The Effect of X-irradiation on RNA Synthesis during
Tryptophan Pyrrolase Induction in Rat Liver

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ABSTRACT

The effect of X-irradiation on $^{32}$P incorporation into both nuclear and cytoplasmic liver RNA of the adrenalectomized rats treated with single or combined inducer, cortisone and L-tryptophan was studied. The single administration of tryptophan as that of cortisone increased the synthesis of nuclear RNA. The tryptophan-induced increase was significantly suppressed by 500 R whole-body X-irradiation, while the cortisone-induced RNA synthesis was not affected by irradiation. No X-ray-induced inhibition of the RNA synthesis was observed in the case of the combined administration of both cortisone and tryptophan, while combined administration of the inducers produced the most marked acceleration of RNA synthesis.

When chromatographic patterns of specific activity of RNA prepared from irradiated animals were compared to those of unirradiated animals, it was found that the synthesis of RNA fractions eluted beyond the absorbancy peak of r-RNA was significantly suppressed by irradiation in the animals treated singly with tryptophan, and that this suppressed synthesis of RNA was not observed in the cortisone induction and was eliminated by the additional administration of cortisone to the tryptophan-treated animals.

INTRODUCTION

Knox and Auerbach$^1)$ found that there was a rise in the liver tryptophan pyrrolase activity following intraperitoneal administration of either cortisone acetate

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or L-tryptophan into adrenalectomized rats. According to Feigelson et al., the linear rise in tryptophan pyrrolase showed no evidence of a lag phase, and reached maximum values of enzyme levels at 4 to 5 hours after injection.

It was demonstrated in a previous paper that 500 R whole body X-irradiation partially inhibited the increase in holo-tryptophan pyrrolase activity which occurred 4 hours following either cortisone or tryptophan administration, while no inhibition was observed in the increased activity of holo-enzyme following the combined administration of both cortisone and tryptophan in adrenalectomized rats. It was further demonstrated with the aid of activator that radiation produced an inhibitory effect on apo-tryptophan pyrrolase formation itself in only the case of tryptophan-induction, and that this effect of radiation was eliminated by the additional administration of cortisone. It was thought that physiological condition of animals was normalized by added hormone.

According to Knox and Auerbach, the increased tryptophan pyrrolase activity of the animals receiving the administration of tryptophan is analogous to the substrate-induced enzyme adaptations frequently encountered in microorganisms. However, Feigelson and Feigelson indicated that there is no accelerated rate of ribonucleic acid (RNA) turnover in any cell fraction of the tryptophan-induced animal liver. Greengard et al. found that actinomycin D abolished the cortisone-mediated rise in the level of tryptophan pyrrolase but did not influence the tryptophan-mediated increase in the level of tryptophan pyrrolase, suggesting that in the tryptophan-induction much of the RNA species necessary for the synthesis of tryptophan pyrrolase was presumably present in the liver before the accumulation of the enzyme was detectable. Therefore, their findings did not coincide with the implication of RNA in bacterial enzyme induction.

The purpose of the present study is to test: (1) Whether the tryptophan administration cannot increase the synthesis of total RNA fractions of nuclei and cytoplasm of rat liver. (2) If the increased synthesis of total RNA fractions of liver induced with tryptophan is not detected, whether the synthesis of individual RNA species is affected by X-irradiation in the tryptophan induction, since it has been known that the synthesis of RNA is easily affected by the physiological condition of animals.

MATERIALS AND METHODS

Wistar male rats (100-160 g, body weight) were used 4 to 5 days after bilateral adrenalectomy to avoid stress-induced adrenocortical secretion. The rats were given whole body X-irradiation (500 R, 180 kVp, 25 mA, 1.0 mm Cu and 0.5 mm Al filters, 80 cm distance, 16.3 R/min). Immediately after irradiation, both single and combined, intraperitoneal administration of inducers, 1 mg of cortisone acetate and 100 mg of L-tryptophan per 100 g body weight, were carried out. Immediately or 2 hours after the administration of inducers, 20 or 100 g body weight was intraperitoneally administered. The animals treated with
both inducer and \(^{32}\text{P}\) were sacrificed at 4 hours following the administration of inducer. The livers were removed and homogenized in 5 volumes of 1% citric acid. Homogenates were filtered through a flannelette. The filtrates were centrifuged at 700× g for 10 minutes. The supernatants were used as cytoplasmic fraction and the sediments were resuspended in 2 ml of 1% citric acid, followed by centrifugation through 40 ml of 1.5 M sucrose at 12,000× g for 2 hours. The sediments were washed with cold 1% citric acid and used as nuclear fraction. Isolation of RNA from both nuclear and cytoplasmic fractions was carried out by the procedure of Reiner et al.\(^5\). Isolated RNA was dissolved in 0.2 M NaCl-0.05 M phosphate buffered saline (pH 6.7). One part of RNA was diluted with distilled water and absorbancy at 260 m\(\mu\) was read with a spectrophotometer. Another part of RNA was sedimented with 10% trichloroacetic acid to measure radioactivity. The remaining RNA, in some cases, was chromatographed on a methylated bovine serum albumin column prepared by the simplified procedure of Sibatani and Mizuno\(^7\). Elution of RNA from the column was carried out by a linear gradient of 0.6 to 1.5 M NaCl in 0.05 M phosphate buffer (pH 6.7). Absorbancy at 260 m\(\mu\) of collected fractions was measured. The fractionated RNA was sedimented with 10% trichloroacetic acid and hydrolyzed in 0.3 \(N\) KOH. Radioactivity of dried samples on aluminum planchette was measured for 30 minutes with an Aloka low-background Geiger counter LBC-22B.

RESULTS AND DISCUSSION

Table 1 summerizes the effects of inducer and X-irradiation on specific activity (cpm/absorbancy) of both nuclear and cytoplasmic RNA 4 hours following the

<table>
<thead>
<tr>
<th>Inducer</th>
<th>X-rays</th>
<th>Nuclei</th>
<th>Cytoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Change by inducer (S.E.)</td>
<td>% Change by irradiation (S.E.)</td>
</tr>
<tr>
<td>---</td>
<td>0 R</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>500 R</td>
<td></td>
<td>122.7 (22.8)</td>
</tr>
<tr>
<td>Cortisone</td>
<td>0 R</td>
<td>138.6 (17.8)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>200 R</td>
<td></td>
<td>119.2 (19.6)</td>
</tr>
<tr>
<td>L-tryptophan</td>
<td>0 R</td>
<td>136.0 (10.6)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>500 R</td>
<td></td>
<td>72.4 (13.4)</td>
</tr>
<tr>
<td>Cortisone &amp; L-tryptophan</td>
<td>0 R</td>
<td>176.7 (5.4)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>500 R</td>
<td></td>
<td>102.0 (3.6)</td>
</tr>
</tbody>
</table>
single or the combined administration of inducers, cortisone and L-tryptophan.

The values for the specific activity of RNA from unirradiated or irradiated animals showed a wide variation among experiments, despite that the procedures of determination of specific activity were identical. However, it should be emphasised that the effects of inducers and X-irradiation on the specific activity were repeatedly and consistently observed to similar extent in every experiments. Therefore, instead of the value of specific activity, that of percentage changes in the specific activity caused by the inducers and irradiation was reported here.

The value of specific activity of RNA following the induction differs widely among the animals receiving the various treatments with inducers. The most marked percentage increase in the specific activity of RNA is observed, irrespective of nuclear or cytoplasmic fraction, in the animals treated with combined cortisone and tryptophan. In the animals treated with single cortisone, an increased specific activity of RNA is observed irrespective of nuclei or cytoplasm. Otherwise, in the animals treated with single tryptophan, an increasing in specific of activity is observed in nuclear RNA fraction with no effect of inducer in cytoplasmic RNA.

The result obtained by us, where the increased synthesis of nuclear RNA is found in the tryptophan induction, is not in agreement with the findings of Feigelson and Feigelson. However, the observed effect of inducer on the nuclear RNA synthesis in the cortisone induction is in good agreement with the results obtained by Feigelson et al. Moreover, our results observed in the cytoplasmic RNA are in good agreement with the findings of Greengard et al. in that there is a 25% increase of cortisone-induced RNA synthesis in the cytoplasmic fraction, while no increase of cytoplasmic RNA synthesis is observed in the tryptophan-induced animals.

The inhibitory effect of radiation on the synthesis of RNA is observed only in the nuclear RNA fraction of animals treated with single tryptophan. This suppressed synthesis coincides with the changing liver apo-tryptophan pyrrolase levels after irradiation. However, the observed effect of radiation is small, though it is apparently detectable. Therefore, to test an intrinsic effect of irradiation on the RNA synthesis under different condition of induction, comparison was next made on the patterns of specific activity of chromatographically separated RNA fractions from unirradiated and irradiated animals.

We previously indicated that the increase of apo-tryptophan pyrrolase activity occurring from 2 to 4 hours following the single administration of tryptophan, i.e. induction proper as indicated by Feigelson and Greengard, was inhibited by irradiation. Therefore, the comparison of the effect of radiation was made on incorporation into RNA in the second 2 hours of induction and then on the patterns of specific activity of the RNA fractions, which had been chromatographically separated.

The effects of inducer and radiation on the synthesis of RNA in the second 2 hours of induction were essentially the same as the effects on that in 4 hours of
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Fig. 1. Chromatographic patterns of specific activity of nuclear and cytoplasmic RNA labelled for 2 hr starting at 2 hr after administration of inducer with 100 µCi ³²P per 100 g body weight. Specific activities were determined as cpm per absorbancy at 260 mµ in each RNA fraction of control (●-●) and irradiated (○-○) animals. (a), (b) and (c), nuclear RNA; (d), (e) and (f), cytoplasmic RNA of the animals treated with cortisone, tryptophan and both cortisone and tryptophan.

induction as shown in Table 1, with the exception that a slightly decreased synthesis of cytoplasmic RNA in the irradiated animals treated with tryptophan was observed.

Figs. 1(a) to (f) show the comparison of patterns of specific activity in each nuclear (Figs. 1(a) to (c)) and cytoplasmic RNA fraction (Figs. 1(d) to (f)) prepared respectively from the unirradiated and the irradiated animals which were treated with either one or both inducers, cortisone and tryptophan. The effect of irradiation on the patterns of specific activity of nuclear RNA synthesized differs significantly among the animals receiving the various treatments with inducers. Specific
activity of fractionated RNA of irradiated, cortisone-induced animals is significantly smaller in an area between transfer RNA (t-RNA) and ribosomal RNA (r-RNA) and rather greater in the other area of RNA fractions than that of unirradiated, cortisone-induced animals (Fig. 1(a)). In the tryptophan-induction, there is a suppression of specific activity of nuclear RNA fractions in the area from t-RNA to the RNA eluted beyond r-RNA after irradiation (Fig. 1(b)). The patterns of specific activity of nuclear RNA of the animals treated with both inducers are not changed by irradiation (Fig. 1(c)). The results in Fig. 1(a) and Fig. 1(b) suggest that there is a basic difference between the effects of irradiation on the cortisone-induced and the tryptophan-induced RNA synthesis, and that the RNA species necessary for the synthesis of tryptophan pyrrolase are presumably present in the nuclear RNA fractions eluted beyond r-RNA of liver. Fig. 1(c), where the suppression caused by irradiation in the synthesis of these RNA fractions is eliminated by the additional administration of cortisone to the tryptophan-treated animals, suggests that the physiologically noxious condition of the animals treated with tryptophan is normalized by cortisone supply.

Otherwise, in cytoplasmic fractions, insignificant effect of irradiation on the synthesis of RNA eluted beyond r-RNA is observed (Figs. 1(d) to (f)).

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REFERENCES