Specific Receptors for Glucocorticoid in the Cytoplasm of the Liver of AH 130 Tumor-bearing Rats

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SUMMARY

Specific receptors for dexamethasone (11β,17α,21-tri hydroxy-9α-fluoro-16α-methyl-1,4-pregnadiene-3,20-dione) in the cytoplasm of the liver from AH 130 (solid type) tumor-bearing rats markedly increased in the advanced stage of tumor growth. The cytoplasmic receptors of the livers of normal and tumor-bearing rats differed in their affinities for dexamethasone, and their apparent equilibrium (dissociation) constants (K) for dexamethasone were 4.0 and 2.6 × 10^-9 M, respectively. The rates of dissociation of dexamethasone-receptor complexes and the heat denaturations of the receptors in the livers of normal and tumor-bearing rats were similar. The glucocorticoid receptors of tumor-bearing rats had slightly higher affinities than did those of normal liver for all the steroids tested. Only a trace amount of receptors for dexamethasone could be detected in the cytoplasm of AH 130 ascites cells.

INTRODUCTION

Glucocorticoid is known to be important in the host-tumor relationship (16, 17, 25, 28). A previous paper (25) from our laboratory reported that the activities of various aminotransferases of liver cytoplasm that are induced by glucocorticoids, such as tyrosine, alanine, and aspartate aminotransferases, increased in the advanced stage of tumor growth. Moreover, it was found that in the advanced stage of tumor growth the hypophyseoadrenocortical function was apparently increased (25), as was the plasma corticosterone level (25, 26).

Recently, there have been many reports of a common mechanism of action of steroid hormones in which first the steroid binds to specific cytoplasmic proteins in the target cell and then the steroid-receptor complex migrates into the nucleus where it associates with an acceptor site in the chromatin (13). The binding of the steroid-receptor complex to the chromatin is thought to act as a modulator of gene expression (6). A similar mechanism has been described for the action of glucocorticoid (2, 3, 5, 14, 15, 19). The binding of glucocorticoid to specific cytoplasmic receptors appears to be the essential initial step in specific enzyme induction mediated by glucocorticoid and involves tissue responsiveness to the hormone (4, 9, 10, 30, 32).

This paper reports some properties of the cytoplasmic receptors of liver from normal and tumor-bearing rats and shows that the receptors for dexamethasone in the cytoplasm of rat liver increased in the advanced stage of tumor growth.

MATERIALS AND METHODS

Animals and Their Treatment. Male albino Donryu rats, weighing 100 to 150 g, were given laboratory chow and water ad libitum and were maintained in an air-conditioned room at approximately 25° with alternate 12-hr periods of light (6 a.m. to 6 p.m.) and dark. Ascites tumor cells (strain AH 130) were used 7 days after transplantation. To obtain AH 130 tumor-bearing rats (solid type), 8 × 10^7 ascites cells were injected s.c. into the right hip of rats weighing 100 to 110 g, and the animals were sacrificed 2 weeks later. Rats with tumors weighing 13 to 17% of the body weight were used as animals with advanced-stage tumors (24, 25) in this investigation. Unless otherwise specified, all rats were adrenalectomized 3 days before sacrifice and were given 0.9% NaCl solution to drink after the operation. The operation was done between 9 and 10 a.m.

Preparation of Cytoplasmic Extracts. Adrenalectomized animals were killed by cervical dislocation between 9 and 10 a.m., and their livers were perfused with 30 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.55) containing 250 mM sucrose, 25 mM KCl, 10 mM MgCl₂, and 1 mM mercaptoethanol (3). The liver was then homogenized in 2 volumes of the same buffer and centrifuged at 700 × g for 10 min. The supernatant fluid was centrifuged at 105,000 × g for 1 hr at 2°C to obtain the cytoplasmic supernatant fraction (cytosol).

Binding Assay. Samples of 0.2 ml of cytosol were incubated for 2 hr at 0°C with various concentrations of [3H]dexamethasone (28 Ci/mmole; The Radiochemical Centre, Amersham, England) with or without 5,000-fold excess of unlabeled glucocorticoid (3). The unbound steroids were removed with dextran-coated activated charcoal (3) by centrifugation in an Eppendorf centrifuge at 8,000 × g for 4 min; then 50 μl of the supernatant were used to determine radioactivity. For this the samples were incubated with 0.3 ml of NCS tissue solubilizer (Amersham/Searle Corp., Arlington Heights, Ill.) for 30 min at room temperature. Then 10 ml of a toluene-based scintillation mixture were added and radioactivity was counted in a Beckman scintillation...
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RESULTS

The activities of various aminotransferases of rat liver cytosol, which are readily induced by glucocorticoids, increased in the advanced stage of AH 130 solid-type tumor growth (Table 1). Similar findings were also observed in the livers of AH 130 ascites-type tumor-bearing rats (25). Although the increase in the activities of these liver enzymes seemed to be controlled by hypersecretion of the adrenocortical hormone (glucocorticoid) (25), we further investigated the possibilities of correlation between the increase in the activity and glucocorticoid receptors in the liver cytosol of tumor-bearing rats in advanced tumor growth.

Chart 1 shows the method used to measure the concentration of specific [3H]dexamethasone-binding sites and affinity of binding proteins (equilibrium constant) for dexamethasone in the cytosol of liver from normal and AH 130 tumor-bearing rats. All animals were adrenalectomized 3 days before experiments to reduce the effect of endogenous glucocorticoid on the binding of [3H]dexamethasone to specific binding sites. Duplicate determinations were made with at least 15 dexamethasone concentrations to minimize variations in the apparent equilibrium constant for the steroid. A plot of the amount of steroid bound against the ratio of bound to free steroid (Scatchard plot) (31) gave a straight line, indicating that there was only 1 class of binding sites in the cytosol of the liver of both normal and tumor-bearing rats (Chart 1B). The Scatchard plots showed apparent equilibrium constants for dexamethasone of 2.6 and 4.0 x 10⁻⁸ M at 0° for the liver cytosol of tumor-bearing rats and normal rats, respectively (Table 2). The value for normal rat liver is consistent with that already reported (3, 15) and is significantly higher than that for tumor-bearing rat liver statistically. As also shown in Table 2, the concentration of specific binding sites for [3H]dexamethasone in the liver cytosol of tumor-bearing rats was higher than that in the liver cytosol of normal rats when evaluated from the Scatchard plot in Chart 1B.

AH 130 ascites cells contained only 0.007 pmol (range, 0.003 to 0.01) of dexamethasone-binding sites per mg of protein.

| Table 1 | Various aminotransferase activities in the cytoplasm of the liver from AH 130 (solid-type) tumor-bearing rats in the advanced stage of tumor growth |
|-------------------|--------------------------------------------------|---------------------------------|-----------------------------------------------|
| Livers*            | Tyrosine aminotransferase (nmol/min/mg protein) | Aspartate aminotransferase (µmol/min/mg protein) | Alanine aminotransferase (µmol/min/mg protein) |
| Normal (6)         | 8.6 ± 2.3  
                       | 1.66 ± 0.27  
                       | 0.34 ± 0.06 |
| Tumor-bearing (7)  | 23.4 ± 3.0  
                       | 3.30 ± 0.39  
                       | 0.54 ± 0.06 |

* Livers from normal and tumor-bearing rats with intact adrenals.
† Numbers in parentheses, number of animals used.
\$ Mean ± S.D.

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Comparison of dexamethasone receptors

Equilibrium (dissociation) constants (K) for dexamethasone and concentrations of dexamethasone-binding sites of liver cytosol receptors were determined from Scatchard plots. p < 0.001 (Student’s t test).

<table>
<thead>
<tr>
<th>Equilibrium constant (nM)</th>
<th>Concentration (pmoles/mg protein)</th>
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<tbody>
<tr>
<td>Normal rats (6)(^a)</td>
<td>4.0 ± 0.5(^d)</td>
</tr>
<tr>
<td>Tumor-bearing rats (8)</td>
<td>2.6 ± 0.2</td>
</tr>
</tbody>
</table>

\(^a\) Equilibrium constants were determined from Scatchard plots (31) of binding data of the type shown in Chart 1B.

\(^b\) Concentration of binding sites were estimated from Scatchard plots of binding data of the type shown in Chart 1B.

\(^c\) Numbers in parentheses, number of animals used.

\(^d\) Mean ± S.D.

Chart 2 shows the rates of dissociation of [\(^3\)H]dexamethasone from [\(^3\)H]dexamethasone-receptor complexes of the livers of tumor-bearing and normal rats at 0°C. As can be seen the rates were the same. The dissociation rate constant was about 1.7 x 10^-4 min^-1 at 0°C.

When receptors were not combined with dexamethasone, the binding activity of receptors from tumor-bearing rats was completely lost on heating at 40°C for 10 min, and incubation of cytosol at 30°C for 10 min resulted in 50% loss of specific dexamethasone-binding activity. This heat lability of the receptors of liver from tumor-bearing rats is similar to that of the thermolabile G protein from normal rat liver reported elsewhere (15). When bound to dexamethasone the receptors were much more heat stable (2, 15, 30). If the dexamethasone-bound receptors from tumor-bearing rats had a different protein conformation from those of normal rats, they might differ in thermostability from the latter. However (Chart 3), their thermostability was essentially similar to the latter.

As described above, the receptors from tumor-bearing animals had a slightly different affinity for [\(^3\)H]dexamethasone from those of normal animals. To study this difference further, we examined the competitive effects of various steroids for [\(^3\)H]dexamethasone-binding sites. Chart 4 shows that the glucocorticoid receptors of tumor-bearing rat liver had higher affinities (Chart 4A) than did those of normal liver (Chart 4B) for all the steroids tested.
that specific receptors for dexamethasone also increased in tumors than in normal rat liver. This could well be due to the level of tyrosine aminotransferase induced 5 hr after i.p. injection of hydrocortisone (5 mg/100 g body weight) was higher in the liver of tumor-bearing rats with advanced tumors than in normal rat liver. This could well be due to the higher concentration of glucocorticoid receptors in the liver cytosol of tumor-bearing rats. In this study, all animals were adrenalectomized 3 days before experiments to reduce the effect of endogenous glucocorticoids. After this treatment, the apparent dissociation constants for dexamethasone and other steroids tested were consistently higher than those of normal liver under the experimental conditions used. Recently, several reports have suggested heterogeneity of the cytoplasmic glucocorticoid-binding proteins of different tissues (18, 19, 23) and a single tissue (23), and Giannopoulos (10) suggested that the glucocorticoid receptors of fetal and adult liver are not identical or that there may be more than 1 type of receptor. However, Feldman (9) could not detect any difference between the glucocorticoid receptors of rat liver present as early as Day 19 to 20 of gestation and those of
adult rat liver. Our results favor the possibility of heterogeneous receptors. However, we used crude extracts and observed only small differences, which could be due to the effects of other factors. Furthermore, there is another binding protein (Binder IB) with an apparent K value of $1 \times 10^{-8}$ M for dexamethasone that was recently found in the liver cytosol of adrenalectomized rats (20, 21), and a difference in the ratio of binding proteins in the livers of tumor-bearing rats and normal rats could cause a difference in their apparent K values. So that this possibility is excluded, experiments are now under way on nuclear binding and on purified receptors from the different sources.

The dissociation rate constants (Chart 2) and heat stabilities of the receptors (Chart 3) from normal and tumor-bearing rats were similar. Therefore, the receptors in the liver of normal and tumor-bearing rats are similar, if not identical, proteins.

It was found that the activities of aminotransferases in the liver cytosol show a biphasic change, first decreasing at the beginning of the tumor-bearing and then increasing at an advanced stage of tumor growth. Studies are also in progress on the participation of glucocorticoid receptors in this biphasic change.

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REFERENCES

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