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CHLOROFORM INDUCTION OF ORNITHINE DECARBOXYLASE ANTIZYME (ODC–AZ) IN MALE RAT LIVER

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Chloroform stimulation of rat hepatic ODC is most dramatic at 18 h following a single injection. Repeated dosing, 1 dose/d for up to 7 d, results in a daily decline in the ability of the liver enzyme to respond 18 h after the final injection. We postulated that this decline was due to an increased synthesis and accumulation of the OCD-AZ protein. ODC-AZ was determined by measuring the inhibition of isolated ODC activity as described by Hayashi and Fujita and modified in our laboratory to use kidney ODC. Male and female Fischer 344 rats were injected daily for 1, 3, or 7 d with 3.0 mmol/kg chloroform. Chloroform induced ODC-AZ activity in males at 3 and 7 d (26% and 37% inhibition of the ODC activity in the incubation medium, respectively). While females exhibited a similar decline in ODC activity after repeated doses, ODC-AZ was not induced. Thus, it would appear that daily exposure of rats to chloroform results in a refractoriness of its induction of ODC activity accompanied by an induction of the ODC-AZ in males. However, in females these two responses were not directly related.

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INTRODUCTION

Several years ago while testing the hypothesis that the induction of ornithine decarboxylase might serve as a molecular marker for tumor promoters, we demonstrated that chloroform was a very potent stimulator of the rat hepatic enzyme (Savage et al., 1982). However, chloroform is not a male rat hepatic tumor promoter. When it was later suggested (Olsen and Russell, 1980) that sustained induction of ornithine decarboxylase was a requirement for tumor promotion, we investigated the response of the rat liver enzyme to chloroform daily dosing (Savage et al., 1982). The maximum response was brought about by a single dose of chloroform. Repeated dosing, namely, one dose per day for a period of up to 7 d, resulted in a daily decline in the ability of the male liver enzyme to respond to the last chloroform dose in the daily dosing regimen as dramatically as in the single-dose routine. This apparent inability to respond as dramatically to a second dose has been demonstrated by other investigators using different chemicals and different tissues (Madhubala and Reddy, 1985) and (Takigawa et al., 1986). Several mechanisms might account for such a "refractory" period. In 1985, Russell reported that phosphorylation, transamidation, inhibition of ODC protein synthesis, or the elaboration of the ODC antizyme might all be potential candidates for decreasing ODC activity. An inverse relationship between ODC and the ODC-AZ had been reported in azo-dye hepatocarcinogenesis (Modena et al., 1983) and the maturation of mouse brain (Laitinen, 1985). Our interests in hepatocarcinogenesis coupled with the chloroform studies on ODC led us to investigate the effects of chloroform on ODC-AZ. Since we have previously established the ODC refractory phenomena (Savage et al., 1982) in male rats with chloroform, the intent of this study was to repeat those experiments in female rats and to determine if the ODC-AZ was present in either sex during the chloroform-induced refractory period.

MATERIALS AND METHODS

Chemicals

Glass-distilled chloroform (without preservative) was purchased from Burdic and Jackson Laboratories (Muskegon, Mich.) and DL-[1-^{14}C]-ornithine monohydrochloride (51.3 mCi/mol) from New England Nuclear (Boston, Mass). DEAE-Cellulose was obtained from Sigma Chemical Company (St. Louis, Mo.), as were other routine biochemicals required for the ODC and ODC-AZ assays.

Treatment of Rats

Male and female Charles River CDF Fischer 344 rats were purchased from Charles River Breeding Laboratories, Inc., Kingston, N.Y. Rats used for experimentation were 10–12 wk old and weighted between 175 and 225 g. All rats were housed in a certified animal facility featuring constant temperature, constant humidity, and 12-h light-dark cycles. Chloroform was administered undiluted by intraperitoneal injection. All rats were injected at 3:00 p.m. and rats were killed at 9:00 a.m. by decapitation.

Preparation and Assay of ODC and ODC-AZ

The ODC activity of a soluble (100,000 × g postmicrosomal supernatant) tissue preparation was determined by measuring the release of $^{14}CO_2$ from DL-[1-^{14}C]-ornithine hydrochloride as described by Hayashi and Fujita (1983). Antizyme was partially purified by DEAE-cellulose chromatography and Sephadex G-75 gel filtration from livers of rats injected intraperitoneally with saline (control), putrescine (positive control), or chloroform (test compound) for varying periods of time as described by Hayashi and Fujita (1983). ODC-AZ activity was determined by measuring in vitro the inhibition of a previously established amount of ODC activity, as described by Hayashi and Fujita (1983) and modified in our laboratory to utilize renal ODC as the enzyme source. Protein determined by Bradford (1976).

RESULTS

Effects of Daily Chloroform Treatment on Rat Hepatic Ornithine Decarboxylase Activity

We have previously demonstrated that the chloroform stimulation of male rat hepatic ODC is most dramatic at 18 h following a single injection. Repeated dosing, at 1 dose/d for up to 7 d, resulted in a daily decline in the ability of the male rat liver enzyme to respond 18 h after the final injection (Savage et al., 1982). The same refractory period prevails when female rats are similarly treated and compared with male rats (data now shown).

Presence of Inhibitory Activity to ODC in Livers of Chloroform-Treated Rats

Dialyzed unfractionated cytosolic preparations of livers from chloroform-treated male and female rats showed no evidence of ODC inhibitory activity. Cytosols were fractionated by DEAE-cellulose chromatography, and fractions from chloroform-treated animals corresponding to ODC-AZ active fractions from the positive control (putrescine-treated) were examined for ODC-AZ activity. Figure 1 illustrates that chloroform treatment resulted in an increase in ODC inhibitory activity in fractions

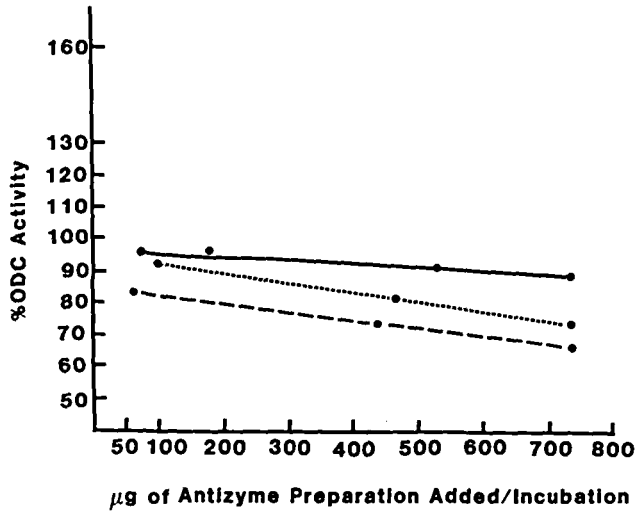

FIGURE 1. Effects of altering protein concentration of ODC-AZ preparation from male rats on ODC activity in vitro. Following DEAE-cellulose chromatography, varying amounts of partially purified ODC-AZ protein derived from pooled male rat livers: control (——), 3 d chloroform (–), and 7 d chloroform (– – –) were added to incubation vessels containing a known amount of ODC activity. Values are means of triplicate determinations (error less than 10%) with pooled ODC-AZ preparations from six male animals at each treatment time. All data points (control, 3 d chloroform, and 7 d chloroform) were statistically independent ($p < 0.05$) when analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test. However, similarly treated female animals produced ODC-AZ activities that exhibited no statistical differences among the three (female control, 3 d chloroform, and 7 d chloroform) groups.

derived from male rat livers only. Similarly prepared fractions from chloroform-treated female rat livers exhibited patterns that paralleled control (saline) animals. Figure 1 further demonstrates that the ODC-AZ activity is increased when increasing amounts of protein are added to the incubation media.

DEAE-Cellulose fractions that contained ODC-AZ activity and corresponding fractions from control preparations (that did not exhibit ODC-AZ activity) were subjected to Sephadex G-75 molecular sieving. ODC-AZ activity was recoverable from positive controls and from chloroform-treated male rats. This recovery also highlighted about a 10-fold increase in the purification of the inhibitory activity. This is evident from the fact that when one-tenth the protein from the appropriate Sephadex G-75 fraction was added to the in vitro ODC-AZ assay, the same amount of inhibition was observed as with the DEAE-cellulose ODC-AZ-active fractions (data not shown).

DISCUSSION

We have demonstrated in this study that female rat hepatic ODC is refractory to simulation by repeated chloroform dosing, as was the previ-

ously reported circumstance with male rats. Additionally we have reported here that while the refractory state in male rats is in some part due to the elaboration of ODC-AZ, this is not the case in the female rat. While it would be interesting to speculate on the possible mechanisms of such a sex difference, conjecture of this type would require additional experiments not currently planned by this laboratory.

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