Effect of Whole-body X-irradiation on the Microsomal Drug-metabolizing Enzyme System in Rat Liver

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(Received, Oct. 1, 1968)

ABSTRACT

Aminopyrine N-demethylase activity in rat hepatic microsomes decreased significantly after whole-body X-irradiation of 500 and 650 R. Process of the phenobarbiturate-induction of aminopyrine N-demethylase was found to be inhibited during quite an early period of the induction. Adrenalectomy caused marked decreases in the enzyme activity and cytochrome P-450 content of hepatic microsomes. Physiological significance of these and other metabolic changes was discussed in relation to the lethality of acutely irradiated animals.

INTRODUCTION

The hepatic drug-metabolizing enzyme system, responsible for the NADPH-dependent oxidative metabolism of many drugs and steroids, seems to play a physiologically important role for detoxication as well as for regulation of the level of active steroid hormones in body fluids. Enzymatic activities for the drug-metabolizing system have been known to be inducible by phenobarbiturate and methylicholanthrene accompanied by the new protein synthesis as an indis-

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pensable process\textsuperscript{4,5}). Moreover, it has been shown that drug-metabolizing enzyme system is under regulatory control of endocrine systems\textsuperscript{6–8}).

Studies on the effects of irradiation on the control system of enzyme synthesis in mammalian cells have presented complicated problems. The hormonal induction of enzyme synthesis was reported to be inhibited by irradiation whereas substrate or dietary induction of several enzymes showed different or no responses\textsuperscript{9,10}).

In the present study, effect of the whole-body X-irradiation on the level and induction of aminopyrine N-demethylase in microsomes of rat liver was examined. In addition, changes in the contents of microsomal cytochromes P-450 and $b_s$ following the irradiation, were determined. Effect of adrenalectomy on the enzyme system was also studied in irradiated animals, since the adrenal function has been shown to be closely correlated with metabolic changes in irradiated animals\textsuperscript{11–13}) as well as with the lethal effect of ionizing radiation\textsuperscript{14,15}).

**MATERIALS AND METHODS**

Six-week-old male Wistar rats were used throughout the present experiments. Adrenalectomy was performed via dorsal route under light ether anesthesia, and the animals were used for the experiment on the fifth day after the operation. All animals were kept in a room of constant temperature (27°C), humidity (60%), and artificial light (12 hours). Food (a standard laboratory chow) and water (for intact rats) or 0.9% NaCl (for adrenalectomized rats) were given ad libitum. Whole-body X-irradiation of 500 R or 650 R in a single exposure was given from a deep therapy X-ray machine operating at 200 kVp and 20 ma, with a 0.5 mm copper plus 0.5 mm aluminum filter, at a dose rate of 20 R/min. For induction of aminopyrine N-demethylase, sodium phenobarbital was administered intraperitoneally, at a dose of 80 mg/kg body weight, at 48 hours prior to sacrifice. To avoid diurnal variation of the drug-metabolizing enzyme activity\textsuperscript{3}), all rats were sacrificed between 8.00 a.m. and 9.00 a.m.

For assay of the aminopyrine N-demethylase activity, livers were homogenized in a glass homogenizer with isotonic KCl containing 10 mM phosphate buffer (pH 7.4). The homogenates were centrifuged at 9,000 x g for 20 minutes, and the supernatant was used for enzyme assay. For determination of cytochromes P-450 and $b_s$, livers were homogenized in isotonic KCl containing 0.1 mM disodium EDTA, and the supernatant after the centrifugation at 9,000 x g for 20 minutes was centrifuged at 105,000 x g for 60 minutes. The resulting microsomal pellet was washed once, and finally resuspended in the isolation medium.

Aminopyrine N-demethylase activity was assayed at 37°C in the incubation system which contained, besides the 9,000 x g supernatant, 10 μmoles of aminopyrine, 0.6 μmoles of NADP, 25 μmoles of glucose 6-phosphate, 25 μmoles of MgSO$_4$, 20 μmoles of semicarbazide, and 25 mM potassium phosphate buffer (pH 7.4) in a total volume of 2.0 ml. Formaldehyde formed was determined by a modified method of Nash\textsuperscript{16}), and the results are expressed as milli-units (mU) per g wet
weight, one milli-unit being defined as the amount of enzyme that yielded one m\(_\mu\)mole of formaldehyde per minute. Contents of cytochromes P-450 and b\(_5\) in the microsomal fraction were determined by the method of Omura and Sato\(^{17}\), and the results are expressed as m\(_\mu\)moles per g wet weight.

RESULTS

The aminopyrine N-demethylase activity in hepatic microsomes decreased after whole-body X-irradiation of 650 R, and reached the minimal level (55\% of that in intact control) on the fifth day postirradiation, then a tendency of recovery in the activity towards the control level was observed with time elapsed (Fig. 1).

In Table 1 are summarized the data on assay of the enzyme activity and cytochrome contents at the fifth day after whole-body X-irradiation in intact and adrenalectomized rats with or without phenobarbiturate injection. Whole-body X-irradiation inhibited, even at a dose of 500 R, the aminopyrine N-demethylase activity in intact rats. Lower enzyme activities were also observed in irradiated, phenobarbiturate-injected rats as compared to the enzyme level of rats treated with phenobarbiturate alone.

However, if the size of inducible fraction (enzyme activity of phenobarbiturate-injected rats minus that of the corresponding control ones) is compared between the intact, unirradiated rat-groups and irradiated ones, there is no significant difference observed (compare values in the parentheses shown in the second column of Table 1).

Adrenalectomy caused marked decrease in the enzyme activity. However, it should be noted that there was no further decrease in enzyme activity caused by irradiation. Size of the phenobarbiturate-inducible fraction of enzyme activity in adrenalectomized rats was somewhat smaller than that in intact rats, but it was not further influenced by irradiation.

Whole-body X-irradiation alone caused little change in contents of cytochrome P-450 in intact rats and in adrenalectomized ones, although this cytochrome is an indispensable component of the aminopyrine N-demethy-lase activity. However, it
was found that induction of this cytochrome by phenobarbiturate was greatly inhibited by whole-body X-irradiation in intact rats but not in adrenalectomized ones (compare values in the parentheses shown in the third column of Table 1).

With respect to cytochrome \( b_s \), there was no significant change by whole-body X-irradiation in both intact rats and adrenalectomized ones. Phenobarbiturate-injection increased the cytochrome \( b_s \) content only slightly.

It has been reported that process of the induction of rat-liver serine dehydrase by administration of an inducer (casein hydrolysate) was initially inhibited by irradiation, becoming resistant at later periods \(^{10}\). In view of these experimental

**Table 1.** Effects of whole-body X-irradiation on the aminopyrine N-demethylase activity and cytochrome contents in hepatic microsomes of intact and adrenalectomized rats.

<table>
<thead>
<tr>
<th>Experimental rat group</th>
<th>Aminopyrine N-demethylase activity (mU/g wet weight)</th>
<th>Cytochrome P-450 (mumoles/g wet weight)</th>
<th>Cytochrome ( b_s ) (mumoles/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (10)</td>
<td>135±6.0 (136)</td>
<td>13.6±0.39</td>
<td>7.5±0.44</td>
</tr>
<tr>
<td>induced (5)</td>
<td>271±9.0</td>
<td>30.2±1.18 (16.6)</td>
<td>8.2±0.51</td>
</tr>
<tr>
<td>500 R (5)</td>
<td>93±8.0</td>
<td>10.3±0.95</td>
<td>6.7±0.24</td>
</tr>
<tr>
<td>650 R (5)</td>
<td>76±7.0</td>
<td>13.6±0.39</td>
<td>8.9±0.34</td>
</tr>
<tr>
<td>500 R, induced (5)</td>
<td>232±10.0 (139)</td>
<td>20.4±1.12 (10.1)</td>
<td>7.9±0.19</td>
</tr>
<tr>
<td>650 R, induced (5)</td>
<td>202±11.5 (126)</td>
<td>18.8±1.26 (5.2)</td>
<td>8.3±0.44</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (5)</td>
<td>85±4.5</td>
<td>7.6±0.32 (99)</td>
<td>5.6±0.21</td>
</tr>
<tr>
<td>induced (10)</td>
<td>184±7.0 (99)</td>
<td>17.5±1.95 (9.9)</td>
<td>7.3±0.51</td>
</tr>
<tr>
<td>500 R (4)</td>
<td>96±11.0</td>
<td>7.2±1.20</td>
<td>9.0±0.92</td>
</tr>
<tr>
<td>500 R, induced (5)</td>
<td>182±8.0 (86)</td>
<td>18.1±1.95 (10.9)</td>
<td>7.2±0.24</td>
</tr>
</tbody>
</table>

Values are the mean±standard error of mean. Numbers of experimental rats are shown in the parentheses. Aminopyrine N-demethylase activity and cytochrome P-450 content shown in the parentheses indicate the size of phenobarbiturate-induced fraction (see text). Irradiated and induced animals received phenobarbiturate injection on the third day post-irradiation, and were sacrificed 48 hours later.

**Table 2.** Effect of whole-body X-irradiation on the induction of aminopyrine N-demethylase by phenobarbiturate injection.

<table>
<thead>
<tr>
<th>Hours between phenobarbiturate injection and irradiation</th>
<th>Aminopyrine N-demethylase activity (mU/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induced, unirradiated (9)</td>
<td>266±10.0</td>
</tr>
<tr>
<td>0 (6)</td>
<td>195±11.5</td>
</tr>
<tr>
<td>3 (6)</td>
<td>275±7.0</td>
</tr>
<tr>
<td>12 (6)</td>
<td>268±7.5</td>
</tr>
</tbody>
</table>
results, the effects of whole-body X-irradiation were examined during an early period of aminopyrine N-demethylase induction (Table 2). Whole-body X-irradiation of 650 R given immediately after the administration of phenobarbiturate depressed the rate of enzyme induction by approximately 30% of that in unirradiated, phenobarbiturate-induced controls. However, irradiation did not cause any change in the rate of enzyme induction when given to the animals later than 3 hours after phenobarbiturate injection.

**DISCUSSION**

Present results clearly demonstrated that the aminopyrine N-demethylase activity of hepatic microsomes is considerably reduced after whole-body X-irradiation. Moreover, it was shown that process of the induction of aminopyrine N-demethylase by phenobarbiturate is also impaired by irradiation.

Kato et al.\(^4\) have reported that there was no significant effect of whole-body X-irradiation on the *in vitro* metabolism of pentobarbiturate and meprobamate in hepatic microsomes of the rat. These authors determined the enzymatic activities only at 48 hours after irradiation, and their results may not be in conflict with the present ones, since a marked depression of the aminopyrine N-demethylase activity was observed during the periods later than 48 hours postirradiation. Moreover, it is possible that the enzyme systems responsible for the metabolism of pentobarbiturate and meprobamate are not influenced by whole-body X-irradiation, since several different enzymatic systems have been reported to participate in the hepatic drug metabolism\(^18,19\).

Induction of drug-metabolizing enzymes by phenobarbiturate involves a new protein synthesis as an indispensable process\(^5\). Pitot et al.\(^20\) have demonstrated that serine dehydroase induction was very sensitive to whole-body X-irradiation as well as to actinomycin D only during an early period of induction but it became completely resistant at 6- and 12-hour after the induction. The present results are perfectly compatible with these observations (cf. Table 2). Therefore it can be concluded that one or more steps in the overall process of aminopyrine N-demethylase induction are inhibited by whole-body X-irradiation.

Death of irradiated animals, if occurred within a week or so after whole-body X-irradiation, has been often referred to as "gastrointestinal death." However, severe defects in metabolism and hormonal environment in the liver and in tissues other than intestine, have been observed in the present study as well as in other ones, as reported from the present authors' laboratory\(^11-13\). Also, it should be recalled here that all of the adrenalectomized animals, but none of the intact control ones, died within a period of 5 days after 650 R\(^12\). These evidences strongly support the view that cause of death during an early postirradiation period can not be attributable only to the gastrointestinal damage.
REFERENCES


