, Springer Verlag, Germany.
21. Mizel, S.B. and Wilson, L. (1972) Biochem. 11, 2573-2578.
22. Loten, E.G. and Jeanrenaud, B. (1974) Biochem. J. 140, 185-192.
23. Redman, C.M., Banerjee, D., Howell, K. and Paladee, G.E. (1975) J. Cell Biol. 66, 42-59.
24. Borisy, G.G. and Taylor, E.W. (1967) J. Cell Biol. 34, 525-534.
25. Wilson, L. (1975) Life Sciences 17, 303-310.
26. Bos, C.J. and Emmelat, P. (1974) Chem. Biol. Interact. 8, 349-361.
27. Jacobs, M., Bennett, P.M. and Dickens, M.J. (1975) Nature 257, 707-709.
28. Behnke, O. (1975) Nature 257, 709-710.