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DIFFERENTIAL EFFECTS OF *N*-METHYL-*N'*-NITRO-*N*-NITROSOGUANIDINE ON CONSTITUTIVE AND HORMONE-INDUCIBLE GENE EXPRESSION IN RAT HEPATOMA CELLS

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SUMMARY

Previous studies have shown that treatment of rat hepatoma cells (the Fao clone of Reuber H-35 cells) with 500 ng/ml of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) causes a 78% decrease in dexamethasone (DEX)-induced tyrosine aminotransferase (TAT) enzyme activity and a concurrent 75% decline in total steroid-induced TAT steady-state RNA levels. To determine if this inhibition was a specific or more general effect on inducible-gene expression, the effects of MNNG on other genes were examined. MNNG had little effect on total DEX or Cd-induced metallothionein (MT) RNA levels when the cells were treated with 1 μM DEX or 3 μM $CdCl_2$ for 4 h. In addition, the carcinogen had no effect on the basal level of MT-specific total RNA, nor did it alter the total RNA levels of the α-tubulin gene. Although attempts were made to measure the levels of the glucocorticoid receptor by both biochemical and molecular methods, receptor levels were too low to quantitate accurately. However, the lack of effect of MNNG on steroid-induced MT RNA levels suggests that the inhibitory effect of the carcinogen was not mediated through alterations in glucocorticoid receptor function. MNNG had no effect on cell number or viability, nor did the carcinogen alter the methylation pattern of the TAT gene as determined from *MspI/HpaII* digests. The results suggest that MNNG mediates its inhibitory effects by a specific interaction with either the TAT gene itself or some other regulatory factor(s) involved in TAT RNA transcription or stability. This effect was relatively gene specific, since expression of the inducible, specialized liver function TAT gene was inhibited by MNNG but expression of the more ubiquitous and inducible MT gene and the constitutively expressed α-tubulin gene were not.

Abbreviations: AFB_1, aflatoxin B_1; DEPC, diethylpyrocarbonate; DEX, dexamethasone; DMSO, dimethylsulfoxide; GRE, glucocorticoid response element; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; MT, metallothionein; PBS, phosphate-buffered saline; TAT, tyrosine aminotransferase; 1 × SSC, 0.15 M NaCl/0.015 M sodium citrate.

Key words: MNNG – Tyrosine aminotransferase – Hepatoma cells – Gene expression – Steroids

INTRODUCTION

Steroids are important regulatory hormones which play a key role in normal cellular differentiation and in the homeostatic regulation of various cellular processes. These hormones mediate their effects through an intracellular receptor. Binding of the hormone to its receptor activates the receptor to a DNA-binding form. The hormone-receptor complex then acts as a transcriptional enhancer to increase the rate of transcription of genes located downstream from the hormone-receptor binding sites on DNA, referred to as the glucocorticoid response element (GRE), resulting in a rapid increase in the RNA levels of specific genes [1–3].

Steroids have proven to be quite amenable to mechanistic studies at the molecular level and their induction of gene expression is probably the best understood eucaryotic gene regulatory system, making this an ideal model in which to study the mechanism(s) by which carcinogens alter the levels of expression of various genes. Previous studies from several laboratories have shown that administration of chemical carcinogens to rats in vivo or in rat cell culture systems causes a marked decrease in the expression of steroid-inducible proteins [4–12]. The in vivo administration of AFB_1 or dimethylnitrosamine to rats resulted in the inhibition of hydrocortisone-induced tryptophan pyrrolase enzyme activity [4,5]. AFB_1 also inhibited the induction of TAT activity by glucocorticoids as well. Since aflatoxin appeared to mediate its inhibitory effects in a manner analogous to actinomycin D, it was suggested that the carcinogen acted at some pretranslational step to prevent protein synthesis [4]. Later studies by Kensler et al. [6,7] provided further evidence for a pretranslational site of carcinogen action. Using in vitro binding studies to measure formation of the steroid hormone-receptor complex and binding of this complex to nuclear DNA, the authors demonstrated that while chemical carcinogens had no effect on the K_d or B_{max} of steroid binding to its receptor, both the K_d and the number of nuclear acceptor sites were decreased when DEX-charged cytosol binding to isolated nuclei was determined.

The inhibition of steroid-inducible TAT gene expression has been demonstrated by several groups [9–11]. Miller and Wogan [10] have recently shown that treatment of rat hepatoma cells with MNNG resulted in a 78% decrease in DEX-induced TAT enzyme activity which was accompanied by a 75% decline in the levels of total TAT-specific RNA. This study provided the first direct evidence that chemical carcinogens may mediate their effects on this gene system by preventing the accumulation of DEX-inducible TAT RNA. A subsequent study has provided evidence that inhibition at a post-transcriptional step may be operative in other cell types [12].

Steroid-responsive genes usually code for enzymes involved in organ-specific specialized cell functions. The expression of these gene products is often altered during the process of neoplastic transformation and these enzymes can serve as markers of cellular differentiation. TAT is a specialized liver enzyme involved in gluconeogenesis and has recently been cloned by Schütz and his colleagues [13,14]. Indeed, the expression of the TAT gene product correlated with the relative differentiation state of the liver or hepatomas studied [15,16].

In this report, I have extended earlier studies on the effects of MNNG on DEX-inducible gene expression in rat hepatoma cells. The results suggest that the effects of the carcinogen were relatively gene-specific, since MNNG inhibited expression of the inducible, specialized liver function TAT gene but had no effect on the expression of either the more ubiquitous and inducible MT gene or the constitutively expressed α-tubulin gene.

MATERIALS AND METHODS

Chemicals

Tissue culture dishes were obtained from Corning Glass Works (Corning, NY); Eagle's minimal essential medium containing non-essential amino acids and L-glutamine were purchased from Whittaker M.A. Bioproducts (Walkersville, MD); fetal bovine serum, trypsin-EDTA solution, 0.4% trypan blue stain, PBS, and penicillin-streptomycin solution were obtained from Gibco Laboratories (Grand Island, NY); MNNG and DEX were purchased from Sigma Chemical Co. (St. Louis, MO); DMSO was obtained from Burdick and Jackson Laboratories, Inc. (Muskegon, MI); and [^{3}H]DEX (49.9 Ci/mmol) and [α-^{32}P]dCTP (3000 Ci/mmol) were purchased from Amersham Corp. (Arlington Heights, IL).

Cells and plasmids

The Fao clone of Reuber H-35 rat hepatoma cells was used because these cells stably maintain many of the specialized liver functions exhibited by differentiated hepatocytes, including the ability to respond to glucocorticoid hormones [16]. Cells were grown in Eagle's minimal essential medium containing non-essential amino acids supplemented with 2 mM L-glutamine and 10% fetal bovine serum in the absence of antibiotics and were maintained at 37 °C in a humidified incubator under a 5% CO_2 atmosphere. Stock cultures were passaged at subconfluent densities by treatment with a trypsin-EDTA solution. The cells were free of any mycoplasma contamination as determined by the Diagnostic Microbiology Laboratory at the Frederick Cancer Research Facility. The medium added to experimental cultures was supplemented with 1% penicillin-streptomycin.

Plasmids pcTAT-2 and pUTAT, cDNA and genomic DNA clones, respectively, of the rat TAT gene [13,17] were provided by Dr. Günther Schütz; p2A10, a cDNA clone of the rat MT gene [18], was provided by Dr. Harvey Herschman; pαT14, a genomic clone of the rat α-tubulin gene [19], was obtained from Drs. Ihor Lemischka and Phillip Sharp; and pRBal117, a

cDNA clone of the rat glucocorticoid receptor gene [20], was provided by Dr. Roger Miesfeld.

Treatment of cells

Fao cells were seeded into 100 mm cell culture dishes. When the cells reached 50–70% confluency, the medium was aspirated from the culture dishes and the cells rinsed once with 5 ml of PBS to remove excess medium. The medium was replaced with 10 ml of PBS and the cells treated with either 10 μl of DMSO alone or 10 μl of DMSO containing a final concentration in the PBS of 500 ng/ml of MNNG. Following incubation in the cell culture incubator for 1 h, the PBS was aspirated and the cells washed twice with 5 ml of PBS. Ten milliliters of fresh medium containing either 0.01% ethanol alone, 0.01% ethanol plus 1 μM DEX, or 3 μM $CdCl_2$ were then added and the cells incubated an additional 2–6 h. The cells were then rinsed once with 5 ml of Ca^{2+}, Mg^{2+}-free PBS and detached by incubation with 1 ml of trypsin-EDTA solution for 5 min. The cells were resuspended in medium and pelleted by centrifugation for 3 min at 800 × *g*. The cell pellets were rinsed once with 5 ml of Ca^{2+}, Mg^{2+}-free PBS and processed as described below. All experimental procedures involving the use of MNNG were carried out under yellow lights.

Isolation of nucleic acids and blot analysis

The cells were treated as described above and then incubated in the presence or absence of inducing agents for either 2 h (for DNA experiments) or 4 h (for RNA experiments). DNA was purified by the procedure described by Perucho et al. [21], and DNA concentrations determined by UV absorbance at 260 nm. The purified DNA samples were digested with a 5-fold excess of restriction enzymes according to the manufacturer's instructions (Bethesda Research Laboratories, Gaithersburg, MD and New England Biolabs, Beverly, MA) and the reactions stopped by addition of EDTA to a final concentration of 10 mM. The restricted DNA samples were fractionated by electrophoresis in a 1% agarose gel with constant recirculation of the gel buffer (23 mM Tris base/10 mM sodium acetate/1 mM EDTA, adjusted to pH 8.3 with glacial acetic acid). The gel was then stained with ethidium bromide and photographed under UV lights. The DNA was denatured by treating the gel with 1.5 M NaCl/0.5 M NaOH, neutralized with 3 M Na acetate (pH 5.5), and transferred to a Biodyne A membrane filter (Pall Ultrafine Filtration Corp., Glen Cove, NY) by the Southern transfer technique [22].

Total cellular RNA was isolated as described by MacDonald et al. [23]. The cell pellets were lysed in 3 ml of a 4 M guanidine isothiocyanate solution and the lysates passed six times through a 20 gauge syringe needle. The 3 ml of cell lysate were layered over 1 ml of a 6.1 M CsCl/25 mM sodium acetate (pH 5.2)/10 mM EDTA solution and total RNA was collected by centrifugation at 127 500 × *g* in a Beckman SW56 rotor for approximately 20 h at 20 °C. The RNA pellets were redissolved in 0.5 ml of DEPC-treated, glass-distilled water and precipitated by centrifugation from a 70% ethanol/0.3 M

sodium acetate (pH 5.2) solution. RNA was dissolved in DEPC-treated water, denatured by treatment with glyoxal, and fractionated by electrophoresis in a 1% agarose gel with constant recirculation of the 10 mM sodium phosphate buffer (pH 7.0). RNA was transferred to Biodyne A membrane filters by the Northern blot procedure described by Thomas [24].

Both Southern and Northern blots were baked, prehybridized, and hybridized as described previously [24], using probes labeled by the random primer labeling technique [25,26]. Following hybridizations, the blots were washed four times with 2 × SSC/0.1% SDS at room temperature for 5–10 min followed by three washes with 0.1 × SSC/0.1% SDS at 50 °C for 15 min. The blots were wrapped in Saran-Wrap and autoradiographed in the presence of an intensifying screen (Cronex Lightning Plus, DuPont, Wilmington, DE) with Kodak X-Omat XAR-5 film at −80 °C. For analysis of Northern blots, the film was preflashed and RNA bands were quantitated by densitometric scanning of the autoradiographs using a LKB Ultroscan XL laser densitometer.

Miscellaneous procedures

For determination of the effects of MNNG on cell cytotoxicity and viability, cells were treated as described above. Following treatment with trypsin, 0.9 ml of the resuspended cells were added to 0.1 ml of a 0.4% trypan blue solution and incubated for 5 min at room temperature. Cell number and viability were determined using a hemacytometer cell counting chamber [27]. For measurement of glucocorticoid receptor levels, dextran-coated charcoal [28] or membrane filter [29] binding assays were used. Protein content was determined by the Lowry procedure [30].

RESULTS AND DISCUSSION

In accordance with previous studies [10], treatment of Fao cells with 500 ng/ml of MNNG for 1 h results in a 40% decrease in total steroid-inducible TAT RNA molecules 4 h after the subsequent addition of 1 μM DEX (Fig. 1A). Although basal levels of TAT-specific transcripts were not detectable in this autoradiograph, it has been shown that MNNG had no effect on the uninduced levels of either TAT enzyme activity or TAT-specific RNA levels 4–6 h after carcinogen treatment [10]. As expected, addition of 3 μM $CdCl_2$ to the cell culture medium for 4 h did not induce TAT RNA species.

In order to determine if this inhibition was a specific or more general effect on inducible-gene expression, the effect of MNNG on other genes was examined. The pcTAT-2 probe was washed off of the blots and the blots reprobed for the constitutively expressed α-tubulin gene and the steroid- and metal-inducible MT gene. Neither MNNG and/or any of the inducing agents had an effect on the RNA levels of the α-tubulin gene (Fig. 2). Staining of a duplicate set of RNA samples with acridine orange [31] showed that the amount of RNA loaded in each lane was relatively constant (Fig. 1B), thus this gene could be used to determine the relative amounts of RNA loaded in

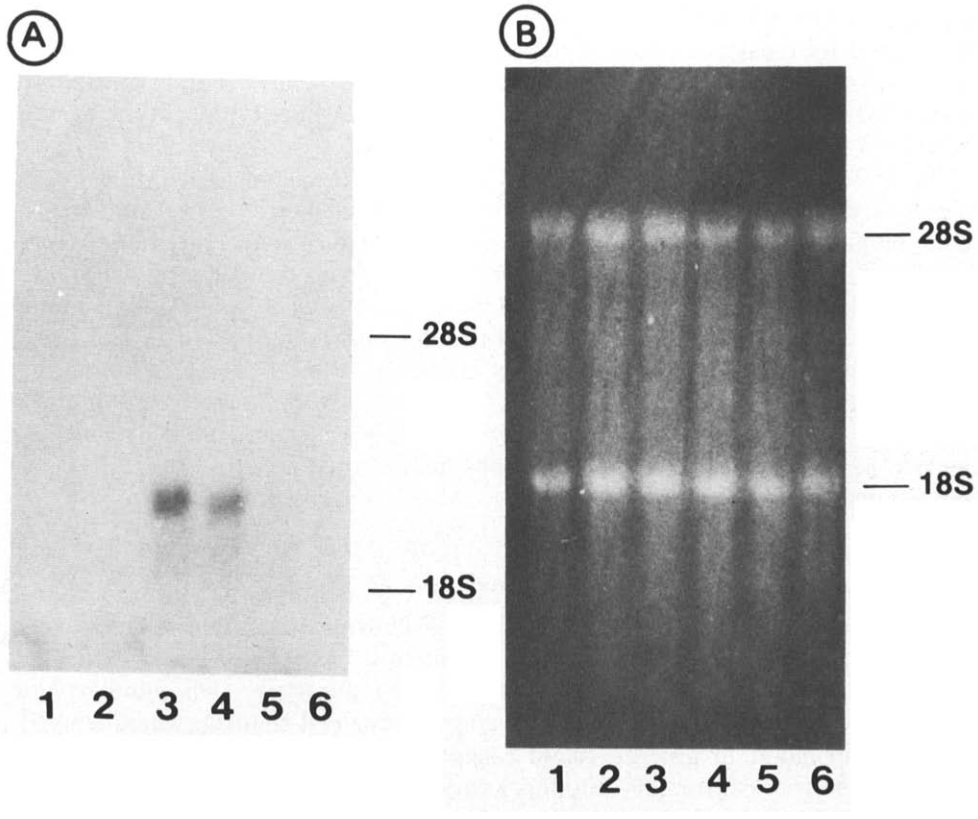

Fig. 1. Inhibition of DEX-inducible TAT RNA levels by MNNG. Fao cells were treated for 1 h with either DMSO or 500 ng/ml of MNNG and then incubated in the presence or absence of 1 μM DEX or 3 μM $CdCl_2$ for 4 h. Twenty micrograms of total RNA were fractionated in a 1% agarose gel, transferred to a nylon membrane filter, and the filter hybridized as described in Materials and Methods. Lane 1, total RNA from DMSO/ethanol-treated cells; lane 2, total RNA from MNNG/ethanol-treated cells; lane 3, total RNA from DMSO/DEX-treated cells; lane 4, total RNA from MNNG/DEX-treated cells; lane 5, total RNA from DMSO/Cd-treated cells; lane 6, total RNA from MNNG/Cd-treated cells. (A) Hybridization with labeled pcTAT-2; (B) acridine orange stained gel.

each lane, and transcript levels for other genes were reported relative to the level of α-tubulin expression. Interestingly, treatment with MNNG had little effect on total DEX- or Cd-induced RNA levels, nor did the carcinogen alter the basal levels of expression of the MT gene (Fig. 3). DEX and Cd caused a 2-fold and 10-fold induction, respectively, of total MT RNA levels both in the absence and presence of MNNG. In addition, as shown in Table I, MNNG had little effect on cell number or viability 6 h after carcinogen treatment, thus demonstrating that the inhibition of DEX-inducible TAT gene expression was not due to the toxic actions of the carcinogen. This dose of MNNG did not cause cytotoxicity in these cells as much as 24 h after carcin-

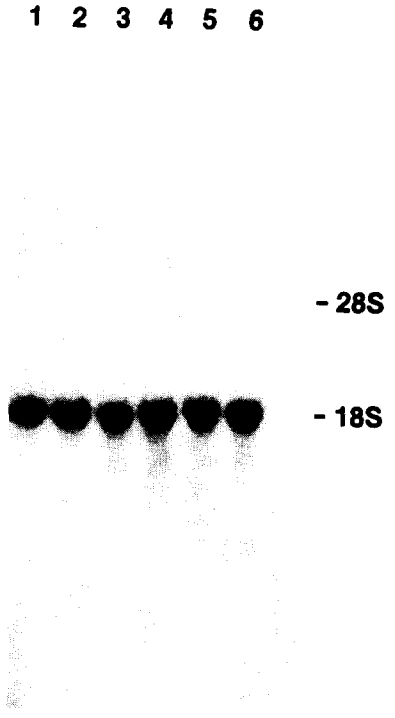

Fig. 2. Total α-tubulin RNA levels in control, MNNG, and inducer-treated cells. Lanes were numbered as described in the legend to Fig. 1; the filter was hybridized with labeled pαT14.

ogen treatment, as demonstrated previously [10]. Attempts to measure the levels of the glucocorticoid receptor in order to detect MNNG-mediated alterations in receptor number or function proved unsuccessful, as receptor levels were too low to measure accurately by either biochemical receptor binding assays or molecular probing of Northern blots with the glucocorticoid-specific plasmid, pRBal117. Receptor binding assays indicated that untreated hepatoma cells had < 100 fmol of receptor per mg protein, thus making it difficult to measure changes in receptor number from these low basal levels. However, the lack of effect of MNNG on DEX-inducible MT gene expression suggests that the carcinogen was not acting to inhibit glucocorticoid receptor function. Support for this conclusion is provided by the studies of Kensler et al. [6], who demonstrated in an in vivo rat model that chemical carcinogens had no effect on either the K_d of DEX binding to receptor or the number of receptors present in the cytosol. Although a more recent study [11] has raised the possibility that AFB_1 and sterigmatocystin may alter glucocorticoid-receptor function and number in rat hepatoma cells, the authors treated cytosolic fractions from the cells with the carcinogens. This may not be an accurate reflection of processes that occur in whole cells or in vivo.

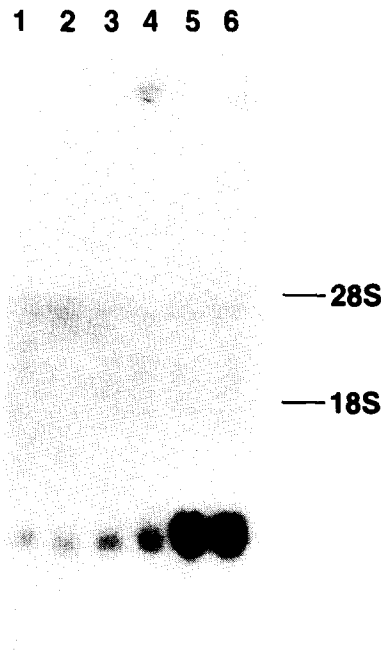

Fig. 3. Lack of effect of MNNG on basal and enhancer-responsive total MT RNA levels. Lanes were numbered as described in the legend to Fig. 1; the filter was hybridized with labeled p2A10.

As a first step toward probing the possible mechanism(s) of MNNG action on DEX-inducible TAT RNA levels, the methylation status of the TAT gene was assessed using the isoschizomeric pair of restriction enzymes, *Msp*I and *Hpa*II. Both enzymes cut DNA at CCGG sequences; however, *Hpa*II is unable to cut at internally methylated cytosine residues while *Msp*I cannot cleave this sequence when the external cytosine is methylated. DNAs were isolated from DMSO- or MNNG-treated cells cultured in the presence or

TABLE I

LACK OF EFFECT OF MNNG ON CELL TOXICITY AND VIABILITY 6 H AFTER TREATMENT

Treatment	Total no. of cells/plate ($\bar{x}$ ± S.D., N = 3)	% Control	% Viable cells ($\bar{x}$ ± S.D., N = 3)
DMSO	5.0 ± 1.3 × 10^6	–	93 ± 1.5
MNNG	5.8 ± 2.9 × 10^6	116	92 ± 3.1

absence of DEX for 2 h and were digested with either *Hind*III alone (Fig. 4, lanes 1 and 2) or *Hind*III plus a second restriction enzyme (Fig. 4, lanes 3–10). The blots were probed with a 940 bp *Eco*RI/*Hind*III genomic fragment spanning the 2nd and 3rd exons of the rat TAT gene [17]. Digestion with *Hind*III alone gave a single 5.2-kb fragment, in good agreement with the 4.4-kb fragment reported by Becker et al. [17]. Double digestion with *Hind*III and the isoschizomers revealed no differences in the methylation patterns resulting from treatment with MNNG and/or DEX relative to solvent-treated samples. However, the TAT gene in these cells appears to be normally methylated in this region of the gene, as indicated by the higher

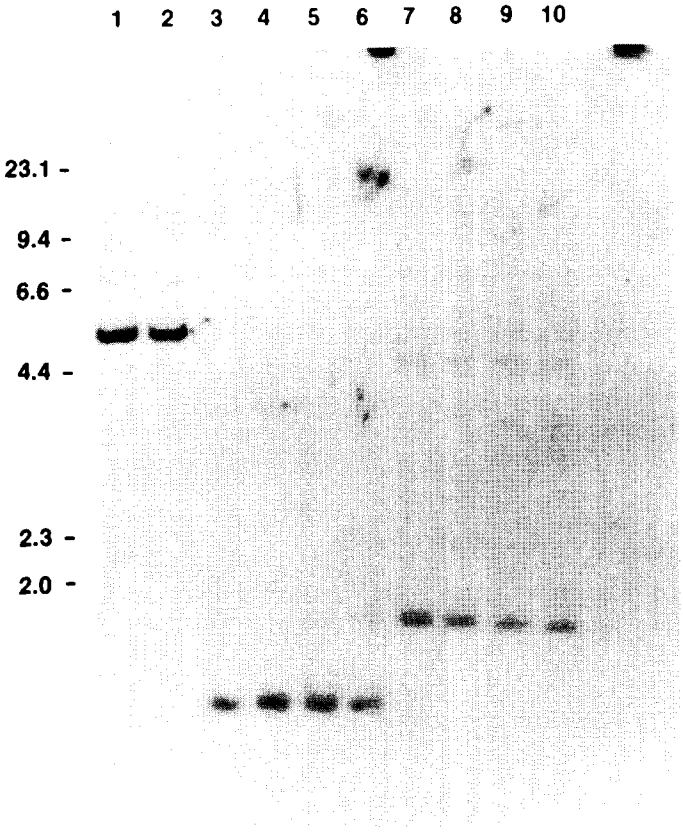

Fig. 4. Southern blot analysis of Fao cell DNA with *Msp*I/*Hpa*II restriction endonucleases. Fao cells were treated for 1 h with either DMSO or 500 ng/ml of MNNG and then incubated in the presence or absence of 1 μM DEX for 2 h. Fifteen micrograms of restricted DNA samples were fractionated in a 1% agarose gel, transferred to a nylon membrane filter, and the filter hybridized with labeled pUTAT as described in Materials and Methods. Lanes 1, 3, and 7 were from DMSO/ethanol-treated cells; lanes 2, 4, and 8 were from MNNG/ethanol-treated cells; lanes 5 and 9 were from DMSO/DEX-treated cells; lanes 6 and 10 were from MNNG/DEX-treated cells. DNA samples were cleaved with the following restriction enzymes: lanes 1 and 2, *Hind*III; lanes 3–6, *Hind*III plus *Msp*I; lanes 7–10, *Hind*III plus *Hpa*II.

molecular weight 1.6-kb band observed in *Hpa*II-digested samples vs. the smaller 1.2-kb band seen in *Msp*I-digested samples.

The results suggest that the inhibition of DEX-inducible TAT gene expression by MNNG is a relatively gene-specific phenomenon. MNNG may mediate this effect through a specific interaction with either the TAT gene itself or some other regulatory factor(s) involved in TAT gene transcription or RNA stability, thus leading to a decrease in the total steady-state levels of TAT RNA accumulation. The specificity of this effect was clearly demonstrated by the data presented, since expression of the DEX-inducible, but not basal, transcripts of the specialized liver function TAT gene were inhibited by MNNG but expression of the basal, DEX-, or Cd-inducible MT gene was not, nor was expression of the constitutively transcribed α-tubulin gene affected. In addition, the carcinogen did not cause any overt cytotoxicity at the concentration used in this study. Interestingly, both the MT and α-tubulin gene products are rather ubiquitously expressed in a wide variety of tissues, compared to the limited, organ-specific expression of TAT in the liver. This suggests that one of the effects of chemical carcinogens in mediating neoplastic transformation may be to down-regulate expression of specialized function genes associated with the differentiated state of the cell. Hormone-responsive genes may be particularly good targets for carcinogen action, since transcriptionally active genes may be preferential targets for these chemicals. Indeed, Berkowitz and Silk [32] have shown that the potent carcinogen *N*-methyl-*N*-nitrosourea caused higher levels of DNA methylation in the actively transcribed regions of rat liver chromatin whereas dimethylsulfate, a relatively weak carcinogen, exhibited a random pattern of methylation. Methylating agents have also been shown to decrease DNA template activity and RNA transport to the cytoplasm [33], and cause a variety of conformational changes in chromatin structure [34,35].

Treatment of mouse embryo cells with thyroid hormone, which enhanced expression of the K-*ras* gene in this cell line, increased the 3-methylcholanthrene-mediated transformation of these cells as a result of an increased activation of K-*ras* to its transforming oncogenic counterpart through a 3-methylcholanthrene-induced point mutation in the K-*ras* gene [36]. Transcriptional activation of the K-*ras* gene locus by hormone treatment rendered the gene more susceptible to carcinogen-mediated damage, possibly by placing the gene locus in a more open conformation. Thus, carcinogens may down-regulate a wide array of organ-specific, differentiation-associated genes because of their relatively high rates of transcription in target tissues and increased susceptibility to genetic damage.

Many hormones have been found to increase gene transcription through their interaction with regulatory receptor proteins at specific DNA sequences called enhancer elements. GREs, which act as enhancer-type sequences to bind steroid-receptor complexes and thus increase downstream gene transcription, have been found in several steroid-regulated genes, including TAT [14]. Courey et al. [37] recently demonstrated that SV-40 enhancer-dependent transcription of the human globin gene was inhibited

when a psoralen-modified SV-40 globin gene recombinant construct was transfected into HeLa cells, thus demonstrating that the positive transcriptional effects of enhancer elements can be inhibited by treatment with alkylating agents. Methylation of specific guanine residues at enhancer sequences associated with hormone-receptor binding sites results in the inhibition of binding of purified steroid hormone-receptor complexes to these DNA fragments [38]. Thus, placement of adducts anywhere between the enhancer and promoter regions of the gene could cause marked decreases in hormone-responsive gene transcription. This would present a rather large target for the chemical carcinogen, and could partly account for the preferential targeting to these types of gene systems. Why some actively transcribed genes are more susceptible to carcinogen-mediated damage than other gene systems is still not known, but could be due to DNA sequence-specific effects or differences in the types and location of DNA regulatory proteins that bind to the DNA.

In addition to direct damage to genomic DNA, chemical carcinogens may alter gene transcription by interfering with the binding of *trans*-acting regulatory proteins to DNA. Several labs have recently identified liver-specific regulatory proteins that control the transcriptional activity of specialized hepatic gene systems [39–41]. If carcinogenic agents acted to inhibit the proper functioning of these proteins, it is conceivable that the transcriptional activity of a whole array of liver-specific genes could be affected. The binding of nuclear regulatory factors to steroid-responsive genes has been demonstrated [42–44].

Future studies will continue to focus on and explore the exact mechanism(s) responsible for the inhibitory effects of MNNG in this gene system. A comparison of the effects of chemical carcinogens on various hormonally regulated gene systems in different organs may provide a clearer picture of the molecular processes governing neoplastic transformation.

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REFERENCES

1 K.R. Yamamoto, Steroid receptor regulated transcription of specific genes and gene networks, Annu. Rev. Genet., 19 (1985) 209–252.

2 G.M. Ringold, Steroid hormone regulation of gene expression, Annu. Rev. Pharmacol. Toxicol., 25 (1985) 529–566.
3 M. Beato, Gene regulation by steroid hormones, Cell, 56 (1989) 335–344.
4 G.N. Wogan and M.A. Friedman, Inhibition by aflatoxin B_1 of hydrocortisone induction of rat liver tryptophan pyrrolase and tyrosine transaminase, Arch. Biochem. Biophys., 128 (1968) 509–516.
5 R.C. Shank, Effect of dimethylnitrosamine on enzyme induction in rat liver, Biochem. J., 108 (1968) 625–631.
6 T.W. Kensler, W.F. Busby, Jr., N.E. Davidson and G.N. Wogan, Aflatoxin inhibition of glucocorticoid binding capacity of rat liver nuclei, Biochem. Biophys. Acta, 437 (1976) 200–210.
7 T.W. Kensler, W.F. Busby, Jr., N.E. Davidson and G.N. Wogan, Effect of hepatocarcinogens on the binding of glucocorticoid-receptor complex in rat liver nuclei, Cancer Res., 36 (1976) 4647–4651.
8 S.T. Perry, S.B. Kulkarni, K-L. Lee and F.T. Kenney, Selective effect of the metallocarcinogen beryllium on hormonal regulation of gene expression in cultured cells, Cancer Res., 42 (1982) 473–476.
9 D.P. Gayda and M.W. Pariza, Effects of carcinogens on hormonal regulation of gene expression in primary cultures of adult rat hepatocytes, Carcinogenesis, 4 (1983) 1127–1131.
10 M.S. Miller and G.N. Wogan, Inhibition of steroid-inducible tyrosine aminotransferase gene expression by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in a rat hepatoma cell line, Carcinogenesis, 7 (1986) 1273–1278.
11 N. Horikoshi, F. Tashiro, N. Tanaka and Y. Ueno, Modulation of hormonal induction of tyrosine aminotransferase and glucocorticoid receptors by aflatoxin B_1 and sterigmatocystin in Reuber hepatoma cells, Cancer Res., 48 (1988) 5188–5192.
12 M.S. Miller, M. Kono and G.N. Wogan, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and benzo[a]pyrene-7,8-diol-9,10-epoxide inhibit glucocorticoid-inducible polyoma virus middle-T gene expression in rat mT-1 cells by a post-transcriptional mechanism, Carcinogenesis, 8 (1987) 1159–1163.
13 G. Scherer, W. Schmid, C.M. Strange, W. Rowe Kamp and G. Schütz, Isolation of cDNA clones coding for rat tyrosine aminotransferase, Proc. Natl. Acad. Sci. U.S.A., 79 (1982) 7205–7208.
14 T. Shinomiya, G. Scherer, W. Schmid, H. Zentgraf and G. Schütz, Isolation and characterization of the rat tyrosine aminotransferase gene, Proc. Natl. Acad. Sci. U.S.A., 81 (1984) 1346–1350.
15 D-P. Huang, A.B. Maine and J-F. Chiu, Changes in hepatic levels of tyrosine aminotransferase messenger RNA during chemical hepatocarcinogenesis, Cancer Lett., 22 (1984) 143–149.
16 J. Deschatrette and M.C. Weiss, Characterization of differentiated and dedifferentiated clones from a rat hepatoma, Biochimie, 56 (1974) 1603–1611.
17 P. Becker, R. Renkawitz and G. Schütz, Tissue-specific DNaseI hypersensitive sites in the 5'-flanking sequences of the tryptophan oxygenase and the tyrosine aminotransferase genes, EMBO J., 3 (1984) 2015–2020.
18 R.D. Andersen, B.W. Birren, T. Ganz, J.E. Piletz and H.R. Herschman, Molecular cloning of the rat metallothionein 1 (MT-1) mRNA sequence, DNA, 2 (1983) 15–22.
19 I. Lemischka and P.A. Sharp, The sequences of an expressed rat α-tubulin gene and a pseudogene with an inserted repetitive element, Nature, 300 (1982) 330–335.
20 R. Miesfeld, S. Rusconi, P.J. Godowski, B.A. Maler, S. Okret, A-C. Wikström, J-Å. Gustafsson and K.R. Yamamoto, Genetic complementation of a glucocorticoid receptor deficiency by expression of cloned receptor cDNA, Cell, 46 (1986) 389–399.
21 M. Perucho, M. Goldfarb, K. Shimizu, C. Lama, J. Fogh and M. Wigler, Human-tumor-derived cell lines contain common and different transforming genes, Cell, 27 (1981) 467–476.
22 E.M. Southern, Detection of specific sequences among DNA fragments separated by gel electrophoresis, J. Mol. Biol., 98 (1975) 503–517.

23 R.J. MacDonald, G.H. Swift, A.E. Przybyla and J.M. Chirgwin, Isolation of RNA using guanidinium salts, Methods Enzymol., 152 (1987) 219–227.
24 P.S. Thomas, Hybridization of denatured RNA transferred or dotted to nitrocellulose paper, Methods Enzymol., 100 (1983) 255–266.
25 A.P. Feinberg and B. Vogelstein, A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity, Anal. Biochem., 132 (1983) 6–13.
26 A.P. Feinberg and B. Vogelstein, Addendum: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem., 137 (1984) 266–267.
27 M.K. Patterson, Jr., Measurement of growth and viability of cells in culture, Methods Enzymol., 58 (1979) 141–152.
28 M. Beato and P. Feigelson, Glucocorticoid-binding proteins of rat liver cytosol. I. Separation and identification of the binding proteins, J. Biol. Chem., 247 (1972) 7890–7896.
29 D.V. Santi, C.H. Sibley, E.R. Perriard, G.M. Tomkins and J.D. Baxter, A filter assay for steroid hormone receptors, Biochemistry, 12 (1973) 2412–2416.
30 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem., 193 (1951) 265–275.
31 G.G. Carmichael and G.K. McMaster, The analysis of nucleic acids in gels using glyoxal and acridine orange, Methods Enzymol., 65 (1980) 380–391.
32 E.M.L. Berkowitz and H. Silk, Methylation of chromosomal DNA by two alkylating agents differing in carcinogenic potential, Cancer Lett., 12 (1981) 311–321.
33 S. Garyfallides, S.A. Kyrtopoulos and C.E. Sekeris, Effects of dimethylnitrosamine on RNA synthesis and metabolism in mouse liver, Cancer Res., 44 (1984) 5110–5117.
34 D.A. Scudiero, S.A. Meyer, B.E. Clatterbuck, M.R. Mattern, C.H.J. Ziolkowski and R.S. Day, III, Relationship of DNA repair phenotypes of human fibroblast and tumor strains to killing by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, Cancer Res., 44 (1984) 961–969.
35 S-C. Cheng, G. Herman and P. Modrich, Extent of equilibrium perturbation of the DNA helix upon enzymatic methylation of adenine residues, J. Biol. Chem., 260 (1985) 191–194.
36 D.L. Guernsey and S.W.C. Leuthauser, Correlation of thyroid hormone dose-dependent regulation of K-ras protooncogene expression with oncogene activation by 3-methylcholanthrene: loss of thyroidal regulation in the transformed mouse cell, Cancer Res., 47 (1987) 3052–3056.
37 A.J. Courey, S.E. Pion and J.C. Wang, The use of psoralen-modified DNA to probe the mechanism of enhancer action, Cell, 45 (1986) 567–574.
38 C. Scheidereit and M. Beato, Contacts between hormone receptor and DNA double helix within a glucocorticoid regulatory element of mouse mammary tumor virus, Proc. Natl. Acad. Sci. U.S.A., 81 (1984) 3029–3033.
39 E.M. Hardon, M. Frain, G. Paonessa and R. Cortese, Two distinct factors interact with the promoter regions of several liver-specific genes, EMBO J., 7 (1988) 1711–1719.
40 M. Jose-Estanyol and J-L. Danan, A liver-specific factor and nuclear factor I bind to the rat *α-fetoprotein promoter*, J. Biol. Chem., 263 (1988) 10865–10871.
41 W. Kugler, U. Wagner and G.U. Ryffel, Tissue-specificity of liver gene expression: a common liver-specific promoter element, Nucleic Acids Res., 16 (1988) 3165–3174.
42 A.F. Stewart and G. Schütz, Camptothecin-induced in vivo topoisomerase I cleavages in the transcriptionally active tyrosine aminotransferase gene, Cell, 50 (1987) 1109–1117.
43 P.B. Becker, S. Ruppert and G. Schütz, Genomic footprinting reveals cell type-specific DNA binding of ubiquitous factors, Cell, 51 (1987) 435–443.
44 M.G. Cordingley and G.L. Hager, Binding of multiple factors to the MMTV promoter in crude and fractionated nuclear extracts, Nucleic Acids Res., 16 (1988) 609–628.