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ORNITHINE DECARBOXYLASE INDUCTION AND POLYAMINE BIOSYNTHESIS BY PHORONE (DIISOPROPYLIDENE ACETONE), A GLUTATHIONE DEPLETOR, IN RATS

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SUMMARY: The administration of Phorone (diisopropylidene acetone, 250 mg/kg, ip.), a glutathione (GSH) depletor, markedly induced (400-fold of the control at 12 hr) ornithine decarboxylase (ODC) in the liver of rats. Parallel to ODC induction there was a marked increase in hepatic putrescine content. Phorone also produced an increase in spermidine content and a decrease in spermine content. The effects of phorone on ODC and putrescine content occurred dose-dependently with more than a 1000-fold increase in ODC activity over the controls at a dose of 500 mg/kg. Pretreatment of rats with buthionine sulfoximine, a GSH depletor by inhibition of biosynthesis, failed to inhibit phorone-mediated induction of ODC. In contrast, pretreatment with GSH, but not post-treatment, blocked the induction of ODC by phorone.

Glutathione (GSH) is ubiquitously distributed in animal tissues, functions directly or indirectly in many important biological phenomena, and plays an important role in drug metabolism and toxicity (for reviews, see 1-4). Various compounds with divergent structures have been shown to conjugate with GSH in the presence of GSH S-transferases, thereby leading to reduce GSH content from animal tissues (1). Among these classes of compounds, α,β-unsaturated carbonyl compounds, particularly diethyl maleate (DEM, 5) and phorone (diisopropylidene acetone, 6), are potent depletors of hepatic GSH content. For this reason, these compounds, especially DEM, has been widely used in order to

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investigate a possible role of GSH-mediated reactions in the metabolism and toxicity of various drugs and chemicals (2).

Concomitant with the depletion of GSH, however, DEM has also been shown to exert various biochemical effects on animal tissues including the induction of heme oxygenase as reviewed by Plummer et al. (2). Additionally, we have recently reported that DEM is able to increase hepatic ornithine decarboxylase (ODC), a rate-limiting enzyme in polyamine biosynthesis (for reviews, see 7-10), in rats (11). Like DEM, phorone was a potent inducer of hepatic heme oxygenase as we have currently reported (12). These findings prompted us to examine further the effect of phorone on hepatic ODC and polyamine contents. In this paper, we describe the induction of ODC and polyamine biosynthesis evoked by phorone, and discuss its possible relation to GSH depletion.

MATERIALS AND METHODS

Phorone was obtained from Wako Pure Chemical Co. Ltd. DL-[1-^{14}C]-Ornithine (49 mCi/mmol) was purchased from Japan Radioisotope Association. Buthionine sulfoximine (BSO), cycloheximide, actinomycin D, putrescine, spermidine, spermine and GSH were obtained from Sigma Chemical Co. All other chemicals were of the highest grade available commercially.

Male Wistar rats, weighing 160-180g, were used in this study. Phorone was dissolved in corn oil and injected ip. at the doses indicated in Fig. and Tables. In some experiments rats were injected ip. with BSO (4 mmol/kg) dissolved in distilled water alkalized with 0.1N NaOH (pH 8.5), with GSH (4 mmol/kg), with cycloheximide (5 mg/kg) or with actinomycin D (2 mg/kg). Rats were killed at appropriate time periods as indicated in Fig. and Tables. The rats were starved for 48 hr before being killed. The livers were perfused in situ with 0.9% NaCl solution, excised, and homogenized (1:4 w/v) with 0.05M sodium/potassium phosphate buffer (pH 7.2) containing 1mM dithiothreitol, 0.1mM EDTA and 40uM pyridoxal phosphate. The preparation of supernatant fraction was performed by differential centrifugation as described previously (11). A portion of the liver was homogenized with 10 vol. of 0.4N $HClO_4$ and centrifuged at 2,000 g for 10 min to precipitate protein. The resulting supernatant fraction was used for the assay of hepatic polyamine contents. Hepatic ODC activity was determined by the method of Russell and Snyder (13) by measuring the liberated $^{14}CO_2$ from DL-[1-^{14}C]-ornithine as described previously (11,14). Hepatic polyamine contents were measured by using gas chromatography equipped with a flamethermoionic detector (Nitrogen-sensitive) according to the method of Yamamoto et al(15). Hepatic GSH content was measured by the method of

Ellman (16) as described by Costa and Murphy (17). Protein concentration was determined by the method of Gornall et al (18).

RESULTS AND DISCUSSION

Fig. 1 shows the time course of the changes in hepatic ODC activity and polyamine contents after an intraperitoneal administration of phorone at a dose of 250 mg/kg. Phorone produced a remarkable increase in ODC activity. An increase in ODC activity began within 4 hr after the administration of phorone, reached a maximum (about 400-fold of the controls) at 12 hr and returned to the control levels by 36 hr. This changing pattern of ODC activity elicited by phorone was very similar to that of heme oxygenase as currently reported (12). Hepatic putrescine content also increased significantly in parallel with the increase of ODC activity. The maximally increased putrescine content (about 50-fold of the controls) was seen at 12 hr after the treatment with phorone. Likewise, spermidine content tended

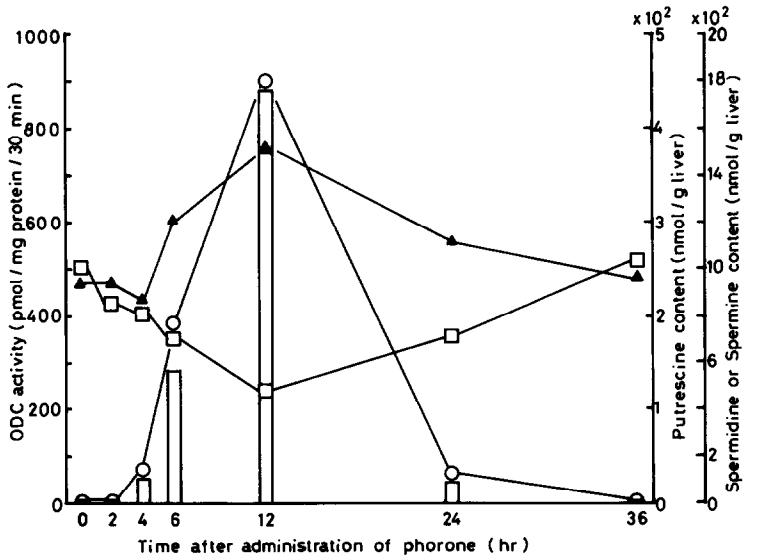

Fig. 1. Time Course of the Effects of Phorone on Hepatic ODC Activity and Polyamine Contents in Rats: Rats were injected ip. with phorone and killed at the times indicated. Vlues are the mean of three to five rats. ▭ ; ODC activity, ○—○ ; putrescine, ▲—▲ ; spermidine, □—□ ; spermine.

Table I. Dose-related Effect of Phorone on Hepatic ODC Activity and Putrescine Content

Treatment	ODC activity (pmol/mg protein/30 min)	Putrescine content (nmol/g liver)
Corn oil	1.80 ± 0.46	1.89 ± 1.83
Phorone (mg/kg)		
62.5	4.56 ± 2.04	5.66 ± 1.33
125	32.0 ± 14.1	32.2 ± 11.9
250	840 ± 100	385 ± 59
500	3159 ± 1231	1488 ± 97

Rats were injected ip. with phorone at the doses indicated and killed 12 hr later. The values are the mean ± S.E.M. for three rats.

to increase, while spermine content decreased with a nadir at 12 hr. The increase of ODC activity seen after the administration of phorone was almost completely inhibited by pretreatment of rats with either actinomycin D or cycloheximide (data not shown). The results indicate that the administration of phorone causes an increased synthesis of ODC mRNA and its subsequent translation into the enzyme protein. From these results, it would be reasonable to state that phorone is a potent inducer of hepatic ODC, a rate limiting enzyme in polyamine biosynthesis (7-10), in the liver of rats.

Dose response effects of phorone on the induction of hepatic ODC and the change in putrescine content were examined at 12 hr after the administration of phorone. As shown in Table 1, phorone was able to produce the induction of ODC and the consequent increase in putrescine content at a dose of 62.5 mg/kg. The highest dose of phorone (500 mg/kg) used in this study induced ODC to about 1,000-fold or more over the control levels. Parallel to this extremely large increase of ODC activity, hepatic putrescine content increased to about 700-fold of the control levels reaching to or over both spermidine and spermine contents, which are generally present at the higher levels compared to putrescine

in animal tissues. These highly increased ODC levels produced by phorone, together with the markedly increased putrescine content is likely to be a unique event, since the agent even at the same or higher dose used in this study has been shown to be non-toxic to mice (6) and also used as a GSH depleting agent in hamsters (19). Thus, it is unlikely that the increased ODC activity elicited by phorone in rats is due to a compensatory response to its probable hepatotoxic effect.

In addition to the present findings on hepatic ODC and polyamine contents, phorone has also been shown to produce the induction of hepatic heme oxygenase (12). The effects of phorone on these hepatic enzymes were very similar to those elicited by DEM (11). The facts suggest that induction of hepatic ODC and heme oxygenase are likely to be susceptible to α,β-unsaturated carbonyl compounds such as DEM and phorone. Thus, experiments were carried out to examine whether the observed effects of phorone are linked to its GSH depleting action. For this purpose, the effects of pretreatment with BSO, a GSH depletor by inhibiting biosynthesis at the step of γ-glutamylcysteine synthetase (20, 21), and pre- or post-treatment with GSH on phorone-mediated induction of ODC and the increase in putrescine content were examined. As shown in Table 2, irrespective of the marked decrease of GSH at the time point examined, BSO alone failed to produce any effects on ODC activity and putrescine content. Pretreatment of rats with BSO also failed to inhibit phorone-mediated induction of ODC and the increase in putrescine content. However, hepatic GSH content was profoundly reduced by treatment of rats with both BSO and phorone as compared to that of separate treatment. In contrast, pretreatment with GSH, but not post-treatment, significantly blocked phorone-induced changes

Table II. Effects of Pretreatment with BSO and Pre- or Post-treatment with GSH on Phorone-mediated Changes in Hepatic ODC Activity, Putrescine and GSH Contents

Treatment	ODC activity (pmol/mg protein/30 min)	Putrescine content (nmol/g liver)	GSH content (μmol/g liver)
Control	6.30 ± 0.5	11.7 ± 2.4	4.43 ± 0.45
Phorone (250 mg/kg)	875 ± 97	645 ± 23	3.72 ± 0.26
BSO (4 mmol/kg)	6.12 ± 1.5	17.9 ± 4.6	2.01 ± 0.19
BSO + Phorone	1079 ± 171	917 ± 128	1.33 ± 0.15
GSH (4 mmol/kg)	7.36 ± 0.41	12.6 ± 3.3	4.97 ± 0.26
GSH + Phorone	9.67 ± 4.6	26.3 ± 8.4	5.35 ± 0.67
Phorone + GSH	703 ± 322	499 ± 125	7.54 ± 0.43

Rats were pretreated ip. with BSO 4 hr before, and pre- or post-treated respectively with GSH 5 min before or 10 min after, the administration of phorone. The animals were killed 12 hr after the administration of phorone. The animals pretreated with BSO alone were killed 16 hr later. The values are the mean ± S.E.M. for three rats.

in ODC activity and putrescine content. In both cases, however, hepatic GSH contents returned to the control levels.

The findings strongly suggest that GSH depletion from the liver by inhibiting its biosynthesis does not simply lead to the induction of ODC. Rather, a rapid and profound depletion of GSH by conjugating with phorone may be a necessary step for triggering the induction of ODC and the subsequent changes in polyamine biosynthesis. This phenomenon may occur very rapidly, since the inhibitory effect of GSH on phorone-mediated induction of ODC was lost within 10 min when GSH was administered to rats pre-dosed with the depletor. The results may also suggest that a GSH-conjugate of phorone is not a possible candidate as an inducer of the enzyme, since the exogenous GSH, which could be available for conjugating with the agent, was able to inhibit the induction of the enzyme. In this respect, phorone itself or its ability to deplete GSH extensively may relate directly or indirectly to the induction of ODC. Thus, it would be of considerable merit to examine further an interrelation between

metabolic fate of GSH depletors, such as phorone and DEM, and the induction of ODC as well as heme oxygenase.

In conclusion, the present results, together with our previous findings (11,12), would add new insight into novel effects of GSH depletors, such as phorone and DEM, on hepatic polyamine and heme metabolism.

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