RAPID LOSS OF CYTOCHROME *P*-450 AND HAEM CAUSED IN THE LIVER MICROSOMES BY THE PORPHYROGENIC AGENT 2-ALLYL-2-ISOPROPYLACETAMIDE

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1. Introduction

Several chemically unrelated drugs stimulate the hepatic formation of porphyrins in the whole animal and in liver cells cultured *in vitro* [1, 2] and enchance the activity of δ-aminolaevulic acid (δ-ALA) synthetase, the rate-limiting enzyme in the biosynthetic pathway of porphyrins and haem [3]. This is thought to result from an interference by the drugs with the feed-back control exercised by haem at the level of the enzyme [3], although the exact mechanism of this effect is not known. One of these drugs, 2-allyl-2-isopropylacetamide (AIA), causes a rapid decrease in the level of cytochrome *P*-450 and haem in rat liver microsomes. This effect, which apparently requires the activity of drug-metabolizing enzymes in liver microsomes, is due to a loss of existing cytochrome *P*-450 and haem rather than an inhibition of their synthesis. Evidence has also been obtained that the haem lost undergoes a change in chemical constitution, probably to certain ill-defined green pigments already described in the liver of animals with experimental porphyria.

2. Experimental

Male albino rats (150–180 g) of the Porton strain were fasted for twenty four hours before being injected with AIA (20 mg/ml of 0.9% NaCl) in the loose subcutaneous tissue of the neck. In some experiments, the animals were treated with phenobarbitone or SKF-525A (2-diethylaminoethyl -diphenyl-propylacetate) before administration of AIA, as already described [4]. The methods followed for the preparation of liver microsomes and for the estimation of microsomal cytochromes have also been described [4].

3. Results and discussion

The effect of a single subcutaneous injection of AIA on the level of cytochrome *P*-450 in the liver microsomes of the rat is shown in fig. 1. The changes in activity of δ-ALA synthetase observed by Marver et al. [5] with the same dose of drug are shown in the same figure for comparison. Cytochrome levels and enzyme activity behaved in a reciprocal fashion; one hour after administration of the drug, a fall in cytochrome level was already apparent, while the enzyme activity began to rise; at forty eight hours, the δ-ALA synthetase activity had returned to normal, and the cytochrome level had risen above its initial value.

A decline in *P*-450 levels following administration of AIA has already been described by Wada et al. [6] and by Waterfield et al. [7] in mice and rabbits, respectively. No attempt was made by these authors to establish whether the decrease was due to inhibition of haem and (or) of cytochrome synthesis, or to loss of existing pigment: Wada et al. [6] suggested that inhibition of haem synthesis was the mechanism involved. Comparison of the rate or *P*-450 decline observed in normal animals (fig. 1) or in phenobarbitone-induced animals (see below) with that obtained after nearly complete inhibition of protein synthesis by cycloheximide [4] indicated, however, that loss of existing pigment was more likely. More direct evidence

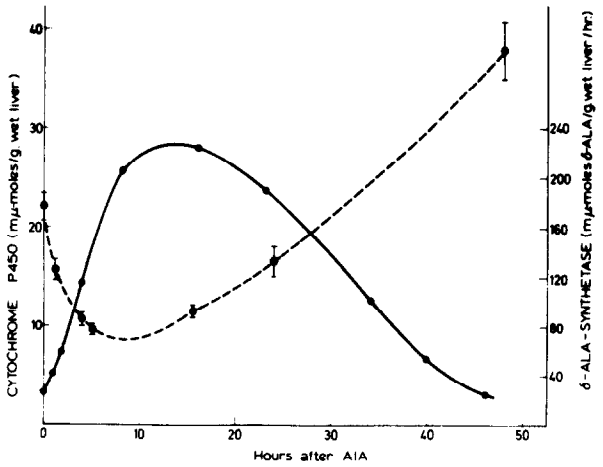

Fig. 1. The effect of a single subcutaneous injection of AIA (400 mg/kg) on microsomal *P*-450 levels (⊛- -⊛) and δ-ALA synthetase activity of the liver homogenate (●——●). *P*-450 levels are given as avergae (± S.E.) of the values observed in four animals. The data on δ-ALA synthetase activity are from Marver's work [5].

for an increased destruction of *P*-450 and haem following administration of AIA is given below.

The decline in cytochrome *P*-450 caused by AIA was affected by treatment known to alter the activity of the microsomal drug-metabolizing enzymes. Pretreatment of rats with phenobarbitone which stimulates drug metabolism in the liver microsomes, greatly increased the loss of *P*-450 caused by AIA, whereas SKF-525A, an inhibitor of drug-metabolizing enzymes afforded protection. Percentage losses of *P*-450 caused by AIA (as compared with appropriate controls) were as follows (with time between drug administration and killing in parentheses): normal animal: 29% (1 hr); 57% (5 hr); Rats given SKF-525A before AIA: 12% (5 hr); Phenobarbitone-induced rats: 70% (1 hr). SKF-525A also partially reversed the increased loss of *P*-450 observed after phenobarbitone treatment. These findings suggested that AIA must be metabolized by the liver microsomes in order to produce its effect on *P*-450. Results compatible with this interpretation were obtained in experiments *in vitro*. When liver microsomes from phenobarbitone-induced rats were incubated with AIA, a loss of cytochrome *P*-450 was observed only when NADPH, the cofactor essential for metabolism of drugs by the liver microsomes, was also present. Some loss of *P*-450 was also observed when the microsomes were incubated in the presence of NADPH, but with no AIA.

In contrast with the marked loss of cytochrome *P*-450 observed after administration of AIA to phenobarbitone-treated rats, the cytochrome b_5 and protein content of the microsomes and the glucose-6-phosphatase activity of the liver were only slightly decreased.

The fall in cytochrome *P*-450 level was accompanied by a loss of microsomal haem and by a characteristic brown-green discolouration of the whole liver, particularly the microsomes. Both loss of haem and degree of discolouration appeared to be related to rate and extent of *P*-450 loss; they were more pronounced in phenobarbitone-treated animals than in normal animals and much less apparent in rats given SKF-525A before AIA. Microsomes incubated with AIA and NADPH were also discoloured at the end of incubation, whereas those incubated with AIA alone were not.

Schwartz and Ikeda [8] described a discolouration of the liver of rats and rabbits given either AIA or the related drug Sedormid and they extracted and partially characterized certain green pigments responsible for this abnormal colour. The significance of these pigments remained obscure. The present results suggest that the abnormal pigments might arise from some chemical change in existing haem as a result of the action of AIA on liver microsomes. To investigate this, the liver haems of phenobarbitone-induced rats were pre-labelled with radioactive δ-ALA prior to AIA administration and the distribution of the label between haem and the fractions known to contain the green pigments [9] was studied, in treated and control rats. Animals killed one hour after AIA administration showed a considerable loss of radioactivity in the haem isolated from their liver homogenates, while a corresponding increase in the radioactivity was recovered in the green pigments fractions [10]. The exact nature of these pigments and the mechanism by which they are produced from haem under the influence of AIA are being investigated. One possibility is that AIA might damage the microsomal structure so that labile pools of microsomal haem (such as the *P*-450 haem) become sensitive to random methine bridge oxidation by endogenously occurring oxidants, such as ascorbate; similarly to the ascorbate-catalysed breakdown of haem *in vitro* [11], this would give rise to a mixture of pre-

dominantly nonphysiological biliverdin isomers, which would not be converted to bilirubin [12] and would tend to accumulate in the liver. Alternatively, the struture of haem may be altered by peroxides produced during the metabolism of the drug or by some reactive metabolite of the drug itself.

The significance of the loss of haem caused by AIA in relation to the induction of δ-ALA synthetase cannot be conclusively assessed at the moment. However, the present results raise the possibility that a drug or an active principle produced during its metabolism may, by changing the chemical constitution of haem, render it unable to fulfil its normal function of feedback control at the level of δ-ALA synthetase.

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