Induction of phenolic ring deiodinase by bovine serum in cultured rat hepatoma cells

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ABSTRACT

Serum induced phenolic ring deiodinase activity in cultured rat hepatoma cells (R117-21B), and this increase of enzyme activity reached a plateau at 12 hr. When the serum concentration in the culture medium was increased to 1.5% from 0.5% where the cells were cultured continuously, a twofold increase in deiodinase activity was observed during 12 hr of pretreatment. This effect reached a plateau above 5.5%. The serum effect on deiodinase activity was slightly different among various serum preparations. Actinomycin D and cycloheximide abolished the induction of the deiodinase activity by serum in rat hepatoma cells.

KEY WORDS thyroid hormone / phenolic ring deiodinase / cell culture / serum effect

A major source of 3,3',5-triiodothyronine (T_3) is the peripheral conversion of thyroxine (T_4) *in vivo* (1-3, 11). Recently, Sorimachi and Robins (15) showed that monkey hepatocarcinoma cells (NCLP-6E) metabolized the thyroid hormones by phenolic and nonphenolic ring deiodination and by sulfation. However, the conversion of T_4 to T_3 was not observed either in the intact cells (15) or in the cell homogenates (16).

We demonstrated that Reuber rat hepatoma cells (R117-21B) metabolized the thyroid hormones by deiodination, by glucuronidation and by sulfation (13) all of which take place commonly *in vivo*, and investigated phenolic ring deiodination in these cell homogenates (12). In this cell line, the conversion of T_4 to T_3 was clearly observed. In the present study, we have used this cell line to investigate whether serum components affect the activity of phenolic ring deiodinase; it is nearly impossible to design this kind of experiments *in vivo* where the tissues are continuously exposed to plasma.

Rat hepatoma cells (R117-21B) were cultured in modified Eagle's minimum essential medium containing 0.5% fetal bovine serum in an atmosphere of 5% CO_2 and 95% air at 37°C (12, 13). After the cells reached confluency, they were pretreated with serum in the presence or absence

of actinomycin D and cycloheximide. The pretreated cells were harvested and then homogenized as reported previously (12). The assay of phenolic ring deiodinase was based on the measurement of the production of $^{125}I^-$ from 3,[3',5'- ^{125}I]triiodothyronine (12); the remaining substrate (rT_3) in the reaction mixtures was removed by ion-exchange resin (AG50W-X4) (16). Labeled iodothyronine (1.1 mCi/ μ g) was purchased from New England Nuclear. The protein concentration of cells was measured by the method of Lowry *et al.* (8).

When rat hepatoma cells (R117-21B) cultured continuously in 0.5% fetal bovine serum were pretreated with 10% calf serum at 37°C, phenolic ring deiodinase activity increased. Fig. 1 shows the relationship between the preincubation period of the cells with serum and the increase of deiodinase activity. The enzyme activity increased gradually until the 12th hour and then reached a plateau at three times the initial value. The increase of the serum concentration to 1.5% raised the deiodinase activity twofold. Higher serum concentration (3.0-5.5%) further increased the activity, and the effect reached a plateau above 5.5%.

A slight difference in the induction of phenolic ring deiodinase activity in the cultured cells was

Fig. 1 Time course of deiodinase activity changes caused by serum. Rat hepatoma cells (R117-21B) were pretreated with 10% serum for stated intervals, and then the cell homogenates (0.24–0.33 mg protein) were incubated with ^{125}I -labeled 3,3',5-triiodothyronine (30 pM) for 1 hr at 37°C. The value is the average of two culture dishes.

observed among various serum preparations (Table 1). However, no clear difference between calf serum and fetal bovine serum was observed, and dialysis of the serum did not show any significant effects on deiodinase activity. On the other hand, heat treatment of serum decreased the serum effect, but a slight induction of deiodinase activity (20–30% of the native serum) still remained. Both insulin and dexamethasone decreased the enzyme activity (to 70–77% of control) after 24 hr of incubation.

The effects of drugs related to protein synthesis on the induction of deiodinase by serum were investigated with actinomycin D or cycloheximide (Table 2). These drugs abolished completely the serum effect at 8 hr, whereas their inhibitory effect was not clear at 4 hr since the serum effect was small.

If we consider that the tissues are exposed to plasma *in vivo*, the serum effect must be involved in controlling the deiodination of the thyroid hormones. The present study clearly revealed that serum increased phenolic ring deiodinase activity in rat hepatoma cells (R117-21B). This serum effect was not due to insulin or glucocorticoid, although both hormones raised the activity of tyrosine aminotransferase (17) and phenylalanine hydroxylase (unpublished data) in this cell line as well as in the parental cell line (6, 10, 14). These two enzymes are induced by serum (4, 5, 14, 18). It seems, therefore, that the induction mechanism of phenolic ring deiodinase is different from that of tyrosine aminotransferase and phenylalanine hydroxylase.

Table 1 Effects of Different Serum Preparations on Deiodinase Activity in Rat Hepatoma Cells (R117-21B)

Serum preparations	Phenolic ring deiodinase activity ($10^{-15}\text{mol } ^{125}\text{I}^-/\text{mg protein/hr}$)
Control	5.3±1.0
Calf serum	
No. 1	14.6±1.2
No. 2	13.8±0.3
No. 3	16.1±1.1
No. 4	16.0±1.4
Fetal bovine serum	
No. 1	14.5±1.5
No. 2	13.7±1.3
Dialyzed bovine serum	13.4±1.5
Boiled serum	
No. 1	7.1±0.2
No. 2	8.0±0.9

The cells cultured continuously in 0.5% fetal bovine serum were pretreated with 10% serum for 24 hr at 37°C. The cell homogenates (0.29–0.42 mg protein) were incubated with 3,3',5'- ^{125}I triiodothyronine (30 pM) for 1 hr at 37°C. The mean±standard deviation is given for 3–6 culture dishes. The serum preparations: calf serum No. 1, Mitsubishi Kasei Institute of Life Sciences; No. 2, Research Institute of Microbiological Diseases, Osaka University Lot. 61011718; No. 3, Grand Island Biological Company Lot. E165319; No. 4, Flow Laboratories Lot. 4020422; fetal bovine serum No. 1, Flow Laboratories Lot. 4055849; No. 2, Flow Laboratories Lot. 4055774; dialyzed bovine serum, Labserum Pty. (Australia); boiled sera No. 1 and No. 2, calf serum No. 1 and fetal bovine serum No. 2 were left in boiling water for 5 min.

Table 2 Inhibitory Effect of Actinomycin D or Cycloheximide on the Induction of Phenolic Ring Deiodinase Activity in Rat Hepatoma Cells (R117-21B) by Serum

	Phenolic ring deiodinase activity (10^{-15} mol 125 I-/mg protein/hr)
Control	4.7 ± 0.9
Actinomycin D	5.5 ± 0.6
Cycloheximide	5.2 ± 1.0
Serum (10%)	9.0 ± 0.6
Actinomycin D	5.2 ± 1.0
Cycloheximide	4.7 ± 1.3

The drug and serum were pretreated simultaneously with the cells for 8 hr at 37°C. The concentrations of actinomycin D and cycloheximide were 7.5 µg/ml and 2.8 µg/ml, respectively, and 10% serum was used. The cell homogenates (0.15–0.40 mg protein) were incubated with 3,[3',5'- 125 I]triiodothyronine (30 pM) for 1 hr at 37°C. The mean $\pm$ standard deviation is given for four culture dishes.

The effect on deiodinase activity in rat hepatoma cells was slightly different among various serum preparations. Even though the difference was very small, this could play an important role in producing T₃ from T₄, since it has been suggested that the peripheral production of T₃ takes place in the liver (7).

It has recently been reported that the preincubation of T₄ with serum, serum albumin or serum γ -globulin accelerated the subsequent reaction which converts T₄ to T₃ in the rat liver homogenates, although no effect was observed when these stimulators, T₄ and the homogenates were incubated simultaneously (9). In our study with intact cells, serum γ -globulin did not induce phenolic ring deiodinase (unpublished data). Our study, which investigated the regulation of the enzyme synthesis, is clearly different from that of Ozawa *et al.* (9), in which the effect of serum and serum proteins on the conversion reaction of T₄ to T₃ was studied. Since actinomycin D and cycloheximide which inhibit RNA synthesis and translation, respectively, inhibited the induction of deiodinase activity by serum, unknown factors in serum might control the deiodinase synthesis.

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