Short Communications

δ -Aminolaevulate Synthetase Induction and Catalase Repression by Porphyrinogenic Drugs

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Porphyrinogenic drugs such as 2-allyl-2-isopropylacetamide and 3,5-diethoxycarbonyl-1,4-dihydrocollidine induce δ -aminolaevulate synthetase activity greatly and also depress catalase activity in experimental animals (Granick & Urata, 1963; Schmid, Figen & Schwartz, 1955; Dematteis, 1967). It is claimed that 2-allyl-2-isopropylacetamide inhibits catalase synthesis (Price, Sterling, Tarantola, Hartley & Recheigl, 1962), although its mechanism of action is not clear. However, drugs such as phenobarbital and hexachlorobenzene do not depress catalase activity (Schmid, Marver & Hammaker, 1966; Ockner & Schmid, 1961). It appeared possible that the depressant effect of the porphyrinogenic drugs on catalase activity may depend on the extent of δ -aminolaevulate synthetase induction. The results obtained in this study reveal that a high induced activity of δ -aminolaevulate synthetase is accompanied by a depression of catalase activity and that overproduction of haem may be the ultimate cause of the depressed enzyme activity.

Adult female rats (100–120 g) and mice (25–28 g) of the local Institute strain were used in these studies. The animals were fed *ad libitum*. The schedule of injections is given in Table 1. The animals were killed by decapitation and δ -aminolaevulate synthetase activity was determined in liver homogenates by the method of Marver, Collins, Tschudy & Recheigl (1966). Catalase activity was determined in the liver preparation made by the procedure of Ganschow & Schimke (1968). The enzyme activity was assayed by the permanganate titration method of Ramachandran & Sarma (1954). Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951), with dry bovine serum albumin as standard.

The results presented in Table 1 indicate that 2-allyl-2-isopropylacetamide and 3,5-diethoxycarbonyl-1,4-dihydrocollidine cause a striking induction of δ -aminolaevulate synthetase in rats and mice as compared with the effects of phenobarbital and hexachlorobenzene. It can be seen also that only the first two drugs depress catalase activity. It has been shown that 3,5-diethoxycarbonyl-1,4-

dihydrocollidine is not as effective in rats as it is in mice as a porphyrinogenic drug (Rao, Padmanaban & Sarma, 1971). Table 1 also shows that this drug does not significantly depress catalase activity in the rat. It was decided to study the interaction between phenobarbital and 2-allyl-2-isopropylacetamide with regard to the induction of δ -aminolaevulate synthetase and depression of catalase activity. It can be seen that phenobarbital pretreatment abolishes the inducing effect of 2-allyl-2-isopropylacetamide on δ -aminolaevulate synthetase and at the same time the depressant effect of the drug on catalase activity is released. If phenobarbital is given along with 2-allyl-2-isopropylacetamide, δ -aminolaevulate synthetase activity is induced to high values and catalase activity is also depressed. It is known that haematin, when administered along with 2-allyl-2-isopropylacetamide, prevents the induction of δ -aminolaevulate synthetase (Waxman, Collins & Tschudy, 1966). Although haematin blocks the induction of δ -aminolaevulate synthetase by 2-allyl-2-isopropylacetamide, depression of catalase activity is still observed (Table 1). This suggested that haematin might cause depression of catalase activity by itself. The results show that haematin as well as bilirubin depress catalase activity. None of the compounds has any effect on catalase activity *in vitro*.

It appears possible that prior treatment with phenobarbital may increase the activity of the drug-metabolizing enzymes, and this may result in a decreased potency of 2-allyl-2-isopropylacetamide to induce δ -aminolaevulate synthetase. However, simultaneous administration of phenobarbital with 2-allyl-2-isopropylacetamide, even with successive treatments, fails to prevent δ -aminolaevulate synthetase induction, and consequently depression of catalase activity is also manifested. It is possible that under the latter conditions drug interactions may operate at the uptake level. A considerable extent of δ -aminolaevulate synthetase induction may be necessary to overproduce haem, the breakdown product of which may depress catalase synthesis. It has been

Table 1. *Effects of porphyrinogenic drugs and haematin on hepatic δ -aminolaevulate synthetase and catalase activities in experimental animals.*

Animals (rats except where indicated) received the following doses of drugs subcutaneously every 12 h: 2-allyl-2-isopropylacetamide (AIA), 400 mg/kg; 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC), 500 mg/kg; hexachlorobenzene, 500 mg/kg; phenobarbital, 80 mg/kg. Pretreatment of rats with phenobarbital was done with three doses at 24 h intervals. Haematin and bilirubin were administered intraperitoneally at a dosage of 20 mg/kg and 10 mg/kg respectively on a similar schedule to that for the drugs. Animals were killed 6 h after each dose. Each value represents the average $\pm$ s.e.m. of four independent determinations, livers from two animals being pooled for each determination. The absolute catalase specific activity for control liver is $350 \pm 11.4 \mu\text{mol of H}_2\text{O}_2$ decomposed/min per mg of protein.

Treatment	No. of doses	...	δ -Aminolaevulate synthetase specific activity (nmol of δ -aminolaevulate/h per g of liver)			Relative catalase specific activity (%)		
			1	2	3	1	2	3
Control			16.6 $\pm$ 2.4	—	—	100	—	—
AIA			90.4 $\pm$ 8.4	195.3 $\pm$ 11.4	224.4 $\pm$ 12.6	85.8	66.2	48.5
Phenobarbital			28.1 $\pm$ 3.8	38.0 $\pm$ 2.8	45.2 $\pm$ 4.1	102.4	99.8	101.5
Phenobarbital-pretreated + AIA			50.4 $\pm$ 8.3	108.0 $\pm$ 8.9	98.9 $\pm$ 4.8	98.1	80.8	69.5
Phenobarbital + AIA (simultaneous)			85.6 $\pm$ 6.8	170.2 $\pm$ 15.6	227.6 $\pm$ 8.9	92.9	68.1	50.6
AIA + haematin (simultaneous)			18.5 $\pm$ 3.0	21.0 $\pm$ 5.2	23.5 $\pm$ 4.6	85.3	70.8	49.6
Haematin			16.4 $\pm$ 1.8	15.8 $\pm$ 4.1	12.4 $\pm$ 1.9	90.8	74.0	51.9
Bilirubin			16.3 $\pm$ 2.5	15.9 $\pm$ 1.4	13.9 $\pm$ 3.1	96.5	85.6	65.8
Hexachlorobenzene			19.4 $\pm$ 3.4	29.8 $\pm$ 5.1	47.2 $\pm$ 4.6	100.0	98.5	92.4
DDC			24.8 $\pm$ 2.9	54.6 $\pm$ 4.9	75.0 $\pm$ 8.6	99.6	94.6	86.8
DDC (mice)			120.8 $\pm$ 10.8	224.3 $\pm$ 21.8	297.6 $\pm$ 28.4	91.6	80.4	62.8

shown that haem can induce microsomal haem oxygenase in the liver, leading to an increase enzymic catabolism of the inducer (Tenhunen, Marver & Schmid, 1970). Phenobarbital by itself causes increased haem synthesis, which gives rise to early labelled bilirubin (Schmid *et al.* 1966). However, phenobarbital does not induce δ -aminolaevulate synthetase to activities as high as that due to 2-allyl-2-isopropylacetamide. The fact that phenobarbital administration by itself fails to depress catalase activity indicates that the enzymic breakdown products of haem may not be available in sufficient concentration and in a free form in the liver to bring about the depression. In this context it is noteworthy that the UDP glucuronyltransferase activity in the liver increases during phenobarbital administration (Mulder, 1970). Haem appears to undergo random non-enzymic cleavage as well, giving rise to unidentified green pigments (Ó Carra & Colleran, 1969).

2-Allyl-2-isopropylacetamide also causes a striking fall in cytochrome *P*-450 concentration, and it has been suggested that the drug may damage the microsomal membrane so that the microsomal cytochromes may be exposed to a random non-enzymic catabolism, giving rise to the green pigments (Dematteis, 1970; Landaw, Callahan & Schmid, 1970). However, the green pigments may not be responsible for depression of catalase activity, since Dematteis (1970) has shown

that in phenobarbital-pretreated rats receiving 2-allyl-2-isopropylacetamide cytochrome *P*-450 degradation, giving rise to green pigments, is more than that in rats receiving 2-allyl-2-isopropylacetamide alone; but the results presented in Table 1 show that phenobarbital pretreatment prevents depression of catalase activity by 2-allyl-2-isopropylacetamide. Our own results (M. R. S. Rao & G. Padmanaban, unpublished work) show that cytochrome *P*-450 degradation due to 2-allyl-2-isopropylacetamide is an independent phenomenon that is not related to overproduction of haem. If enzymic cleavage of the overproduced haem leads to depressed catalase activity, then it has to be assumed that in 2-allyl-2-isopropylacetamide administration, even though the non-enzymic cleavage of the overproduced haem appears to be predominant, a sufficient concentration of the enzymic breakdown product of haem is available in a free form in the liver for the manifestation of the depression phenomenon. It is also possible that 2-allyl-2-isopropylacetamide itself may be responsible for the depression of catalase activity and that the release of depression by phenobarbital pretreatment may be due to a rapid metabolism of 2-allyl-2-isopropylacetamide. But the fact that haematin and its breakdown product also can depress catalase activity significantly and also the non-additive effect of 2-allyl-2-isopropylacetamide and haematin rules out this possibility,

implicating the overproduction of haem as a necessary criterion. This is further supported by the distinct correlation observed between the extent of δ -aminolaevulate synthetase induction and depression of catalase activity obtained with different drugs.

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