Dietary Induction of Some Enzymes of Amino Acid Metabolism following the Acute Administration of Aminoazo Dyes

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SUMMARY

The dietary responses of 5 enzymes: serine dehydratase [l-serine hydrolase (deaminating), EC 4.2.1.13], histidase (L-histidine ammonia-lyase, EC 4.3.1.3), ornithine-δ-transaminase (L-ornithine:2-oxoacid amino transferase, EC 2.6.1.13), tryptophan pyrrolase (L-tryptophan:oxygen oxidoreductase, EC 1.13.1.12), and tyrosine-α-ketoglutarate transaminase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) were investigated in the livers of rats given injections i.p. of the hepatocarcinogen 3'-methyl-4-dimethylaminoazobenzene, its noncarcinogenic isomer 2-methyl-4-dimethylaminoazobenzene, or the activated carcinogen N-benzoyloxy-4-monomethylaminoazobenzene. The responses were compared to the corresponding responses in control rats. Induction was effected by the forced feeding of casein hydrolysate to protein-depleted animals. The administration of each of the compounds 3 hr prior to the intubation of casein hydrolysate completely inhibited the dietary response of histidase and partially repressed that of serine dehydratase. The metabolic response of ornithine-δ-transaminase was partially repressed by the acute administration of 3'-methyl-4-dimethylaminoazobenzene and 2-methyl-4-dimethylaminoazobenzene, but not by N-benzoyloxy-MAB. Tryptosine-α-ketoglutarate transaminase induction presented an anomalous situation; the injection of 3'-methyl- or 2-methyl-4-dimethylaminoazobenzene 3 hr before enzyme induction had no statistically significant effect upon the induced enzyme level, while N-benzoyloxy-4-monomethylaminoazobenzene injected at the same time significantly increased the induced enzyme level. A complete or partial inhibition by 3'-methyl-4-monomethylaminoazobenzene of the induction of serine dehydratase, histidase, and ornithine-δ-transaminase was observed following injection of the dye 16 or 3 hr before, at the same time as, or 3 and 8 hr after the initial intubation of casein hydrolysate. The injection of the dye 16 hr before the start of enzyme induction appeared to inhibit the induction of tyrosine-α-ketoglutarate transaminase; at no time did any of the dyes investigated have a significant effect on the adaptive response of tryptophan pyrrolase. A comparison of these results with the known times of template stability for each of these enzymes suggests that the 3'-methyl-4-monomethylaminoazobenzene exerts its effect during the translational period of enzyme synthesis.

INTRODUCTION

Previous experiments have amply demonstrated the presence of altered enzyme control mechanisms in the livers of rats chronically fed various hepatocarcinogens (1, 3, 6, 7, 9, 17—20). In general, these alterations have consisted of losses or decreases in the adaptive responses of specific enzymes and bear a close resemblance to the altered responses of hepatomas. Relatively few studies, however, have been performed investigating the acute effects of carcinogen administration on enzyme induction and subsequent correlation of these findings with those seen after chronic feeding of these compounds. To date, experiments designed to study modifications of cellular adaptation by hepatocarcinogens have tended to emphasize the actinomycin D-like effects of the carcinogens. Thus, the inhibition by aflatoxin of the hydrocortisone induction of hepatic tryptophan pyrrolase and tyrosine transaminase (24), or RNA polymerase in vitro (5), and of RNA synthesis in vivo (2, 23) were all found to be produced in a manner similar to the action of actinomycin D. Also consistent with an actinomycin D-like role by hepatocarcinogens was the observation that the administration of the hepatocarcinogens aflatoxin B1, N-hydroxy-2-acetylaminofluorene, and 3'-methyl-DAB led to marked losses in the nuclear RNA content of rat liver (22). More recent evidence, however, has shown that such losses could not be observed in the livers of rats fed the potent hepatocarcinogen 4'-fluoro-4-dimethylaminoazobenzene (8). The present experiments compare the altered adaptive responses of 5 amino acid-metabolizing enzymes following the acute administration of the hepatocarcinogen 3'-methyl-DAB and its noncarcinogenic isomer 2-methyl-DAB, with alterations previously observed during the chronic feeding of these 2 compounds (18) and provide evidence that such effects may not be directly attributable to an actinomycin D-like action of the carcinogen.

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MATERIALS AND METHODS

Young, adult male rats (170 to 190 g) (Holtzman Rat Co., Madison, Wis.) were used in these experiments. They were housed in Wahman galvanized wire-mesh cages and given food and water ad libitum. Upon arrival at this laboratory, the rats were placed on a semisynthetic 0% protein diet for 5 days to lower the hepatic levels of the amino acid-metabolizing enzymes. The rats were then fasted overnight for the period immediately preceding the dietary induction. At 0 time (6:00 a.m.), 4 to 6 rats from the control group were killed by decapitation; their livers were quickly removed and homogenized in 4 volumes of 0.2 M KCl containing 0.1 M Tris buffer (pH 8.2), 10⁻³ M EDTA, and 10⁻⁴ M dithiothreitol with a Polytron homogenizer. Approximately 3 ml of each homogenate were collected and stored at −70° for the later assay of serine dehydratase, histidase, ornithine transaminase, tryptophan pyrrolase, and tyrosine transaminase. The remaining control and experimental rats were intubated by stomach tube with a dose of a 33% suspension of enzymatically hydrolyzed casein (2 ml/100 g body weight); their livers were quickly removed and homogenized in 4 volumes of 0.2 M KCl containing 0.1 M Tris buffer (pH 8.2), 10⁻³ M EDTA, and 10⁻⁴ M dithiothreitol with a Polytron homogenizer. Approximately 3 ml of each homogenate were stored at −70° for the later assay of ornithine transaminase. Ten-mi portions of each homogenate were centrifuged at 100,000 × g for 1 hr, and the individual clear supernatants were collected and stored at −70° for the later assay of serine dehydratase, tyrosine transaminase, tryptophan pyrrolase, and histidase. The remaining control and experimental rats were intubated by stomach tube with a dose of a 33% suspension of enzymatically hydrolyzed casein (2 ml/100 g body weight); the intubations were repeated 6 and 12 hr later. Groups of 4 to 6 rats were sacrificed at 6, 12, and 18 hr, and their livers were treated as above. The experimental rats were given injections i.p. of 2.0 ml of corn oil containing 5 to 50 mg of 3'-methyl-DAB (Eastman Chemicals, Rochester, N. Y.), 50 mg of 2-methyl-DAB (m.p., 67–68°) (10), or 2.0 mg of the highly toxic N-benzyloxy-MAB (m.p., 89–91°) (16) at times varying from 72 hr before the 1st intubation of casein hydrolysate (−72 hr) to 8 hr after the 1st intubation (+8 hr). Whenever an aminoazo dye was injected, 2 ml of corn oil without dye was similarly injected into control rats and both groups were treated as above. Since the time of corn oil administration exerted no effect on the induction of any of the enzymes tested, the results from all the control groups were pooled for Table 1. Since the injection of rats at +8 hr, i.e., 8 hr following the 1st intubation of casein hydrolysate, might not have permitted sufficient time to elapse to show altered induction in rats killed at +18 hr, additional control and experimental groups were added to this series. These latter groups received an extra intubation of casein hydrolysate at +18 hr and were sacrificed at +24 hr. In 1 experiment, the inhibitory effects of the aminoazo dyes on the tryptophan induction of the amino acid-metabolizing enzymes were studied. Groups of rats were given injections of 2 ml of corn oil containing 25 mg of 3'-methyl-DAB, 25 mg of 2-methyl-DAB, or no dye 15 hr prior to the p.o. intubation of 80 mg of tryptophan in 4.0 ml of distilled water at 0 time (6:00 a.m.); the intubations were repeated at 6 and 12 hr. Groups of 4 to 5 control rats were killed at 0, 6, 12, and 18 hr; similar groups of experimental rats were killed at 6, 12, and 18 hr. All subsequent operations were carried out as described above.

Enzyme Assays. Histidase (14), serine dehydratase (14), ornithine transaminase (11), tyrosine transaminase (15), and tryptophan pyrrolase (17) were determined by previously described procedures, and their activities were expressed as @ moles product produced/g liver/hr ± S.E. During the course of these experiments, the reaction rates of each of all these enzymes except ornithine transaminase were digitized (15) by linking a digital readout to the automated combination unit previously described (14). Serine dehydratase and tyrosine transaminase determinations were found to be unaffected by this improvement, but histidase and tryptophan pyrrolase assays were found to give slightly, but significantly, elevated values. In Table 1, as well as in the text, the base level indicates a 0 time hepatic enzyme level; the induced level refers to the hepatic enzyme level 6 hr after the start of casein hydrolysate intubation.

RESULTS

The adaptive responses of serine dehydratase, histidase, and ornithine transaminase normally seen in the livers of rats given

<table>
<thead>
<tr>
<th>Enzyme level</th>
<th>Compound injected</th>
<th>Dose (mg)</th>
<th>Serine dehydratase</th>
<th>Histidase</th>
<th>Ornithine transaminase</th>
<th>Tryptophan pyrrolase</th>
<th>Tyrosine transaminase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>None</td>
<td></td>
<td>152 ± 27b (4)</td>
<td>15.5 ± 0.8 (5)</td>
<td>15.8 ± 2.4 (5)</td>
<td>13.3 ± 2.2 (5)</td>
<td>87 ± 20 (5)</td>
</tr>
<tr>
<td>Induced</td>
<td>None</td>
<td></td>
<td>503 ± 117 (5)</td>
<td>35.6 ± 2.7 (5)</td>
<td>34.0 ± 6.7 (5)</td>
<td>37.5 ± 4.3 (5)</td>
<td>187 ± 54 (5)</td>
</tr>
<tr>
<td>Induced</td>
<td>3'-Methyl-DAB</td>
<td>25</td>
<td>91 ± 30 (5)</td>
<td>13.9 ± 1.1 (5)</td>
<td>11.1 ± 0.4 (5)</td>
<td>36.4 ± 6.0 (3)</td>
<td>243 ± 14 (5)</td>
</tr>
<tr>
<td>Induced</td>
<td>2-Methyl-DAB</td>
<td>25</td>
<td>159 ± 48 (5)</td>
<td>13.7 ± 0.4 (5)</td>
<td>13.6 ± 2.9 (5)</td>
<td>282 ± 28 (5)</td>
<td></td>
</tr>
</tbody>
</table>

The base level indicates the 0 time enzyme level; the induced level indicates the hepatic enzyme level of serine dehydratase, histidase, ornithine transaminase, and tryptophan pyrrolase 18 hr after the initial intubation of casein hydrolysate and the hepatic level of tyrosine transaminase 6 hr after the initial intubation of casein hydrolysate.

Enzyme activities are expressed as @ moles product formed/g liver/hr ± S.E.; numbers in parentheses, number of rats/group.
multiple doses of casein hydrolysate appeared to be significantly diminished following injection with 50 mg of the hepatocarcinogen 3'-methyl-DAB or its noncarcinogenic isomer 2-methyl-DAB (Charts 1, 2, and 3). Although some response by ornithine transaminase and serine dehydratase was found in rats given injections of either aminoazo dye, that of histidase was completely lost. The i.p. injection of 2.0 mg of the activated carcinogen N-benzoyloxy-MAB at 3 hr led to a complete suppression of the histidase induction (Chart 2); this compound also appeared to repress partially the adaptive response of serine dehydratase, but had no effect on the dietary induction of ornithine transaminase (Charts 1 and 3). Except for serine dehydratase, no significant differences could be observed in the extent of inhibition of the adaptive responses produced by 3'-methyl- or 2-methyl-DAB. Following injection at —3 hr, none of the 3 dyes investigated appeared to exert a repressive effect on the metabolic response of tryptophan pyrrolase or tyrosine transaminase (Charts 4 and 5); indeed, each of the dyes seems to increase the adaptive response of tyrosine transaminase to casein hydrolysate, with 2.0 mg of N-benzoyloxy-MAB exerting a significantly greater effect than 50 mg of either 3'-methyl- or 2-methyl-DAB. Investigations on the effects of the dose of 3'-methyl-DAB on the dietary induction of each of the 5 enzymes showed that 25 mg of dye administered 3 hr prior to the 1st intubation of casein hydrolysate was just as effective as 50 mg in suppressing the induction of serine dehydratase, histidase, and ornithine transaminase. A dose of 15 mg partially inhibited serine dehydratase induction, but it had no effect on the responses of histidase or ornithine transaminase. Doses of 5 and 10 mg were without effect on the induction of serine dehydratase, histidase, and ornithine transaminase. None of the doses of 3'-

Chart 1. The effects of acute doses of 3'-methyl-DAB, 2-methyl-DAB, and N-benzoyloxy-MAB on the dietary induction of rat liver serine dehydratase. The animals were given injections of 50 mg of 3'-methyl-DAB, 50 mg of 2-methyl-DAB, or 2.0 mg of N-benzoyloxy-MAB 3 hr prior to enzyme induction. Induction was effected by multiple intubations of casein hydrolysate over an 18-hr period, as described in "Materials and Methods." Points, average of enzyme assays performed on the livers of 4 to 6 rats. Vertical lines, S.E.

Chart 2. The effects of acute doses of 3'-methyl-DAB, 2-methyl-DAB, and N-benzoyloxy-MAB on the dietary induction of rat liver histidase. The format and experimental details are identical to those in Chart 1.

Chart 3. The effects of acute doses of 3'-methyl-DAB, 2-methyl-DAB, and N-benzoyloxy-MAB on the dietary induction of rat liver ornithine-6-transaminase. The format and experimental details are identical to those in Chart 1.
methyl-DAB tested had any effect on the adaptive response of tryptophan pyrrolase. Similarly, 25- or 50-mg doses of 3'-methyl-DAB had no effect on the induction of tyrosine transaminase, while the lower doses of 5, 10, or 15 mg appeared to increase the adaptive response of this enzyme slightly.

Previous studies from this laboratory have indicated the existence of a time-dependent sensitivity of enzyme induction to acute doses of actinomycin D (13). When 25 mg of 3'-methyl-DAB were administered 16 hr before the initial dose of casein hydrolysate, a complete loss of the metabolic responses of serine dehydratase, histidase, ornithine transaminase, and tyrosine transaminase occurred. It would seem that such an early administration of aminoazo dye permitted greater derangement of the adaptive machinery of the liver, since injection of 3'-methyl-DAB at −3, 0, or +3 hr had no effect on the response of tyrosine transaminase, and it only partially repressed that of serine dehydratase and ornithine transaminase. In addition, the hepatic level of ornithine transaminase in rats given injections at −16 hr was found to diminish during the 18-hr induction period. The injection of 3'-methyl-DAB at the same time as or 3 hr after the initial intubation of casein hydrolysate led to only a decrease, and not a loss, of the adaptive response of histidase. When 3'-methyl-DAB was injected at +8 hr, the 18-hr and 24-hr levels of histidase and ornithine transaminase appeared to be depressed when compared to the corresponding levels in control liver; similarly, the 18-hr level of serine dehydratase was significantly lower ($p < 0.01$) in the livers of rats given injections of dye at +8 hr than in the control animals. At no time investigated did the acute injection of 3'-methyl-DAB appear to have any effect on the adaptive response of tryptophan pyrrolase. Following the injection of 2.0 mg of N-benzoyloxy-MAB 72 hr prior to the start of enzyme induction, the induced levels of serine dehydratase, histidase, ornithine transaminase, tryptophan pyrrolase, and tyrosine transaminase were found to be $1204 \pm 126$, $30.9 \pm 2.2$, $73.0 \pm 6.1$, $19.6 \pm 3.6$, and $682 \pm 105$, respectively, indicating that no permanent alteration in the metabolic responses of these enzymes had been produced by this regimen. Both 3'-methyl-DAB and 2-methyl-DAB administered at −15 hr completely suppressed the tryptophan induction of serine dehydratase, histidase, and ornithine transaminase (Table 1). As usual, the metabolic response of tryptophan pyrrolase appeared to be unaffected by the injection of 3'-methyl-DAB. Tyrosine transaminase appeared to show only a slight response to the intubation of tryptophan ($p = 0.13$), while this response was highly significant ($p < 0.01$, $< 0.01$) in the livers of rats given injections of tryptophan and 25 mg of 3'-methyl- or 2-methyl-DAB, respectively.

**DISCUSSION**

Two major conclusions may be drawn from these experiments: (a) acute injections of aminoazo dyes exert different effects on enzyme induction than those seen after chronic administration of the same compounds (17–20), and (b) on the basis of the results of earlier studies (13), acute injections of azo dyes appear to affect protein synthesis at the translational level. The differences between the acute and chronic modes of administration of azo dye were seen in the inductions of serine dehydratase, tyrosine and ornithine transaminases. The response of serine dehydratase, which is not
changed by the chronic administration of 3′-methyl-DAB (18), is totally lost in the livers of rats given injections of this dye 16 hr prior to enzyme induction and is significantly diminished in the livers of rats given injections 3 hr before, at the same time as, or 3 and 8 hr after the 1st intubation of casein hydrolysate. Similarly, tyrosine transaminase levels after dietary alteration remained normal in rats fed 3′-methyl-DAB for 5 weeks (18) but were lost in rats given injection of dye 16 hr before enzyme induction. The casein-induced increase in ornithine transaminase was completely lost, both in the livers of rats chronically fed 3′-methyl-DAB and in the livers of rats given injections 16 hr before enzyme induction; however, later injections of 3′-methyl-DAB only diminished the final level of enzyme induction. The response of ornithine transaminase to the acute and chronic modes of dye administration. Histidase and livers of rats chronically fed 3′-methyl-DAB; the response of histidase was completely lost in the rats given injections 16 or 3 hr before induction; that of tryptophan pyrrolase remained unaffected. A comparison of the effects of 3′-methyl-DAB and 2-methyl-DAB on the adaptive increases of the amino acid-metabolizing enzymes demonstrated even further differences between the acute and chronic modes of dye administration. During chronic feeding, the carcinogen 3′-methyl-DAB exerted a more dramatic inhibition of enzyme adaptation than did the noncarcinogen 2-methyl-DAB (18), following acute administration; however, both compounds produced essentially the same effects.

Previous studies have tended to emphasize an actinomycin D-like effect of various carcinogens (21, 24), i.e., the carcinogens appeared to act at the transcriptional rather than at the translational level of enzyme synthesis. The present studies suggest an interference by 3′-methyl-DAB, and perhaps 2-methyl-DAB, on the translation of the enzyme-coding message. With ornithine transaminase and serine dehydratase, the observed induced level following dye injection was always equal to the normal induced level expected about 6 hr following dye administration. It thus appears that about 6 hr was required for sufficient dye to be absorbed and “activated” to exert an inhibitory effect on enzyme induction. However, this effect by 3′-methyl-DAB was noted even at times (+3 and +8 hr) when the templates synthesizing serine dehydratase and ornithine transaminase were presumably stable (13), i.e., the induction of these enzymes was insensitive to actinomycin D. Similarly, injections of 3′-methyl-DAB at −3 hr still gave only a partial repression of these 2 enzymes, similar to that observed following injection at +3 and +8 hr. Previous experiments, however, had established that from 0–3 hr the induction of serine dehydratase and ornithine transaminase was sensitive to actinomycin D. Thus, it would appear that, at least in the present experiments, the inhibition of enzyme induction by the acute administration of 3′-methyl-DAB resembles that of puromycin more than that of actinomycin D. Such a puromycin-like effect would, of course, mask a potential actinomycin D-like role of the carcinogen. One similarity to actinomycin D noted in the present investigations was the “paradoxical” induction of tyrosine transaminase, previously reported by Garren et al. (4) with actinomycin D following the injection of N-benzoyloxy-MAB 3 hr before the start of enzyme induction.

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REFERENCES

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