INTERACTION BETWEEN DEXAMETHASONE RECEPTOR COMPLEXES AND ISOLATED NUCLEI FROM ZAJDELA HEPATOMA AND RAT LIVER CELLS

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For translocation of hormone-receptor complexes (HRC) from the cytosol of the hepatoma and rat liver into the nuclei temperature activation of the HRC is necessary. It was shown by saturation analysis that compared with the amount of HRC which normal liver nuclei bind from the homologous cytosol of the same tissue (3 pmoles/mg DNA), hepatoma nuclei bind only one-twentieth as much HRC from homologous hepatoma cytosol (0.15 pmole/mg DNA) but twice as much HRC from heterologous liver cytosol (5.6 pmoles/mg DNA). The order of the association constants (K_a) for HRC with the acceptor sites in the nuclei of the hepatoma and liver is practically identical. It is suggested that inhibition of HRC translocation from the cytosol of hepatoma cells into their nucleus is the probable reason why these cells do not respond to hormone.

KEY WORDS: dexamethasone, hormone-receptor complex, acceptor.

The present writers [1] and other workers [5] have found receptors for dexamethasone with the same affinity for it as the cytosol receptors of normal rat liver, in the cell cytosol of hormone-resistant Zajdela ascites hepatoma. However, these receptors are from three to five times more numerous in normal liver than in the tumor.

In the investigation described below the mechanism of the subsequent stages of action of the hormone in the normal and tumor cells – temperature activation of the hormone-receptor complex (HRC) and its translocation into the nucleus – was studied in vitro.

EXPERIMENTAL METHOD

The [1,2-^{3}H]dexamethasone (D) used in the work, with a specific radioactivity of 10 Ci mmole, was from the Radiochemical Center, Amersham, England; nonradioactive D from Sigma, USA; Tris-(hydroxymethyl)-aminomethyl from Serva, West Germany; Norit A charcoal from Serva, West Germany; dextran (mol. wt. 80,000) from Fluka, Switzerland. The remaining reagents, of the chemically pure grade, were of Soviet origin.

Male Wistar rats weighing 150-200 g were used. Adrenalectomy was performed on the animals of the control group 3-4 days before the experiment. Zajdela ascites hapatoma was grafted intraperitoneally [1] and the cells were harvested on the sixth day after transplantation. All the operations for isolation of the cytosol and nuclei from the rat liver and hepatoma were carried out in the cold. The following buffer solution was used: A) 0.02 M Tris-HCl, pH 8.0, 0.25 M sucrose, 5 mM $MgCl_2$, 1.5 mM dithiothreitol; B) 0.02 M Tris-HCl, pH 8.0, 2.3 M sucrose, 5 mM $MgCl_2$, 1.5 mM dithiothreitol (ρ=1.275 for the control and ρ=1.265 for the hepatoma).

The cytosol of the liver and hepatoma cells and the HRC of these cytosols were obtained by the method described previously [1]. The cytosols were incubated for 2-3 h at 4°C with [^{3}H]D only in increasing concentrations of 5×10^{-10} to 1×10^{-8} M or with 5×10^{-9} M [^{3}H]D with the addition of 2.5×10^{-6} M nonradioactive D (nonspecific binding). The unbound hormone was removed by adsorption on charcoal coated with dextran (a suspension of 2% charcoal + 0.2% dextran). The cytosol with HRC was used for the investigation.

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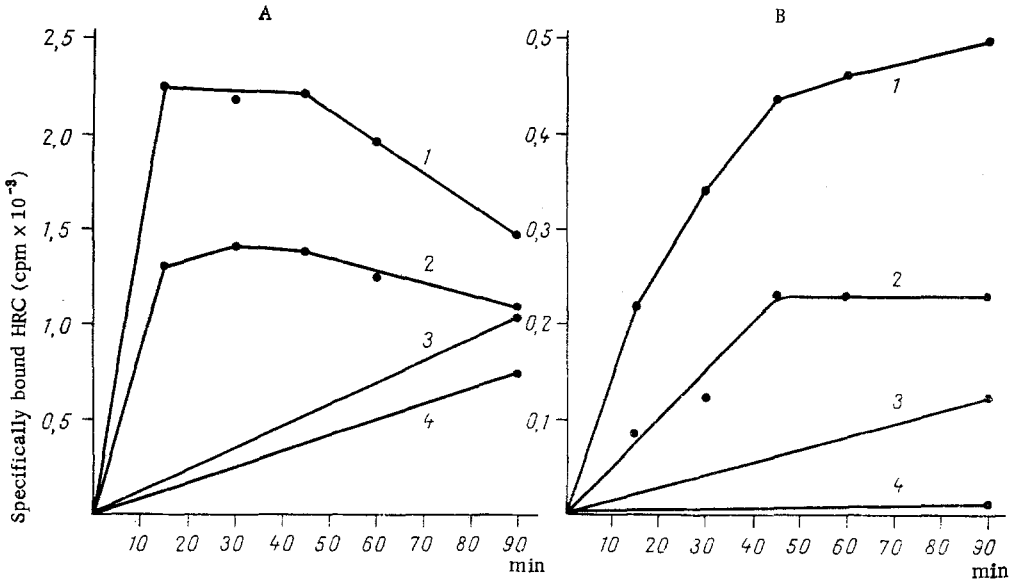

Fig. 1. Kinetics of binding of [^{3}H]dexamethasone-receptor complexes from liver and hepatoma cytosol with rat liver nuclei. Abscissa, time (in min); ordinate, quantity of [^{3}H]HRC (in cpm/mm × 10^3) specifically bound with nuclei. HRC specifically bound with nuclei determined from difference between total and nonspecific binding of [^{3}H]HRC with nuclei. A) HRC from liver cytosol incubated in presence of liver nuclei at 20°C (1) and 0°C (3); HRC incubated alone without additions at 20°C (2) and 0°C (4); B) HRC from hepatoma cytosol incubated in presence of rat liver nuclei at 20°C (1) and 0°C (3); HRC from hepatoma cytosol incubated alone without addition at 20°C (2) and 0°C (4).

Nuclei were obtained by Chauveau's method [4]. The protein/DNA ratio in the liver and hepatoma nuclei varied from 2 to 4 in both cases.

Protein was determined by Lowry's method [9] or spectrophotometrically by means of an empirical equation: protein concentration (in mg/ml) = $1.45 \times E_{280} - 0.74 \times E_{260}$ [7]; DNA in the nuclei was determined by Burton's method [3].

To bind the HRC with the nuclei, usually 0.1 ml of a suspension of nuclei (1×10^8 nuclei/ml) was added to 0.5 ml of cytosol.

Temperature activation of HRC was carried out at 20°C at different times from 0 to 90 min by two methods: a) HRC alone were activated without any additions, b) HRC were activated in the presence of liver cell nuclei. After activation (in the first case nuclei were added) all the samples were incubated at 0°C for 30 min. The nuclei were then sedimented, washed twice with 1 ml buffer A to remove HRC adsorbed by the outer membranes of the nuclei, the nuclei were suspended in 0.5 ml water, after which they were transferred to counting flasks, to which 8 ml of scintillation fluid was added. Specific binding of HRC by nuclei was estimated from the difference between the total and nonspecific binding of D.

Translocation of HRC into homologous and heterologous nuclei after temperature activation of the mixture at 20°C for 30 min was studied in different combinations: 1) liver cytosol + liver nuclei; 2) liver cytosol + hepatoma nuclei, 3) hepatoma cytosol + hepatoma nuclei, and 4) hepatoma cytosol + liver nuclei. After incubation the nuclei were washed, as described above, and the radioactivity was counted.

The association constants (K_a) and the number of specific binding sites (n) in the cytosol and nuclei were calculated by Rosenthal's method [10] in Scatchard plots [11].

Radioactivity was counted in ZhS-8 dioxane scintillator on a Mark II (Nuclear Chicago, USA) scintillation counter. The results were calculated by a special program on an Olivetti (Italy) computer.

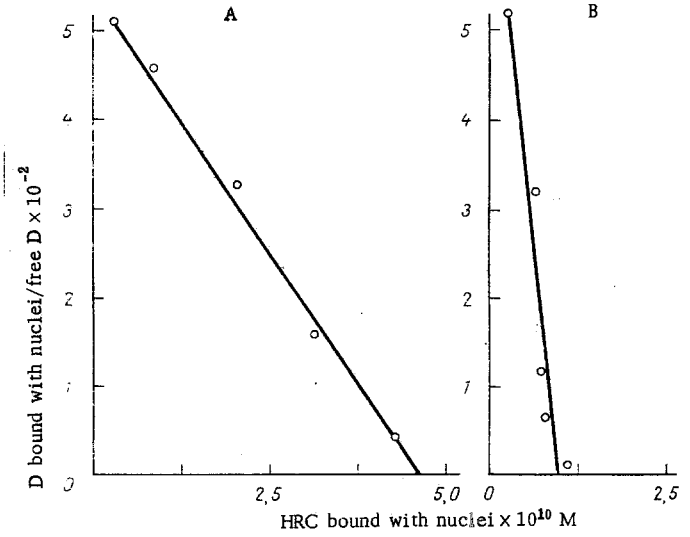

Fig. 2. Specific binding of [^{3}H]HRC by liver (A) and hepatoma (B) cell nuclei. Abscissa, quantity of [^{3}H]HRC bound with nuclei (in moles $\times 10^{-10}$ mg nuclear DNA); ordinate, ratio of bound [^{3}H]HRC to unbound ^{3}H-HRC (in units $\times 10^{-2}$). Cytosol obtained with HRC, suspension of homologous nuclei added, sample activated at 20°C for 30 min, then cooled, and radioactivity counted in nuclei (see "Experimental Method"). Specific binding of [^{3}H]D by nuclei calculated by Rosenthal's method [10] in Scatchard plots [11].

EXPERIMENTAL RESULTS

To study interaction between HRC and the nuclei, a suspension of rat liver nuclei was added to cytosol containing labeled HRC as described in "Experimental Method." At 20°C so-called activation of HRC takes place, after which binding of HRC with the nuclei increased (Fig. 1). As Fig. 1 also shows, activation of HRC in the presence of nuclei led to a subsequent almost twofold increase in the binding of HRC with the nuclei compared with that observed after activation of HRC alone. The acceptor sites of the nucleus, by binding the activated HRC, may perhaps have stabilized them. Binding reached a maximum for the liver after 15-20 min, and subsequent incubation at 20°C actually led to a decrease in binding. Similar data for rat liver have been obtained by other workers [2]. In the case of hepatoma a maximum occurred after 46-60 min, the binding curves flattened out on a plateau, and no decrease was observed. Analysis of temperature activation of hepatoma and liver HRC thus showed that, despite certain differences in the character of the binding curves, activation was an essential stage in interaction of the hormone with the tumor cells, just as with the normal cell.

To determine the physicochemical characteristics of the nuclear acceptor sites translocation of HRC into the nucleus was investigated as described in "Experimental Method." Analysis of the specific binding curves showed that this binding of HRC by nuclei of both types was due to specific acceptor molecules of the same class (Fig. 2).

The apparent K_a for HRC with the acceptor sites of homologous nuclei remained of the same order of magnitude as K_a for dexamethasone with cytosol protein: 0.9×10^8 M^{-1} for nuclei and 3.7×10^8 M^{-1} for cytosol of the liver, 4.7×10^8 M^{-1} for nuclei and 3.9×10^8 M^{-1} for cytosol of the hepatoma. However, the number of acceptor sites determined in the hepatoma nuclei was only one-twentieth (0.15 pmole/mg DNA) of their number in the liver nuclei (3 pmoles/mg DNA) in the case of homologous binding. During heterologous translocation of HRC from liver cytosol into hepatoma nuclei, i.e., during incubation of the liver cytosol with the hepatoma nuclei, the number of acceptor sites determined in the hepatoma nuclei was almost twice (i.e., 5.6 pmoles mg DNA) that observed in the liver nuclei after incubation with the same liver cytosol (3 pmoles/mg DNA; Table 1). Other workers who studied binding of a glucocorticoid-receptor complex by nuclei of hormone-sensitive

TABLE 1. Values of Association Constants and Number of Specifically D-Binding Macromolecules in Cytosol and Nuclei of Hepatoma and Liver Cells during Direct and Crossed Translocation of HRC into Nuclei

Material tested	No. of expt.	$K_a \times 10^8\ M^{-1}$	$n \times 10^{-12}$ mole/mg DNA
Hepatoma cytosol	5	3,9	0,13
Liver cytosol	6	3,7	0,6
		$K_a \times 10^8\ M^{-1}$	$n \times 10^{-12}$ mole/mg DNA
Hepatoma nuclei (after incubation with HRC of hepatoma cytosol)	5	4,7	0,15
Liver nuclei (after incubation with HRC of hepatoma cytosol)	3	4,4	0,16
Hepatoma nuclei (after incubation with HRC of liver cytosol)	3	1,1	5,6
Liver nuclei (after incubation with HRC of liver cytosol)	6	0,9	3,0

*In each experiment 5-7 animals were used.

and hormone-resistant cultures of tumor cells observed a similar picture. In the cytosol of hormone-resistant cells they found no receptors for the synthetic glucocorticoid triamcinolone acetonide. However, nuclei of these cells bound HRC from the cytosol of hormone-dependent cells just as well as homologous nuclei [6].

When HRC from hepatoma cytosol were incubated with liver nuclei the number of acceptor sites in the liver nuclei remained at just as low a level as in the hepatoma nuclei after their incubation with hepatoma cytosol: 0.16 and 0.15 pmoles/mg DNA respectively (Table 1).

It can be concluded from these direct and crossed translocation experiments that hepatoma nuclei themselves are not defective compared with liver nuclei in the number of acceptor sites determined in them. Meanwhile nuclear membranes of the hepatoma evidently present no obstacles to the translocation of HRC into the nucleus.

The following conclusions can thus be drawn from these experiments: 1) the cell cytosol of hormone-resistant Zajdela ascites hepatoma contains glucocorticoid receptors which form a complex with the hormone, although they are between 3 and 5 times more numerous in the normal cell; 2) HRC from hepatoma cytosol, just as in the liver also, requires temperature activation to enable its subsequent translocation into the nucleus in vitro; 3) the number of acceptor sites in hepatoma nuclei is the same as in liver nuclei.

The writers suggest on the basis of these results that in the cytosol of Zajdela ascites hepatoma cells the stage of translocation of HRC into the nucleus is blocked. This view is also shared by Lin and Webb [8], who observed a 75% reduction in binding of the dexamethasone-receptor complex by isolated nuclei from the cells of a Novikoff hepatoma.

LITERATURE CITED

1. L. M. Dmitrieva, A. G. Volchek, V. B. Rozen, et al., Biokhimiya, 41, 1851 (1976).
2. M. Beato and P. Feigelson, J. Biol. Chem., 247, 7890 (1972).
3. K. Burton, Biochem. J., 62, 315 (1956).
4. J. Chauveau, J. Moule, and C. Rouiller, Exp. Cell. Res., 11, 317 (1956).
5. N. Defer, B. Dastugue, J. Kruh, et al., FEBS Lett., 45, 179 (1974).
6. N. W. Garroway, D. N. Orth, and R. W. Harrison, Endocrinology, 98, 1092 (1976).

7. N. M. Kalckar, J. Biol. Chem., 167, 461 (1947).
8. S. L. H. Lin and T. E. Webb, Cancer Res., 37, 1763 (1977).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1975).
10. H. E. Rosental, Analyt. Biochem., 20, 525 (1967).
11. G. Scatchard, Ann. New York Acad. Sci., 51, 660 (1949).

EFFECT OF DL-TRYPTOPHAN ON INDUCTION OF TUMORS BY ACRYLIC PLASTIC

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Plastic disks implanted subcutaneously in rats induced sarcomas in 11 of 27 animals (40.7%) surviving until the appearance of the first tumor (12.3 months). No tumors were found at the sites of implantation of powdered plastic. After prolonged administration of DL-tryptophan tumors developed around the disks in six of 19 rats (31.5%) after 13.8 months and around the powder in two of five rats surviving until 18.7 months. In some animals of this group pre-sarcomatous changes were discovered around concentrations of powder.
KEY WORDS: acrylic plastic; tumor induction; tryptophan; fibrosarcoma.

The view is held [3, 4] that the ability of plastic disks to induce tumors can be explained by their ability to deposit endogenous carcinogenic agents, possibly tryptophan metabolites, on themselves. As confirmation of this hypothesis the results of experiments to study implantation of shredded cellophane, previously incubated with urine of intact rats [1], are cited; in 11 of 31 rats which survived 11.5 months sarcomas developed. Under ordinary conditions shredded cellophane did not induce tumors.

Potentiation of the action of several chemical carcinogens by administration of a "tryptophan diet" has been demonstrated by a number of investigations [8, 9]. The effect of tryptophan on the induction of tumors by polymers has not previously been studied.

The object of this investigation was to study the induction of tumors by plastic implants during loading with DL-tryptophan.

EXPERIMENTAL METHOD

Experiments were carried out on 66 noninbred albino rats reared by the writers, weighing 140-160 g, into which the plastic ethacryl (a copolymer of methyl and ethyl esters of methacrylic acid with the methyl ester of acrylic acid) was implanted under ether anesthesia. The powdered plastic, with a particle size of 20-300 μ, was introduced in a dose of 800 mg beneath the skin of the right flank, and a plastic disk of the same weight, measuring $20 \times 20 \times 1$ mm, was introduced subcutaneously into the left flank. The animals of group 1 (17 females and 17 malles) were given 100 mg DL-tryptophan in 0.5 ml sunflower oil per os through a tube five times a week, starting from the day after the operation until the end of their life. The rats of group 2 (16 females and 16 males) received 0.5 ml sunflower oil with their food five times a week. The animals were kept under observation until they died naturally. The material was treated histologically and paraffin sections were stained with hematoxylin-eosin and with picrofuchsin by Van Gieson's method.

EXPERIMENTAL RESULTS

Changes in the connective tissue around the implanted plastic in the rats of group 2 corresponded to those described by other workers [2, 11]. Around the disks during the first days after the operation a picture of

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