Inhibition by ultraviolet irradiation of the glucocorticoid induction of tyrosine aminotransferase in bromodeoxyuridine-treated H-35 hepatoma cells in culture

Donald E. Bushnell

McArdle Laboratory for Cancer Research

Madison, Wisconsin 53706

Received October 18,1976

SUMMARY:

Low doses of ultraviolet irradiation at 254 nm inhibited the dexamethasone induction of tyrosine aminotransferase (E.C. 2.6.1.5) in H-35 Reuber (H-4-II-E) hepatoma cells exposed to bromodeoxyuridine for approximately two generations of growth. The cells were transferred to a serum-free medium for the hormone induction studies so that growth inhibition would not be a factor in the experiments. Doses of 90 ergs/mm^2 inhibited the glucocorticoid induction and resulted in a decline in tyrosine aminotransferase in both basal- and steroid-induced levels of the enzyme. Comparison of the inhibition of the glucocorticoid induction by low doses of ultraviolet irradiation at 254 nm in thymidine-treated versus bromodeoxyuridine-treated cells suggested that bromodeoxy-uridine-treated cells were more sensitive to low doses of irradiation. The basal activities of two other enzymes, alkaline phosphatase and leucine aminopeptidase, were only slightly inhibited 6 hours following irradiation of bromodeoxy-uridine-treated cells. These results suggest that studies using ultraviolet irradiation of monolayer cell cultures to study different mechanisms of hormonal regulation of cellular processes may provide an alternative approach for studies on modulation of gene expression.

INTRODUCTION

Ultraviolet irradiation has mutagenic and oncogenic effects on mammalian cells (1). The mechanism of these biological alterations is thought to be related to DNA damage and the "faulty" repair of DNA damage (2,3). Thus, ultraviolet irradiation studies have emphasized the characterization of the chemical nature and location of the DNA lesions and the repair systems that attempt to restore DNA structure to its original form (3). Presumably, the damage to

DNA and cell constituents by ultraviolet irradiation will alter some aspects of gene expression until such damage is corrected.

Bushnell et al. (1974) proposed a model system to study the alteration of hormonal regulation of enzyme activity by ultraviolet irradiation at 254 nm (4). This model system requires a carefully designed experimental approach so that meaningful data can be collected. First, all cells should be exposed to the same amount of irradiation at a defined wavelength. In previous studies monolayer cell cultures on plastic plates were exposed to a germicidal lamp with a primary ultraviolet output of 254 nm for the amount of time necessary to expose the cells to a defined amount of irradiation (4). Second, the cells in initial studies should be in a nondividing state so that any changes in gene expression will be changes in the total preexisting cell population. In the present experiments, serum was removed for at least 18 hours to stop cell division. Third, low doses of irradiation that allow translation to continue at near-normal levels should be used to permit detection of transcription alterations (4). Fourth, the enzyme (or protein) studied should be under hormonal control and factors regulating its activity should be investigated prior to the study.

Extensive investigations have been made on the regulation of tyrosine aminotransferase (E.C. 2.6.1.5) in the hepatoma cell lines H-35 and HTC. Steroids appear to induce enzyme activity by a transcriptional mechanism, while insulin appears to induce activity by a posttranscriptional mechanism (5,6,7,8,12). The effect of ultraviolet irradiation on steroid and insulin induction have been reported previously (4). In the present study, cells were exposed to bromodeoxyuridine for about two generations so that the cells would be in an altered stage of differentiation (Dr. James Gurr, personal communication) and be photosensitized to ultraviolet irradiation effects

Table I - Effects of ultraviolet irradiation on the steroid induction of tyrosine aminotransferase in H-35 cells pretreated with bromodeoxyuridine.

Treatment dose ergs/mm^2	*Units tyrosine aminotransferase per plate		
	basal	plus dexamethasone	fold induction
0	0.70	3.94	5.6
0	0.70	3.75	5.4
20	0.50	2.24	4.5
20	0.49	2.31	4.7
40	0.40	1.73	4.3
40	0.39	1.53	3.9
60	0.36	1.25	3.4
60	0.34	1.26	3.7
90	0.27	0.71	2.6
90	0.32	0.63	2.0

*Each value represents a single plate.

Cells were grown in bromodeoxyuridine as described in Materials and Methods. Cells were transferred to serum-free medium for 18 hours prior to ultraviolet irradiation. Fresh serum-free medium was added back to cultures and 10^{-7} M dexamethasone added to appropriate plates. Cells were harvested 6 hours later and assayed for tyrosine aminotransferase activity.

on DNA (14). This paper reports the effects of ultraviolet irradiation on the steroid and insulin induction of tyrosine aminotransferase under the conditions specified above in cells grown in bromodeoxyuridine.

METHODS AND MATERIALS

Culturing of H-35 (H-4-II-E) cells as monolayer on plastic plates was carried out as previously described with some modifications (Bushnell et al., 1974). During growth of the cells in serum, thymidine or bromodeoxyuridine was added at 5 µg/ml for 48 hours prior to shift of cultures to S-77 +GA medium lacking serum. The protocol was developed by Dr. James Gurr, and the incorporation of BrdU into cellular DNA has been verified by isotope incorporation studies (Gurr and Potter, in preparation). Cells were cultured an additional 18 hours to minimize cell replication, after shifting to a serum-free medium. Ultraviolet irradiation of cells has been described previously (4). Cells were harvested and lysed as described (4). Tyrosine amino-

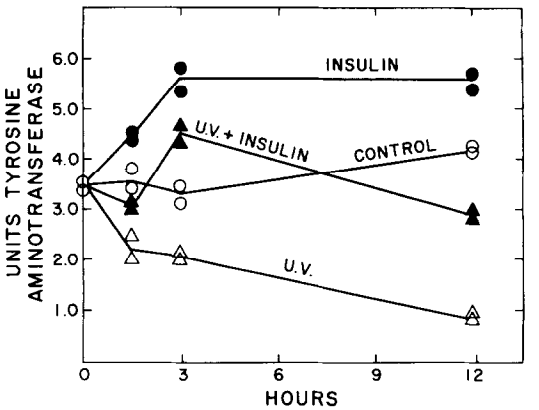

Figure 1.

Cells were grown in BrdU as described in Materials and Methods. Cells were then transferred to serum-free medium and dexamethasone (10^{-7} M) was added. After 18 hours, the medium was aspirated and appropriate cultures were irradiated with 90 ergs/mm^2 of ultraviolet light (254 nM). Fresh serum-free medium was added and insulin was added to appropriate control and irradiated cells. Each point is the units of enzyme activity per plate.

---

transferase was assayed by the method of Diamondstone (15) and protein was determined by the method of Lowry (16). Alkaline phosphatase was assayed in 0.5 ml of 0.75M 2-amino-2-methyl-1-propanol, pH 10.3, containing 12.5 nM p-nitrophenyl phosphate as substrate. Reactions were carried out at 37°C for 15 minutes and the reaction terminated by addition of 5 ml of 0.05N NaOH (18). Absorbance at 405 nM was measured to determine the amount of p-nitrophenol liberated. Leucine aminopeptidase was determined using a reagent kit (Sigma Chemical Co., St. Louis, MO). Aliquots of cell extracts were incubated for 1 hour at 37°C in 1 ml of phosphate buffer, pH 7.1 containing 0.02% L-leucyl-β-naphthylamide and the amount of β-naphthylamide released through hydrolysis was determined.

RESULTS

Ultraviolet irradiation at 254 nm of cells exposed to BrdU during growth inhibited the steroid induction of tyrosine aminotransferase activity of cells in serum-free medium (Table I). Basal activities of this enzyme also declined following irradiation.

The steroid induction of tyrosine aminotransferase was almost completely inhibited by 90 ergs/mm^2 of irradiation and resulted in a decline of steroid-induced activities of the enzyme in cells containing bromodeoxyuridine in their DNA (Figure 1). Addition of insulin to steroid-induced BrdU-treated cells resulted in further increase of tyrosine aminotransferase activity. Ultraviolet irradiation (90 ergs/mm^2) caused a rapid decline in tyrosine-aminotransferase activity even in the continued presence of dexamethasone, while insulin blocked the decline in enzyme activity up to 12 hours after treatment. Enzyme activity in irradiated cells cultured in a medium containing insulin was several fold higher than that in irradiated cells cultured without insulin.

Table II - Effects of ultraviolet irradiation on leucine aminopeptidase and alkaline phosphatase activity in H-35 cells pretreated with bromodeoxyuridine.

Dose ergs/mm^2	Units Leucine aminopeptidase*	Units alkaline phosphatase*
0	8.2 ± 1.8	11.4 ± 1.4
20	7.0 ± 1.3	12.4 ± 1.7
40	7.6 ± 2.8	11.1 ± 0.2
60	6.2 ± 1.2	10.5 ± 0.7
90	6.2 ± 1.0	9.6 ± 1.0

*Each value represents the mean of 4 plates with the indicated standard deviation.

Cells were grown and transferred to serum-free medium as described in Table I. Cultures were irradiated and fresh serum-free medium was added back. Cells were harvested 6 hours later and assayed for leucine aminopeptidase and alkaline phosphatase.

Table III - Effects of ultraviolet irradiation on the steroid induction of tyrosine aminotransferase in H-35 cells pretreated with thymidine or bromodeoxyuridine

Treatment		Units tyrosine aminotransferase*	
Dose (ergs/mm^2)	Dexamethasone (10^{-7}M)	Tdr	BrdU
0	-	0.80	0.54
0	-	0.74	0.49
0	+	13.5	5.97
0	+	14.1	5.71
0	+	13.1	5.87
0	+	12.9	5.92
20	+	11.4	3.65
20	+	12.3	3.80
40	+	10.0	3.00
40	+	9.95	3.12
40	+	9.07	3.02
40	+	10.1	3.09

*Each value represents the activity per individual plate.

Cells were handled as described in Table I with the exception that some cultures were treated with thymidine instead of bromodeoxyuridine.

Effects of 90 ergs/mm^2 on activities of alkaline phosphatase and leucine aminopeptidase in bromodeoxyuridine-treated cells indicated that these enzyme activities remained near initial values up to 6 hours following irradiation (Table II). The activity of these enzymes appears to be noninducible by either glucocorticoids or insulin in this cell line (Bushnell, unpublished results). These enzymes were selected since damaged cells leak these enzymes from the intracellular compartment to the extra cellular compartment (18). Also, these enzymes appear to have long half-lives compared with the short half-life of tyrosine aminotransferase (Bushnell, unpublished results).

Table III shows that very low doses of ultraviolet irradiation inhibited the steroid induction of tyrosine-aminotransferase activity in thymidine- and bromodeoxyuridine- treated cells. When compared with thymidine-treated cells, the bromodeoxyuridine-treated cells appeared to be more sensitive to very low doses of irradiation. Control experiments with plastic cover between the germicidal lamp and the cells indicated that wavelengths longer than 300 nM given off by the lamp were not effective in inhibiting the steroid induction under the conditions used in these experiments (not shown).

DISCUSSION

Investigations of hormonal regulation of the enzyme tyrosine aminotransferase in cultured hepatoma cells developed into a model system for the study of gene expression largely due to the work of Tomkins and Kenney, and their associates (5,7,10,13). The present report utilizes some of the knowledge concerning regulation of tyrosine transaminase to demonstrate the possibilities for development of model systems to study the effects of ultraviolet irradiation on modulation of gene expression. The exact mechanism by which ultraviolet light inhibits the steroid induction of tyrosine-amino-transferase activity of control and bromodeoxyuridine-treated cells has not been determined.

Presumably, low doses of ultraviolet irradiation at 254 nM damaged the structure of DNA. This damage may have inhibited synthesis of messenger RNA for tyrosine aminotransferase whose half life is thought to be short (7). This inhibition could have resulted from premature release of newly synthesized incomplete RNA nucleotide chains or from abnormal binding of RNA polymerase (17). Alternatively,

synthesis of an RNA that regulates transcription, translation, or degradation of tyrosine aminotransferase could have been inhibited.

In contrast to actinomycin D which blocked the steroid induction of tyrosine aminotransferase but caused "superinduction" of steroid induced enzyme levels, ultraviolet irradiation of bromodeoxyuridine treated H-35 cells blocked the steroid induction and caused decline in enzyme activity following irradiation of steroid treated cells (5,13). Thus ultraviolet irradation did not appear to block inactivation or degradation of tyrosine aminotransferase as has been reported following actinomycin D treatment (7,10). Further investigations on the effects of ultraviolet irradiation of RNA synthesis and enzyme synthesis in H-35 cells are now being carried out and the results suggest that use of ultraviolet irradiation to alter regulation of gene expression in monolayer cultures of mammalian cells may be a useful molecular probe for studies on nuclear regulation of enzyme levels. The current research agrees with earlier studies on the effects of bromodeoxyuridine on the steroid induction of tyrosine aminotransferase in cultured hepatoma cells (19). The effects of ultraviolet irradiation on regulation of tyrosine aminotransferase in BrdU treated hepatoma, however, extend the earlier work and indicate that studies using the combination of treatments may be utilized as a molecular probe for studies on the structural and regulatory genes for tyrosine aminotransferase.

ACKNOWLEDGMENTS

The work reported here was supported by Grants TO1-CA-5002 and CA-07175. The author thanks Professor Van R. Potter for use of his laboratory facilities and J. Becker and H. Brania for aid in use of the tissue culture laboratory. The author also thanks Dr. James Gurr for his helpful discussion during the course of this study.

REFERENCES

1. Setlow, R. B. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 3363-3366.
2. Regan, J. D. and Setlow, R. B. (1974) Cancer Res. 34, 3318-3325.

3. Trosko, J. E. and Chu, E. H. Y. (1975) In: Advances in Cancer Research, Vol. 21 (G. Klein and S. Weinhouse, eds). Academic Press, New York, 391-425.
4. Bushnell, D. E., Yager, J. D., Becker, J. E., and Potter, V. R. (1974) Biochem. Biophys. Res. Comm. 57, 949-956.
5. Bushnell, D. E., Becker, J. E., and Potter, V. R. (1974) Biochem. Biophys. Res. Comm. 56, 815-821.
6. Butcher, F. R., Bushnell, D. E., Becker, J. E., and Potter, V. R. (1972) Exp. Cell Res. 74, 115-123.
7. Steinberg, R. A., Levinson, B. B., and Tomkins, G. M. (1975) Cell 5, 29-35.
8. Emanuel, J. R. and Gelehrter, T. D. (1975) Biochem. Biophys. Res. Comm. 63, 825-831.
9. Wagner, K., Roper, M. D., Leichting, J. W., and Wicks, W. D. (1975) J. Biol. Chem., 231-239.
10. Kenney, F. T., Lee, K. L., Stiles, C. D., and Fritz, J. E. (1973) Nature New Biol. 246, 208-210.
11. Reel, J. R., Lee, K. -L., and Kenney, F. T. (1970) J. Biol. Chem. 245, 5800-5805.
12. Lee, K. -L., Reel, J. R., and Kenney, F. T. (1970) J. Biol. Chem. 245, 5806-5812.
13. Tomkins, G. M., Gelehrter, T. D., Granner, D. K., Martin, D. W., Samuels, H. S., and Thompson, E. B. (1969) Science 166, 1474-1480.
14. Weintraub, H. In: Cold Spring Harbor Symposia on Quantitative Biology (1973) Vol. 38, 247-256.
15. Diamondstone, T. I. (1966) Ann. Biochem. 16, 395-401.
16. Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
17. Mantieva, V. L. and Arion, V. Ya. (1969) J. Mol. Biol. 3, 231-237.
18. Searcy, R. L. (1969) Diagnostic Biochemistry. McGraw-Hill, New York.
19. Stellwagen, R. H. and Tomkins, G. M. (1971) J. Mol. Biol. 56, 167-182.