BIOCHEMICAL LETTERS

Induction of 5-aminolaevulinate synthase in chick embryo liver by propylisopropylacetamide does not involve destruction of cytochrome P-450

Haem biosynthesis in the liver is regulated by the activity of 5-aminolaevulinate synthase (see Elder, 1980, for a review). This enzyme can be induced by a number of chemicals. A current view of the mechanism of induction suggests that inducing chemicals deplete a regulatory haem pool which normally represses synthesis of the enzyme (Elder, 1980). Depletion of the regulatory haem pool has been attributed to destruction of haem (e.g., by 2-allyl-2-isopropylacetamide) and to increased incorporation of haem into induced cytochrome P-450 (De Matteis, 1978). Recently, Lim *et al.* (1980) reported in this journal that, in chick embryo liver, the non-allyl containing isopropylacetamide, 2-propyl-2-isopropylacetamide, as well as allyl-isopropylacetamide, induce 5-aminolaevulinate synthase and produce initial decreases in hepatic microsomal cytochrome P-450. They proposed, therefore, that all chemicals which induce synthesis of 5-aminolaevulinate synthase do so by causing an initial depletion of cytochrome P-450 haem. In contrast, Krupa *et al.* (1974) found that allyl-, but not propyl-, isopropylacetamide caused detectable decreases in chick embryo hepatic cytochrome P-450.

Here we report that we are unable to confirm the findings of Lim *et al.* (1980). Thus, at different times up to 2.5 h after injection, propylisopropylacetamide did not detectably decrease microsomal cytochrome P-450 levels, although 5-aminolaevulinate synthase increased 4–8-fold in this period. These data were obtained from different experiments in which pooled livers were assayed 0.75, 1.5, 2 and 2.5 h after injection of the drug (2 mg per egg) or solvent (dimethyl sulphoxide). In an attempt to enhance our ability to detect a propylisopropylacetamide-mediated decrease in cytochrome P-450, ferrochelatase activity, and therefore haem synthesis, was inhibited by administering a low dose (0.15 mg/egg) of dicarbethoxydihydrocollidine 8 h before killing (Cole *et al.*, 1979; Rifkind, 1979);

during the final 2 h, the embryos were treated with either propylisopropylacetamide or solvent. No detectable decrease in cytochrome P-450 occurred under these conditions. However, in agreement with Lim *et al.* (1980), we did observe a decrease in cytochrome P-450 and an increase in 5-aminolaevulinate synthase 2 h after administration of a high dose of dicarbethoxydihydrocollidine (2 mg/egg).

These findings were sent to the authors of Lim *et al.*, (1980) and they now report (L.-K. Lim, G. Srivastava, J. D. Brooker, B. K. May & W. H. Elliott, personal communication) that in repeating their experiments with propylisopropylacetamide, they have not observed a reproducibly significant drop in cytochrome P-450 and are investigating why these results differ from those they reported earlier.

The differences between the original findings of Lim *et al.* (1980) and our present findings are probably due to the presence of haemoglobin in their microsomes. Cytochrome P-450 in our experiments was measured by the method of Omura & Sato (1964) using microsomes washed with 0.15 M-KCl to remove excess haemoglobin [indicated by the almost total absence of a peak at 420 nm in the CO-difference spectra (Sinclair *et al.*, 1979)]. In contrast, the microsomes assayed by Lim *et al.* (1980) were not washed with KCl. We have found that haemoglobin bound to unwashed chick hepatic microsomes (1) causes a shift in the 450 nm peak, (2) distorts the symmetry of the peak, and (3) when present in large amounts, causes underestimation of cytochrome P-450.

Using similar techniques to ours, Giger & Meyer (1981) have not detected the decrease in cytochrome P-450 following phenobarbital administration reported by Lim *et al.* (1980). However, Giger & Meyer (1981) could demonstrate a decrease in cytochrome P-450 following allylisopropylacetamide administration.

We conclude that propylisopropylacetamide does not cause a detectable decrease in cytochrome P-450 yet it produces rapid induction of 5-aminolaevulinate synthase. The mechanism underlying the latter effect remains to be elucidated.

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