Selective Effect of the Metallocarcinogen Beryllium on Hormonal Regulation of Gene Expression in Cultured Cells

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ABSTRACT

Effects of the metallocarcinogen beryllium on regulation of gene expression were assessed by analysis of hormonal regulation of synthesis of tyrosine aminotransferase in beryllium-treated hepatoma cell cultures. Cell growth was not affected by exposure of the cells to 1 μM BeSO₄ throughout their 4- to 5-day growth cycle. In cells pretreated in this way, the induction by glucocorticoids was specifically impaired, the extent of induced enzyme synthesis being reduced about 50%. Inductions by insulin or cyclic adenosine 3':5'-monophosphate were not influenced by the metal. The results suggest that low concentrations of beryllium selectively interfere with regulatory mechanisms controlling transcriptional events in gene expression.

INTRODUCTION

Beryllium is recognized as being both toxic and carcinogenic; acute exposure causes chronic lung disease in humans (21) and liver necrosis in rats and rabbits (1), while long-term exposure elicits pulmonary carcinomas and bone sarcomas in experimental animals (22). These and other adverse biological effects of the metal have been reviewed recently (18). In cultured fibroblasts and rat liver-derived cells, high concentrations of beryllium (0.15 to 2.5 mm) bring about inhibition of synthesis of DNA, cessation of mitosis, and cell death (2, 3, 19). A striking feature of these studies in cell cultures is the finding that beryllium has a specific affinity for binding in the cell nucleus (5), binding which has recently been localized to a specific class of non-histone nuclear proteins (15).

In animal-based studies on the mechanism of beryllium toxicity, Witschi has demonstrated inhibition of the induced synthesis of enzymes required for liver regeneration (23) as well as of those responding to hormones and other environmental stimuli (24), leading him to suggest that beryllium may interfere with genetic transcriptional processes. To evaluate this possibility as well as to probe further into what may be the molecular basis of carcinogenicity of beryllium, we have initiated studies of the effects of this metal on regulation of gene expression in cultured rat hepatoma cells wherein synthesis of tyrosine aminotransferase (EC 2.6.1.5) is regulated by several hormones, each acting by a discrete mechanism. These phenomena require a cell capable of unimpaired synthesis of nucleic acids and proteins, and here we have limited our analyses to very low concentrations of beryllium where these cellular functions were not markedly influenced. Under these conditions, we observe a significant inhibition of enzyme synthesis induced by glucocorticoids, presumably acting at the level of transcription, but we find no effect on the apparently posttranscriptional inductions elicited by insulin and cAMP.

MATERIALS AND METHODS

Cell Culture. Rat hepatoma cells of the H-35 line, developed originally by Pitot et al. (16) and maintained in our laboratory for some years, were grown (unless otherwise indicated) in 75-cm² Falcon flasks in Eagle's minimal medium enriched with 4 times the usual concentration of vitamins and amino acids, 20% fetal calf serum, and gentamicin, 10 μg/ml. A cloned derivative of H-35 termed KRC-7 and selected by Leichtling et al. (11) for improved responsiveness to hormones was generously made available to us by Dr. W. D. Wicks, and these cells were grown under the same conditions. Prior to experiments, the cells were routinely placed in nonenriched minimal (holding) medium for 24 hr; in the absence of serum, the cells remain viable but do not grow. Beryllium treatment involved supplementation of both growing and holding media with an aqueous solution of BeSO₄.

In our initial analyses of the effects of beryllium on hydrocortisone-mediated induction of tyrosine aminotransferase, we detected no effect of 1 μM BeSO₄ when added with the steroid and no effect if beryllium was present during the entire 24-hr holding period in serum-free medium prior to steroid treatment. However, inhibition of induction became apparent if beryllium was present for an additional 24-hr period after cells were in growth medium and was both more reproducible and more extensive as the period of exposure was extended further. Accordingly, effects of beryllium on hormonal regulation were analyzed in cells exposed to 1 μM BeSO₄ throughout their growth cycle (4 or 5 days) as well as during the holding period and the interval of hormone treatment. Since enzyme levels and the response to hormonal inducers varied appreciably over the time period in which these experiments were done, data from representative experiments are presented; each was repeated a number of times and by several investigators.

Analyzes. At designated intervals after hormone treatment, cell monolayers were rinsed with 0.14 w NaCl·10 mm phosphate (pH 7.4) and then scraped into this solution, centrifuged, and washed before being lysed in a solution (1 to 2 ml/flask) containing 50 mm K-PO₄ (pH 7.6), 5 mm α-ketoglutarate, 10 μM pyridoxal phosphate, and EDTA and dithiothreitol, 1 mm each. After 3 cycles of freeze-thawing in liquid N₂ and a 37° bath, lysates were centrifuged at 105,000 × g for 30 min, and the supernatants were used for analyses. Tyrosine aminotransferase was assayed as described before (6), using the product measurement of Diamondstone (4); for those preparations containing very low enzyme levels (specific activity <10 units/mg protein), the duration of the assay was extended from the usual 10 min to 20, 40, or 60 min as required. One unit of aminotransferase activity is the amount catalyzing formation of p-hydroxyphenylpyruvate, 1 mmol/min, at 37°.

Proteins were estimated by the method of Lowry et al. (12).

For measurement of rates of enzyme synthesis, the cells were exposed to [3H]leucine (5 μCi/ml, 50 Ci/mmol) for 15 min and then

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The abbreviation used is: cAMP, cyclic adenosine 3':5'-monophosphate.
collected, lysed, and centrifuged as above, pooling the cells from 8 similarly treated flasks. Aliquots of supernatant fractions were taken for determination of radioactivity in total soluble proteins by the method of Mans and Novelli (13), and the remainder (1.5 ml) was supplemented with carrier aminotransferase and immunoprecipitated with a slight excess of specific aminotransferase antiserum. Immunoprecipitates were washed and analyzed after electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate as described by Lee and Nickol (9), the radioactivity of the 2 gel slices containing the m.w. 50,000 enzyme subunits being taken as the specific aminotransferase radioactivity.

RESULTS

Preliminary experiments revealed that exposure of H-35 cells for 24 hr to beryllium at concentrations of 5 μM or higher resulted in overt toxicity with cells becoming detached from culture flask surfaces. At 1 μM, no toxicity was apparent, and all subsequent experiments were done at this level. The growth of H-35 cells was not influenced by beryllium at this concentration except perhaps for a slightly prolonged lag period before logarithmic growth was established. KRC-7 cells grew somewhat more slowly, reaching confluence in 6 to 7 days, and their growth was similarly unaffected by 1 μM BeSO₄ (Chart 1A). Since exposure of the cells to hormonal inducers was routinely done after a 24-hr "holding" period in serum-free medium, the possibility of a beryllium effect on growth under these conditions was also examined. Treatment of H-35 cells with 1 μM BeSO₄ throughout the growth cycle which included a 24-hr interval in serum-free medium indicates no significant effect of the metal on growth under these conditions (Chart 1B).

The effect of beryllium pretreatment on the induction of tyrosine aminotransferase by hydrocortisone in both H-35 and KRC-7 cells is illustrated in Chart 2. Induced enzyme synthesis was inhibited in both cell lines, beryllium-treated cells typically being able to synthesize only about 50% of the amount of enzyme synthesized in response to the steroid in untreated cells. Small effects of long-term beryllium treatment on the basal level of the enzyme were sometimes apparent, as in Chart 2, suggesting a minor and variable impairment of the synthesis of this rapid-turnover enzyme [half-lives of the enzyme and its mRNA are about 1.5 hr in these cells (7)]. The metal did not affect transaminase activity when added directly to the enzyme assay.

The beryllium actually diminishes the synthetic response to the steroid hormone is documented in the pulse-labeling measurement of this parameter presented in Table 1 and Chart 3. Six hr after steroid addition, specific aminotransferase synthesis was accelerated about 9-fold in untreated cells; in this experiment, labeling of the total soluble proteins appeared to be atypically reduced somewhat by the hormone. Steroid-induced enzyme synthesis was increased to only about one-half this extent in cells pretreated with beryllium in accordance with effects on the amount of enzyme induced; this effect is illustrated in the electrophoretic analysis of immunoprecipitates presented in Chart 3. Beryllium had no detectable effect on synthesis of the total soluble proteins or on the uninduced rate of aminotransferase synthesis.

Induction by insulin was found to be unusually effective in KRC-7 cells, the aminotransferase level rising 5- to 6-fold over an 8-hr period after a single addition of insulin to culture media.

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Chart 2. Induction by hydrocortisone in control and beryllium-treated hepatoma cells. Data are averages of duplicate flasks of similarly treated cells.

Table 1

| Treatment       | Amino- | Amino- | Radioactivity |
|-----------------| transferase | transferase | soluble proteins |
|                 | activity (units/mg protein) | (cpm) | (cpm/mg) |
| None            | 6.4 | 380 | 45,480 |
| Beryllium       | 5.6 | 370 | 46,000 |
| Hydrocortisone  | 52.2 | 3,590 | 36,640 |
| Beryllium + hydrocortisone | 28.6 | 2,310 | 45,740 |
Beryllium Effect on Gene Expression

Unlike the parent H-35 cells which require multiple additions of this hormone for a sustained induction response (17). These cells, like currently available H-35 cells, have retained a somewhat limited capacity for induced synthesis of tyrosine aminotransferase in response to N⁶,O²⁻-dibutyryl cyclic adenosine 3',5'-monophosphate, a 2-fold increase in the enzyme occurring over a 4-hr period after a single addition of the cyclic nucleotide, after which the enzyme returns quickly to the basal level. Acceleration of enzyme synthesis by either insulin or N⁶,O²⁻-dibutyryl cyclic adenosine 3',5'-monophosphate was undiminished by long-term beryllium treatment of the cells (Chart 4). Similar results with these inducers were seen in H-35 cells.

Some noncarcinogenic metals were tested to ascertain if effects comparable to those of beryllium could be detected. Iron proved to be incompatible with required components of the medium and could not be adequately tested; aluminum had no effect on any parameter examined at concentrations as high as 1 mm. The toxic, noncarcinogenic metal selenium caused rapid detachment of the cells at concentrations above 100 μM; between 100 and 10 μM, little overt toxicity was apparent. If cells were pretreated with selenium in this range for 24 hr and then washed free of the metal before addition of inducing hormones, there was no effect. However, if selenium was present during the induction response, inductions by hydrocortisone and by insulin were both inhibited and to similar extents (Chart 5). This metal also caused some reduction in the basal aminotransferase level. Induction by cAMP or derivatives was not tested in these experiments.

**DISCUSSION**

These initial findings concerning effects of the metallocarcinogen beryllium on hormonal regulation of gene expression may have significant implications for the study of gene expression as well as to the mechanisms of its carcinogenicity. It is important that the concentration of beryllium used did not affect growth, indicating that the cellular capacity to replicate DNA or to synthesize the normal complement of RNAs and proteins required for maintenance and growth was not impaired. Indeed, even the basal level of tyrosine aminotransferase, requiring continual replacement of 50% of both the enzyme and its mRNA each 1.5 hr, was not affected or only slightly reduced after 5 to 6 days of exposure to the metal. The only impairment detected was a reduced capacity to respond to hormonal regulators of gene expression, and this impairment was limited to the specific mechanisms involved in steroid-mediated regulation.

The mechanisms of induced synthesis of tyrosine aminotransferase by glucocorticoids, insulin, or cAMP are not yet entirely clear. Earlier studies indicating that the steroid-mediated induction involves transcriptional or related processes leading to de novo production of mRNA (10) have now been supported by evidence for glucocorticoid-induced increases in the mRNA assessed by translation assay (14), and there is clear evidence in other experimental systems that steroids act at or close to the transcriptional level in controlling gene expression (e.g., Ref. 20). Thus, our data are entirely in accord with the earlier suggestion of Witschi (23) implicating transcriptional events in beryllium toxicity. The mechanisms of induction by insulin and by cAMP are more obscure and currently somewhat controversial; we continue to favor the view expressed...
earlier (10) that these regulators act by improving the translational capacity of existing mRNAs (for a discussion of this topic, see Ref. 8). The specificity of the effect of beryllium on glucocorticoid-mediated induction is important in this regard, since it clearly delineates this mechanism from those involved in inductions by insulin or cAMP, the latter being completely unaffected by long-term beryllium treatment.

At present, we can only speculate as to how beryllium may interfere with steroid-mediated regulation of gene expression. The requirement that we observe for long-term treatment with beryllium can probably be understood in terms of the slow uptake of this metal by cells in cultures described by Skilleter and Paine (19). From a mechanistic point of view, it is especially significant that this uptake is localized to nuclei (5) and specifically to a discrete class of non-histone nuclear proteins (15). Future studies of beryllium-binding nuclear proteins and their relationship to processes involved in mRNA production may thus contribute toward detailing the mechanism of regulation of gene expression by steroid hormones.

Alterations in gene expression are typical of cancer cells; the loss of capacity to regulate genes would thus be expected to be an early and perhaps fundamental component of the cascade of molecular dysfunctions leading to cancer. The subtle effect of beryllium described here could well be related, then, to the carcinogenicity of this metal in chronic, low-dose exposures, for the treated cells retain their capacity to grow, divide, and otherwise maintain their metabolic requirements, but their responsiveness for the treated cells retain their capacity to grow, divide, and otherwise maintain their metabolic requirements, but their functional capacity of existing mRNAs (for a discussion of this topic, see Ref. 8). The specificity of the effect of beryllium on glucocorticoid-mediated induction is important in this regard, since it clearly delineates this mechanism from those involved in inductions by insulin or cAMP, the latter being completely unaffected by long-term beryllium treatment.

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REFERENCES


