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Retinoids increase transglutaminase activity and inhibit ornithine decarboxylase activity in Chinese hamster ovary cells and in melanoma cells stimulated to differentiate

(growth/differentiation/transamidation/G_1-specific enzyme regulation/polyamine biosynthesis)

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ABSTRACT **Transglutaminase (TGase; R-glutaminyl-peptide:amine γ-glutamyltransferase, EC 2.3.2.13) and ornithine decarboxylase (ODCase; L-ornithine carboxy-lyase, EC 4.1.1.17) activities were measured after the addition of retinoid analogs to Chinese hamster ovary (CHO) cells released from quiescence and Cloudman S91 (CCL 53.1) mouse melanoma cells stimulated to differentiate with α-melanocyte-stimulating hormone (MSH, melanotropin). In both cell culture lines, we detected a biphasic increase in TGase activity and a single peak of ODCase activity within 7 hr after release or stimulation. Retinoid analogs altered the expression of the initial TGase peak in both CHO and melanoma cells. Retinol increased the activity of TGase 1 hr after release in CHO cells, and the activity remained elevated until hr 4. A broad peak of TGase activity also occurred after the addition of α-difluoromethylornithine, an irreversible inhibitor of ODCase, and after addition of α-difluoromethylornithine plus retinol. In mouse melanoma cells, retinoic acid plus MSH markedly enhanced the activity of the initial TGase peak compared to MSH alone. Retinoic acid alone also increased TGase activity biphasically in these cells without the addition of MSH. These studies suggest that retinoid effects that increase TGase activity may alter the ODCase expression in proliferation and differentiation.**

Transglutaminases (TGases; R-glutaminyl-peptide:amine γ-glutamyltransferase, EC 2.3.2.13) have recently been implicated as the enzymes that conjugate putrescine and possibly spermidine and spermine to glutamine residues of proteins (1–11). In regenerating rat liver, the most striking change occurred in putrescine-protein conjugates, which fluctuated in a biphasic manner with maximal nuclear levels 12- and 25-fold above those of sham-operated controls at 4 and 42 hr after hepatectomy, respectively (5). Conjugated spermidine and spermine increased 3- and 2-fold, respectively, within 4 hr and remained high throughout the 48-hr study. An increase in the specific activity of nuclear TGase paralleled the increase of putrescine-protein conjugate(s).

Recent evidence suggests that the putrescine-protein conjugate may be putrescine attached to glutamine residues of ornithine decarboxylase (ODCase; L-ornithine carboxy-lyase, EC 4.1.1.17), the rate-limiting enzyme in polyamine biosynthesis and also the enzyme responsible for the formation of putrescine (7, 8). Purified ODCase serves as an acceptor protein for putrescine in the presence of TGase purified from guinea pig liver (7) and, in regenerating rat liver, [^{14}C]putrescine was selectively conjugated to one detectable protein, ODCase (8). The enzyme that appeared to be responsible for the conjugation was TGase because γ-glutamyl[^{14}C]putrescine was obtained after proteolytic digestion (8). *In vitro*, the transamidation of putrescine to ODCase resulted in a stoichiometric decrease in enzymatic activity (8), and in regenerating rat liver the modified protein was detected in nucleolar fractions (4, 6, 8). In this laboratory we are attempting to clarify the process by which the modification of ODCase by its product putrescine may modify the nucleolar transcriptional processes.

Because of the many reports that analogs of vitamin A inhibit ODCase expression in growth-stimulated systems (12–20), we wondered whether retinoids were altering the conjugation rate of ODCase through effects on TGases. In Chinese hamster ovary (CHO) cells, the most effective retinoid has been shown to be retinol (14, 15, 18), whereas many cells are more sensitive to retinoic acid (17, 21, 22). We have found previously that there are two G_1 peaks of TGase activity in CHO cells released from quiescence (23). In melanoma cells stimulated to differentiate by the addition of α-melanocyte-stimulating hormone (MSH, melanotropin), peaks of TGase activity occur at 1 and 5 hr after the addition of the differentiating agent (9). The addition of retinol to CHO cells resulted in a significant increase of TGase within 1 hr at a time when TGase activity was actually decreasing in control cultures. TGase activity remained elevated through 3 hr and then declined in a manner similar to control cultures. Further, retinol administration actually resulted in a significant decrease in the second peak of TGase at 5 hr. Addition of α-difluoromethylornithine (CHF_2-Orn), an irreversible inhibitor of ODCase, or retinol plus CHF_2-Orn, also resulted in an early and broader peak of TGase activity. In melanoma cells during logarithmic growth, the addition of *trans*-retinoic acid alone resulted in a biphasic increase in TGase activity with peaks at 2 and 5 hr after addition. The addition of MSH alone increased TGase at 1 and 5 hr, whereas the addition of *trans*-retinoic acid plus MSH resulted in a markedly stimulated TGase activity at 1 and 2 hr compared to MSH alone or retinoic acid alone. Therefore, in both cell lines, retinoids attenuated the induction of ODCase that occurred immediately after the first peak of TGase activity.

MATERIALS AND METHODS

Materials. [2,3-^{3}H(N)]Putrescine dihydrochloride (21.9 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels), L-[1-^{14}C]ornithine (60 mCi/mmol), and Omnifluor scintillant were purchased from New England Nuclear. Putrescine hydrochloride, dithiothreitol, dimethylcasein, pyridoxal phosphate, phenylmethylsulfonyl fluoride, L-ornithine, calcium chloride, *trans*-retinol, and

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Abbreviations: CHF_2-Orn, α-difluoromethylornithine; CHO cells, Chinese hamster ovary cells; MSH, α-melanocyte-stimulating hormone (melanotropin); ODCase, ornithine decarboxylase; TGase, transglutaminase.

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all-trans-retinoic acid were purchased from Sigma. These retinoids were subjected to gas chromatographic analysis before their use to ascertain their purity. The silica gel-impregnated glass fiber sheets (ITLC) were from Gelman Instrument (Ann Arbor, MI). McCoy's medium 5A, Ham's F-10 mixture, penicillin, and streptomycin were purchased from GIBCO. Fetal calf serum and horse serum were purchased from KC Biologicals. NCS tissue solubilizer was purchased from Amersham/Searle. MSH, actinomycin D, and cycloheximide were purchased from Calbiochem. CHF_2-Orn was a gift from Merrell-Dow Research Center (Cincinnati, OH).

Cell Culture Procedures. Cloudman S91 NCTC 3960 (CCL 53.1) mouse melanoma cells were grown in Ham's F-10 mixture in monolayer cultures at 37°C in a humidified 5% CO_2/95% air atmosphere. Ham's F-10 mixture was supplemented with 10% horse serum and 2% fetal calf serum, penicillin at 100 units/ml, and streptomycin at 100 μg/ml. The doubling time of the mouse melanoma cells was 28–30 hr under these conditions. Cell numbers were determined by using a hemacytometer. Mouse melanoma cells were trypsinized and replated at least 12 hr previous to the addition of MSH. The activity of tyrosinase, the rate-limiting enzyme in melanin synthesis, was measured as an index of differentiation (24, 25). Retinoic acid solutions were prepared in ethanol (0.1%) under red light and used immediately. Control cell populations received an equivalent amount of ethanol, which was found to have no effect on growth parameters.

CHO cells were grown in McCoy's medium 5A in monolayer cultures at 37°C in a humidified 5% CO_2/95% air atmosphere. McCoy's medium was supplemented with 10% fetal calf serum, penicillin at 100 units/ml, and streptomycin at 100 μg/ml. Cell doubling times were 13–15 hr under these conditions. Cell numbers were determined by using a hemacytometer. CHO cells were synchronized by release of quiescent cultures as described (26). In cultures released from quiescence, synchrony was routinely 60–65%, as assayed by flow microfluorometric measurements of cellular DNA content (15). Retinol solutions were prepared in dioxane under red light and used immediately. Control cell populations received an equivalent amount of dioxane (0.5%), which was found to have no effect on growth parameters, as described (15). All cells were handled under red light when retinoids were used. Dose responses to retinoid analogs of TGase and ODCase for the two cell lines are shown in Table 1. Although a high concentration of retinol was required in CHO cells to increase TGase activity and inhibit ODCase expression, this concentration was nontoxic as assessed by colony formation (15).

Table 1. Effects of retinoid analogs on the early peaks of TGase and ODCase activities in mouse melanoma cells stimulated to differentiate with MSH and quiescent released CHO cells

Retinoid analog	Conc., μM	Activity, pmol/hr per 10^6 cells	
		TGase	ODCase
	Mouse melanoma cells		
trans-Retinoic acid	Control	360	322
	0.01	450	354
	0.10	462	278
	1.0	480	228
	CHO cells		
Retinol	Control	1,560	100
	50	1,690	85
	80	1,700	60
	120	2,060	50

TGase Assay. TGase activity was assayed as the Ca^{2+}-dependent incorporation of [^{3}H]putrescine into trichloroacetic acid-precipitable protein. Cells were harvested by scraping, sedimented, and sonicated (twice for 30 s) in 50 mM Tris·HCl, pH 8.3. The assay mixture contained 50 mM Tris·HCl at pH 8.3, 30 mM NaCl, 10 mM dithiothreitol, 5 mM $CaCl_2$, 2.5 mg dimethylcasein per ml, 0.2 mM putrescine, and 0.4 μM [^{3}H]putrescine in a final volume of 200 μl. The reaction was initiated by addition of a 10,000 × *g* supernate of a lysate of 10^6 cells. Over 90% of TGase was in the cytosolic fraction in the CHO cells, and the amount in the particulate fraction did not alter as cells progressed through the cell cycle. In melanoma cells, supernatant TGase activity was always higher than the total homogenate, suggesting the presence of an inhibitor in the particulate fraction. Therefore, this paper reports the cytosolic fluctuations of the enzyme in response to MSH. The reaction mixture was incubated for 20 min at 37°C. A 50-μl aliquot was then spotted on silica gel-impregnated glass fiber strips (ITLC) and chromatographed in 5% trichloroacetic acid/0.2 M KCl as described (5). Each time point represents duplicate determinations of four separate cell lysates. Radioactivity of the trichloroacetic acid precipitate was assessed by scintillation counting in 6 ml of toluene/Omnifluor. Activity was linear for 60 min with respect to enzyme and substrate concentrations. In this chromatographic system, the reaction product [^{3}H]putrescine covalently conjugated to protein remains at the origin while free [^{3}H]putrescine moves with the solvent front. Analysis of 1-cm segments of chromatographed ITLC strips showed that, in the presence or absence of added protein, more than 99.8% of the free diamine moved away from the origin to the upper 2 cm of the strip. Recovery of radiolabeled protein at the origin was slightly better than that achieved with the filter paper procedure (27). Background radioactivity was determined in assay mixtures that did not contain the cell lysate. This was always 200–300 cpm and was subtracted from the assays.

ODCase Assay. ODCase activity was determined by measuring the liberation of $^{14}CO_2$ from L-[1-^{14}C]ornithine as previously described with minor modifications (28). At the times indicated, cells were collected by scraping with a rubber policeman and sonicated in 0.5 ml of 50 mM Na_2H/KH_2PO_4, pH 7.2, containing 0.1 mM EDTA, 1.0 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 5 mM NaF, and 0.03 mM pyridoxal phosphate. Two 200-μl aliquots of a 10,000 × *g* supernate from each sample were incubated for 60 min at 37°C in the presence of 0.5 mM L-[^{14}C]ornithine. The pyridoxal phosphate-independent release of $^{14}CO_2$ was determined at each sampling time by incubating an equivalent amount of excess pooled supernatant in the presence of 4-bromo-3-hydroxylbenzyloxyamine dihydrogen phosphate (28), and the amount of release was subtracted from the sample values. The reaction was terminated by addition of 0.25 ml of 1 M citric acid; the incubation was continued for another 15 min, and the CO_2 evolved was trapped by 20 μl of NCS on a filter paper suspended above the reaction. The filter paper was placed in 6 ml of toluene/Omnifluor, and radioactivity was determined in a liquid scintillation spectrometer.

RESULTS

Effects of Retinol and CHF_2-Orn on TGase Activity in CHO Cells. Fig. 1 illustrates that, in CHO cells released from quiescence, there was a biphasic increase in TGase activity as a function of G_1 progression, with maxima occurring at 3 and 5 hr. Also, after release there was a marked drop in TGase activity within the first 2 hr. The early drop was not seen in cells that received retinol, CHF_2-Orn, or a combination of retinol and CHF_2-Orn. The biphasic expression of TGase was not due to

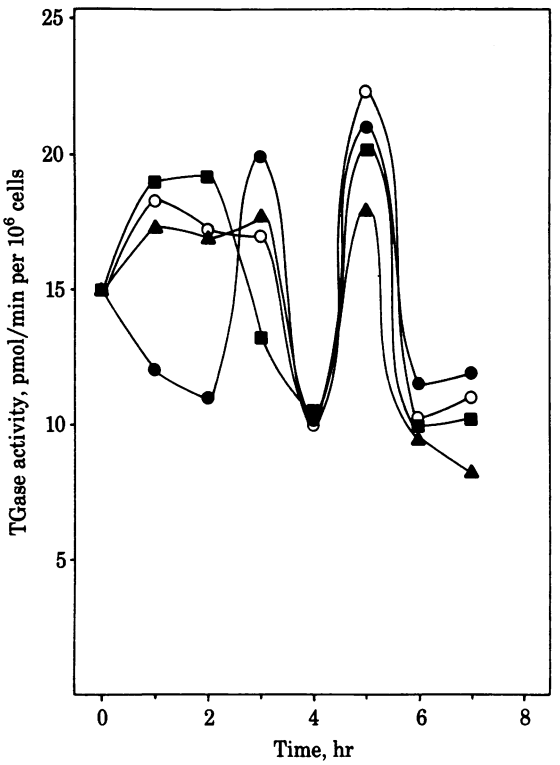

FIG. 1. TGase activity in CHO cells released from quiescence (●) and effects of retinol (87 μM) (▲), retinol plus CHF_2-Orn (10 mM) (■), and CHF_2-Orn alone (○) on TGase activity. This is a representative experiment which was repeated with quadruplicate determinations of TGase at each time point. Each experiment was within ±10% of the mean of the quadruplicate values of the other. The second peak sometimes occurred at 6 hr but was of the same magnitude.

two separate populations of cells progressing through cell cycle because nearly identical data were obtained for TGase excursions in CHO cells after mitotic shake-off, in which a greater than 95% synchrony has been documented (23). The TGase increases at 3 and 5 hr were not inhibited by cycloheximide administration or actinomycin D administration (Table 2). In fact, actinomycin D appeared to enhance the excursions of enzyme activity at certain time points.

Effects of Retinol and CHF_2-Orn on ODCase Activity in CHO Cells. There was a marked induction of ODCase activity that was maximal between 4 and 6 hr, expressed after the first peak of TGase and continuing to increase until the second peak of TGase activity was detected (Fig. 2). The maximal activity of ODCase detected at 4–5 hr, 160–180 pmol/hr per 10^6 cells, was inhibited approximately 65% by the administration of retinol. The administration of CHF_2-Orn resulted in almost total inhibition of ODCase expression, and the combination of retinol and CHF_2-Orn completely inhibited any detectable increase in ODCase after release from quiescence. The induction of ODCase activity was decreased significantly by cycloheximide and by low-dose actinomycin D, indicative of transcriptional regulation (Table 2). In some experiments, ODCase activity was low at 4 hr and maximal at 5 hr, and TGase peaks occurred at 3 and 6 hr after release from quiescence (23). In all cases, the magnitudes of the increases were similar.

Table 2. Effects of inhibitors of RNA and protein synthesis on activities of TGase and ODCase in CHO cells released from quiescence

Enzyme	Time, hr	Activity, pmol/hr per 10^6 cells		
		Control	Cycloheximide	Actinomycin D
TGase	3	1,060	1,020	
	5	1,260	780	
	6	920	1,500	
ODCase	4–5	160–180	40	
TGase	3	1,080		1,110
	6*	1,120		1,260
ODCase	5	140		50

The times shown represent peaks of enzyme activities after release from quiescence. The second peak of TGase activity detected at 5 hr after release from quiescence was delayed to 6 hr by the administration of cycloheximide (5 μg/ml). Actinomycin D (0.04 μg/ml) enhanced the peak activities of TGase in these cells. Both cycloheximide and actinomycin D inhibited the activity of ODCase.

* In these experiments, the second peak of TGase activity occurred at 6 hr. ODCase activity was low at 4 hr and was maximal at 5 hr.

Effect of Retinoic Acid on TGase Activity in Melanoma Cells After MSH. In Cloudman S91 melanoma cells stimulated to differentiate by the addition of MSH to logarithmically growing cultures, there was a greater than 2-fold increase in TGase activity within 1 hr; the activity then rapidly declined, with a second peak occurring 5 hr after the addition of MSH. When retinoic acid was added at the time of MSH addition, there was a greater than 3-fold increase at 1 hr, and the activity remained elevated more than 3-fold at 2 hr, again with the second peak being similar to that for MSH addition alone (Fig. 3). The addition of retinoic acid alone resulted in a small but significant increase in TGase activity detectable at 2 hr and a second peak of TGase at 5 hr, which was again comparable to that detected after MSH alone or a combination of MSH plus retinoic acid. The addition of cycloheximide abolished the first peak of TGase activity at 1 hr but only shifted the second peak to 1 hr later, suggesting that the first peak is translational in nature whereas the second peak may be related to an activation phenomenon, which has been shown to occur for the circulating TGase, factor XIII (1). Actinomycin D did not alter the peak excursions but delayed the first peak 1 hr and enhanced the second peak significantly at 5 hr (Table 3). Cells without addition of MSH or

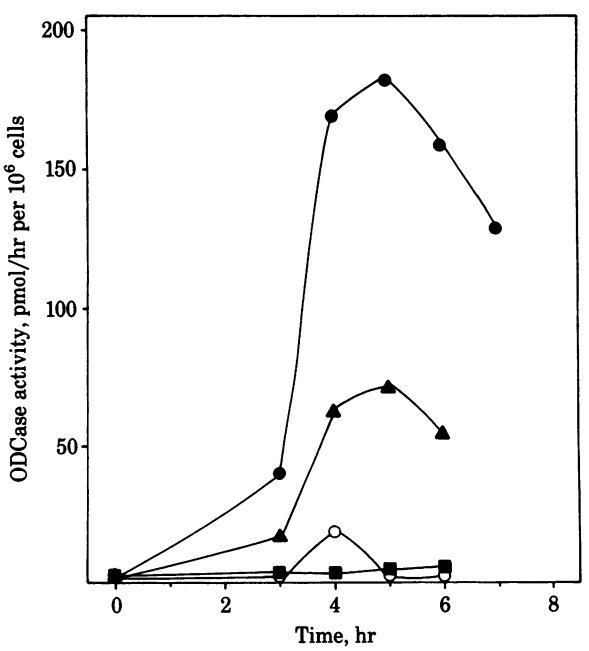

FIG. 2. ODCase activity in CHO cells after quiescent release (●) and effect of retinol (87 μM) (▲), CHF_2-Orn (10 mM) (○), and retinol plus CHF_2-Orn (■) on ODCase activity in quiescent released CHO cells. This experiment was conducted twice with quadruplicate determinations at each time point.

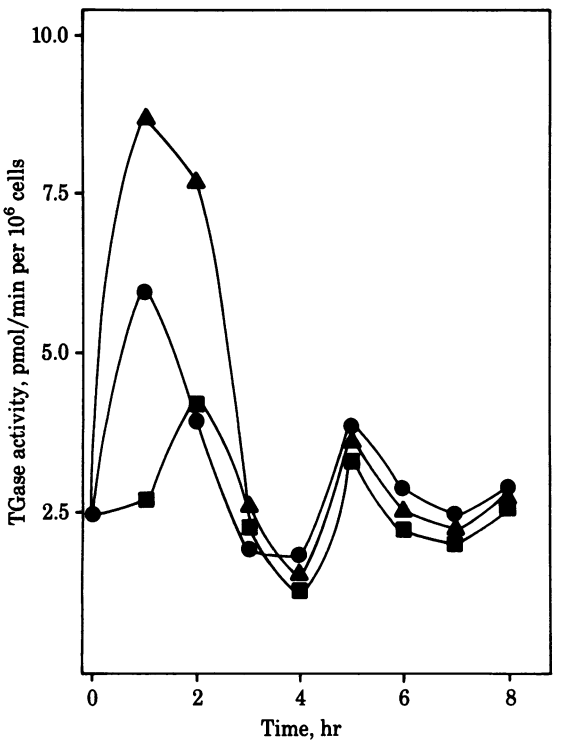

FIG. 3. TGase activity in Cloudman S91 mouse melanoma cells, logarithmic phase, stimulated to differentiate with MSH (0.1 μM) (●), retinoic acid (83 μM) plus MSH (▲), or retinoic acid alone (■). These experiments were repeated and quadruplicate determinations were done at each time point.

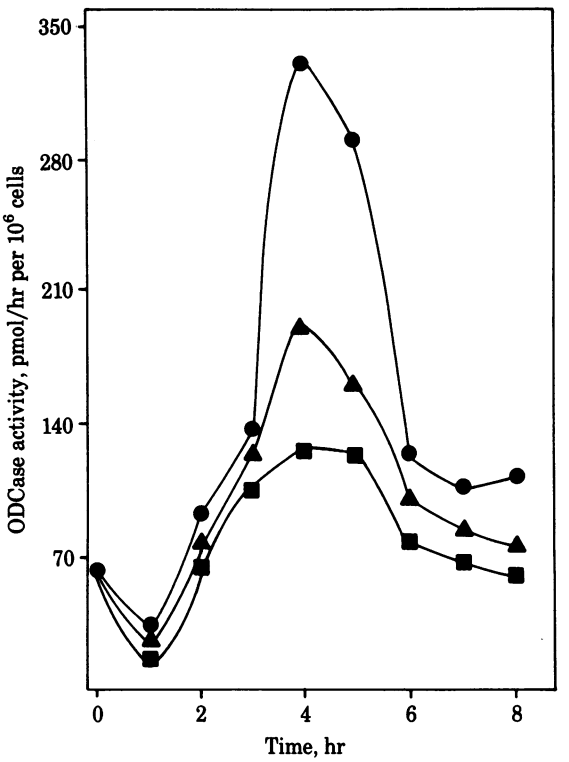

FIG. 4. ODCase activity in logarithmic phase Cloudman S91 mouse melanoma cells after stimulation with MSH (0.1 μM) (●), MSH and retinoic acid (83 μM) (▲), and retinoic acid alone (■). This experiment was conducted twice with quadruplicate determinations at each time point.

retinoic acid or both showed no fluctuation of TGase activity throughout the time studied.

Effect of Retinoic Acid on ODCase Activity in Melanoma Cells After MSH. Fig. 4 illustrates that, in Cloudman S91 mouse melanoma cells stimulated to differentiate by addition of MSH to logarithmic phase cells, ODCase activity increased to a peak 5-fold above the control level. This single peak of activity occurred 4 hr after MSH, with maximal ODCase activity detected prior to the increase of the second peak of TGase activity. When the cells were exposed to retinoic acid plus MSH at zero hour, the increase in ODCase activity was attenuated by 50%. In cells exposed only to retinoic acid at zero hour, ODCase increased to an activity approximately one-third of the activity with MSH alone. ODCase activity was abolished by the addition of cycloheximide with MSH at zero hour. The addition of actinomycin D with MSH at zero hour resulted in a 30% inhibition at this concentration (Table 3).

Table 3. Effects of cycloheximide and actinomycin D on TGase and ODCase activities in Cloudman S91 mouse melanoma cells in logarithmic growth phase stimulated to differentiate by addition of MSH

		Activity, pmol/hr per 10^6 cells		
Enzyme	Time, hr	MSH	Cycloheximide + MSH	Actinomycin D + MSH
TGase	1	360	130	
	2	240	100	
	5	220	160	
	6	170	230	
ODCase	4	330	50	
TGase	1	370		190
	2	240		380
	5	250		340
	6	170		190
ODCase	4	330		150

The times shown represent peaks of enzyme activities after MSH application. TGase activity at 1 hr was inhibited by cycloheximide (5 μg/ml). The second peak was shifted to 1 hr later by cycloheximide. Actinomycin D (0.04 μg/ml), on the other hand, delayed the 1-hr peak by 1 hr and enhanced the second peak of activity. Both cycloheximide and actinomycin D attenuated ODCase activity.

DISCUSSION

CHO cells stimulated to proliferate and melanoma cells stimulated to differentiate show rapid increases, in an inverse manner, of two enzymes, TGase and ODCase. That is, the peak of ODCase activity directly follows the decline of the first peak of TGase activity. The second peak of TGase activity is coincident with the decline of ODCase activity at 6 hr in CHO cells and at 5 hr in melanoma cells. In CHO cells, we found that 8-bromo-cyclic AMP stimulated a 12-fold increase in ODCase activity in G_1 of the cell cycle (23) but had no effect on the TGase excursions. This suggests that control of the two enzymes is mediated by separate hormonal pathways. Because TGase requires Ca^{2+} for activity, it seems likely that it may be regulated by hormones that selectively alter calmodulin and other Ca^{2+}-mediated events in the cell cycle (29).

Retinoid analogs altered the expression of the initial TGase peak in both CHO and melanoma cells. In CHO cells, retinol resulted in an increase from 1 through 3 hr, compared to a drop from 1–2 hr and a peak at 3 hr in quiescent released cells. A broad peak of activity also occurred after administration of CHF_2-Orn and CHF_2-Orn plus retinol. This is evidence that CHF_2-Orn may affect events other than the direct irreversible inhibition of ODCase (30–32). This may be related also to the enhancement of ODCase inhibition by the combination of CHF_2-Orn and retinoic acid in neuroblastoma cells (12).

The ability of retinoids to enhance the magnitude of the first peak of TGase in both differentiating tumor cells and CHO cells and to inhibit ODCase activity in both cell lines is compatible with the conjugation of ODCase by putrescine via a TGase-catalyzed reaction with a concomitant decrease in activity (7, 8). Alternatively, retinoids may alter the ability of cyclic AMP-dependent protein kinase to translocate to the nucleus because we have shown previously that retinol does not alter the activation of cyclic AMP-dependent protein kinase in CHO cells in which ODCase expression is inhibited (14, 15, 18).

TGase activity in the two cell lines appears to be regulated by activation and translational processes. In CHO cells, actinomycin D and cycloheximide do not markedly alter the biphasic expression of TGase during G_1 progression, suggesting activation of the enzyme; actinomycin D actually significantly enhances the activity at certain times (23). This contrasts to ODCase activity, which is inhibited to some extent by either actinomycin D or cycloheximide in both cell lines. In melanoma cells, the initial peak of TGase activity is reduced by cycloheximide and shifted to 1 hr later by actinomycin D. The second peak of TGase activity is not affected by inhibitors of RNA or protein synthesis.

The ability of retinoids to inhibit proliferation and enhance differentiation (21, 22, 33–35) may be related to their ability to inhibit ODCase activity and to rapidly increase the activity of TGase (9, 23). Transformed cells are known to have lower TGase activity than normal cells and be less differentiated (36), but the ability of retinoids to alter TGase activity in cell cycle and in response to a differentiating hormone has not been reported previously. In line with other reports, the retinoid alone actually increases ODCase activity (18). Retinoids alone also result in a small increment in TGase activity in melanoma cells, suggesting a complex interaction of retinoids with hormones such as MSH. Isolation and characterization of putrescine-protein conjugates should be informative.

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1. Folk, J. E. & Chung, S. I. (1973) *Adv. Enzymol.* **38**, 109–191.
2. Folk, J. E., Park, M. H., Chung, S. I., Schrode, J., Lester, E. P. & Cooper, H. L. (1980) *J. Biol. Chem.* **255**, 3695–3700.
3. Haddox, M. K. & Russell, D. H. (1980) *Eur. J. Cell Biol.* **22**, 112 (abstr.).
4. Haddox, M. K. & Russell, D. H. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 643 (abst.).
5. Haddox, M. K. & Russell, D. H. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1712–1716.
6. Haddox, M. K. & Russell, D. H. (1981) *J. Cell Physiol.* **109**, 447–452.
7. Russell, D. H. (1981) *Biochem. Biophys. Res. Commun.* **99**, 1167–1172.
8. Russell, D. H. (1981) *Med. Biol.* **59**, 286–295.
9. Scott, K. F. F., Meyskens, F. L., Jr., & Russell, D. H. (1981) *Pharmacologist* **23**, 127 (abst.).
10. Williams-Ashman, H. G., Beil, R. E., Wilson, J., Hawkins, M., Grayhack, J., Zunamon, A. & Weinstein, W. K. (1980) *Adv. Enzyme Regul.* **18**, 239–258.
11. Williams-Ashman, H. G. & Canellakis, Z. N. (1980) *Physiol. Chem. Phys.* **12**, 457–472.
12. Chapman, S. K. (1980) *Life Sci.* **26**, 1359–1366.
13. Fish, L. A., Baxter, C. S. & Bush, J. A. (1981) *Toxicol. Appl. Pharmacol.* **58**, 39–47.
14. Haddox, M. K. & Russell, D. H. (1979) *Cancer Res.* **39**, 2476–2480.
15. Haddox, M. K., Scott, K. F. F. & Russell, D. H. (1979) *Cancer Res.* **39**, 4930–4938.
16. Kensler, T. W., Verma, A. K., Boutwell, R. K. & Mueller, G. C. (1978) *Cancer Res.* **38**, 2896–2899.
17. Paranjpe, M. S., DeLarco, J. E. & Todaro, G. J. (1980) *Biochem. Biophys. Res. Commun.* **94**, 586–591.
18. Russell, D. H. & Haddox, M. K. (1981) *Ann. N.Y. Acad. Sci.* **359**, 281–297.
19. Verma, A. K., Rice, H. M., Shapas, B. G. & Boutwell, R. K. (1978) *Cancer Res.* **38**, 793–801.
20. Verma, A. K., Shapas, B. G., Rice, H. M. & Boutwell, R. K. (1979) *Cancer Res.* **39**, 419–425.
21. Chytil, F. & Ong, D. E. (1976) *Nature (London)* **260**, 49–51.
22. Lotan, R. (1980) *Biochim. Biophys. Acta* **605**, 33–91.
23. Scott, K. F. F. & Russell, D. H. (1982) *J. Cell Physiol.* **112**, in press.
24. Pawelek, J. M. (1976) *J. Invest. Dermatol.* **66**, 201–209.
25. Wong, G., Pawelek, J., Sansone, M. & Morowitz, J. (1974) *Nature (London)* **248**, 351–354.
26. Stubblefield, E. & Klevecz, R. R. (1965) *Exp. Cell Res.* **40**, 660–664.
27. Lorand, L., Campbell-Wilkes, L. K. & Cooperstein, L. (1972) *Anal. Biochem.* **50**, 623–631.
28. Russell, D. H. & Snyder, S. H. (1968) *Proc. Natl. Acad. Sci. USA* **60**, 1420–1427.
29. Whitfield, J. F. (1980) in *Handbook of Experimental Pharmacology*, ed. Szekeres, L. (Springer, Berlin), Vol. 54, pp. 267–317.
30. Bacchi, C. J., Nathan, H. C., Hunter, S. H., McCann, P. P. & Sjoerdsma, A. (1981) *Science* **210**, 332–334.
31. Mamont, P. S., Duchesne, M. C., Grove, J. & Bey, P. (1978) *Biochem. Biophys. Res. Commun.* **81**, 58–66.
32. Seiler, N., Danzin, C., Prakash, N. & Koch-Weser, J. (1978) in *Enzyme-activated Irreversible Inhibitors*, eds. Seiler, N., Jung, M. J. & Koch-Weser, J. (Elsevier/North-Holland, Amsterdam), pp. 55–71.
33. Bollag, W. (1970) *Int. J. Vit. Res.* **40**, 299–314.
34. Meyskens, F. L., Jr. (1980) *Ariz. Med.* **37**, 84–86.
35. Sporn, M. B. (1977) *Nutr. Rev.* **35**, 65–69.
36. Birckbichler, P. J., Orr, G. R., Conway, E. & Patterson, M. K. (1977) *Cancer Res.* **37**, 1340–1344.