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Effect of piperonyl butoxide on hepatic δ-aminolevulinic acid synthetase activity in mice

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Piperonyl butoxide, a widely used insecticide synergist, is a well-known inhibitor of mammalian hepatic microsomal drug-metabolizing enzymes, both *in vivo* and *in vitro* [1–4], probably by combining with cytochrome P-450 to form an inactive form of cytochrome P-450 [5, 6]. There are marked species differences in sensitivity to piperonyl butoxide, with mice being very sensitive and humans and rats being less sensitive [7]. However, several reports have shown that the effect of piperonyl butoxide on hepatic drug-metabolizing enzymes is biphasic; acute inhibition is followed by increased activity [8, 9]. In addition, long-term feeding of piperonyl butoxide causes phenobarbital-like induction of hepatic drug-metabolizing enzymes as well as a general hypertrophy in the liver, such as proliferation of the smooth endoplasmic reticulum [10, 11].

Because of the key role played by hemoproteins in the drug-metabolizing enzyme system, it was of interest to examine the effect of piperonyl butoxide on hepatic δ-aminolevulinic acid synthetase (ALA synthetase) activity, the first enzyme in haem synthesis. The induction of ALA synthetase by a wide variety of foreign chemicals has been demonstrated in laboratory animals [12–14], and a relationship between the induction of ALA synthetase and the alterations in the drug-metabolizing enzyme activity has also been suggested by several investigators [15–17].

In the present study, piperonyl butoxide was shown to significantly stimulate hepatic ALA synthetase activity in mice. The biochemical nature of this stimulation was investigated, and the implications of alteration of the hepatic drug-metabolizing enzyme system are discussed.

Piperonyl butoxide (96 per cent purity) was a gift from Dr. A. Hayashi, Tokyo Medical and Dental University. All other chemicals were of reagent grade and were purchased commercially.

Male ddY mice, weighing 25–28 g and fed a commercial diet, were used. The mice were fasted for 20 hr prior to synergist treatment. The mice were injected intraperitoneally with different dose levels of piperonyl butoxide dissolved in corn oil, and sacrificed at the times indicated. Control mice were injected with corn oil. Livers were rapidly excised and homogenized in 3 vol. of 0.9% NaCl containing 10 mM Tris–HCl buffer (pH 7.4) and 0.5 mM EDTA using a Potter–Elvehjem homogenizer with a Teflon pestle. ALA synthetase activity was assayed by the method of Marver *et al.* [18] using total homogenate as the enzyme source. The reaction mixture contained 200 μmoles glycine, 20 μmoles EDTA, 0.4 μmole pyridoxal phosphate, 150 μmoles Tris–HCl buffer (pH 7.2), and 0.5 ml homogenate, in a final volume of 2 ml. The reaction mixture was shaken in a metabolic incubator for 60 min at 37°, and the reaction was stopped by the addition of 0.5 ml of 25% trichloroacetic acid solution. The ALA produced was converted to a pyrrole by condensation with acetylacetone, and the product was isolated using a Dowex-1-acetate column. Then, the pyrrole compound derived from ALA was determined colorimetrically by reaction with Ehrlich-Hg reagent as reported previously [19].

The time course of ALA synthetase activity after the administration of piperonyl butoxide at 100, 300 and 500 mg/kg dose levels is shown in Fig. 1. A significant increase in ALA synthetase activity was observed by the administration of piperonyl butoxide at all three dose levels. The increase in ALA synthetase activity due to piperonyl butoxide administration occurred somewhat dose dependently, and in each case a peak value was reached within 16 hr after the synergist treatment. These values corresponded to 2.3, 3.5 and 4.5 times that of the control levels for 100, 300 and 500 mg/kg dose levels, respectively. Treatment of mice with a dose of 50 mg/kg produced a slight increase in ALA synthetase activity, and a dose of 800 mg/kg produced an increase in enzyme activity similar to that seen with the 500 mg/kg dose when measured 16 hr after piperonyl butoxide was given. The elevated ALA synthetase activity was sustained for as long as 24 hr after piperonyl butoxide treatment.

Contrary to the marked increase in ALA synthetase activity due to the administration of piperonyl butoxide, hepatic drug-metabolizing enzyme activity, when measured as aminopyrine *N*-demethylase activity, was profoundly inhibited for 16 hr after the synergist treatment, and returned

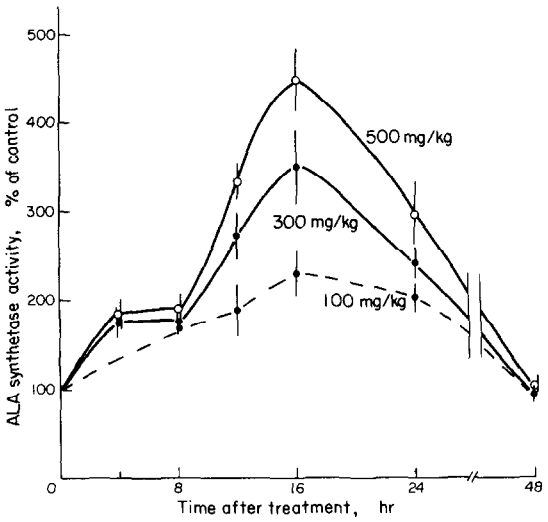

Fig. 1. Time course of ALA synthetase activity after administration of piperonyl butoxide. Fasted mice were injected intraperitoneally with a single dose of 100, 300 and 500 mg/kg piperonyl butoxide and were sacrificed at the time indicated. The mice were injected with the synergist and then fasted when the activity of the 48-hr interval was determined. Values are expressed as per cent of control activity at each time period. Control values ranged from 51.4 to 73.5 nmoles ALA/g of liver/hr during the time course study. The vertical bars are standard errors of the mean of three to five mice.

Table 1. Effect of pretreatment with cycloheximide and hemin on the increase in ALA synthetase activity caused by the administration of piperonyl butoxide*

Treatment	ALA synthetase activity (nmoles ALA/g liver/hr)
Control	61.5 ± 6.6
Piperonyl butoxide	209.4 ± 15.4
Cycloheximide	46.7 ± 3.9
Cycloheximide + piperonyl butoxide	106.5 ± 8.8
Hemin	39.5 ± 3.8
Hemin + piperonyl butoxide	91.1 ± 7.2

* Fasted mice were used. Cycloheximide (20 mg/kg, i.p.) was injected into mice 1 hr before and 8 hr after the administration of piperonyl butoxide (300 mg/kg, i.p.). Hemin (20 mg/kg, i.p.) was injected into mice 1 hr before the synergist. The mice were sacrificed 16 hr after the administration of the synergist. Values are the mean ± S.E. of four to five mice.

nearly to control levels by 24 hr, as reported by other investigators (data not presented).

The results of the present study indicate that the administration of piperonyl butoxide to mice causes a reciprocal relationship between ALA synthetase activity and drug-metabolizing enzyme activity in the early stages.

The increase in ALA synthetase activity caused by the administration of piperonyl butoxide may have been due to an increase in enzyme protein rather than to activation or stabilization of pre-existing enzyme. As shown in Table 1, pretreatment of mice with cycloheximide prevented, to some extent the increase in ALA synthetase caused by piperonyl butoxide only. In addition, pretreatment with hemin prior to the administration of piperonyl butoxide prevented to some extent the increase in ALA synthetase activity. Several studies have shown that hemin prevents drug-induced increase in ALA synthetase activity, presumably by acting as a feed-back repressor of the enzyme [20–22]. This result also supports the concept that piperonyl butoxide stimulated an increased synthesis of the enzyme.

It has been reported that a wide variety of chemicals with divergent structures are able to induce ALA synthetase [12–17, 23, 24]; however, it is not clear how these chemicals induce ALA synthetase. It has been suggested that several chemicals may elicit the increase in ALA synthetase through the decreased concentration of cytochrome P-450, mainly due to the destruction of the haem moiety, thereby decreasing the normal feed-back control [25, 26].

With respect to the effect of piperonyl butoxide on cytochrome P-450 content, Matthews *et al.* [8] reported the apparent decrease in cytochrome P-450 of mouse liver shortly after the administration of piperonyl butoxide. Although it is not clear whether the decreased content of cytochrome P-450, due to the administration of piperonyl butoxide, is accompanied by a decrease in haem content, it might be possible to indicate that the decreased cytochrome P-450 is compensated for by the induction of ALA synthetase, thus leading to the increased availability of haem content in the liver. This contention may be also supported by the fact that piperonyl butoxide can form an inactivate form of cytochrome P-450 [5, 6]. However, a detailed study will be needed to prove this possibility.

On the other hand, the rather persistent increase in ALA synthetase caused by piperonyl butoxide treatment may, in turn, lead to the increase in cytochrome P-450 content in the later stages that was observed by Matthews *et al.* [8]. Thus, the biphasic effect of piperonyl butoxide on hepatic drug-metabolizing enzymes, as well as on cytochrome P-450 content, which was observed by several investigators [8, 9], could be interpreted by the present finding to mean that piperonyl butoxide induced ALA synthetase.

Consistent results have also been obtained by the repeated administration of piperonyl butoxide to fed mice. When mice were treated with piperonyl butoxide in a dose of 300 mg/kg daily for 3 days, ALA synthetase activity was increased in a similar extent to that observed with a single administration of piperonyl butoxide to fasted mice, although the measurable activity in fed mice was lower than that of fasted mice.

There is evidence that piperonyl butoxide is metabolized by the hepatic drug-metabolizing enzyme system [27–29]. An experiment was carried out to examine whether the metabolism of piperonyl butoxide is causually involved in the induction of ALA synthetase, using the modifiers of drug-metabolizing enzymes. The experimental conditions were similar to those described in Table 1. It was found that pretreatment of mice with SKF 525-A, an inhibitor of drug-metabolizing enzymes, prevented to some extent the increase in ALA synthetase activity caused by piperonyl butoxide alone, although SKF 525-A itself increased the enzyme activity about 1.9 times that of the control, as observed by Marver [30] in rats. On the other hand, pretreatment with phenobarbital, an inducer of drug-metabolizing enzymes, for 3 days did not enhance the increase in ALA synthetase activity due to the administration of piperonyl butoxide. Administration of phenobarbital alone increased the enzyme activity about 2 times that of the control. Because of the increase in ALA synthetase activity brought about by the administration of phenobarbital or SKF 525-A alone under these experimental conditions, it would be difficult to indicate that metabolism of piperonyl butoxide is causually involved in the induction of ALA synthetase.

Although the mechanisms involved in the induction of ALA synthetase due to piperonyl butoxide administration remain to be experimentally shown, the present findings provide information on a specific pharmacological effect of the synergist on mouse liver.

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