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Cytochrome P-450-dependent H_2O_2 production demonstrated in vivo

Influence of phenobarbital and allylisopropylacetamide

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By administration of allylisopropylacetamide, an inhibitor of cytochrome P-450, we demonstrated that cytochrome P-450 is involved in the production of H_2O_2 during aminopyrine metabolism and phenobarbital induction in both the unanaesthetized guinea pig and rat. In the guinea pig we also found evidence for the existence of a basal cytochrome P-450-dependent H_2O_2 production, i.e. in the absence of exogenous substrate. Catalase participates in the decomposition of H_2O_2 produced in the endoplasmic reticulum where cytochrome P-450 is localized.

Cytochrome P-450 *H_2O_2 production* *Aminopyrine* *Phenobarbital* *Allylisopropylacetamide*

1. INTRODUCTION

In vitro research has mentioned H_2O_2 production through the intermediary of cytochrome P-450 [1–8]. In vivo, Mannering et al. [9] demonstrated changes in H_2O_2 production during metabolism of ethylene glycol by measuring changes in methanol metabolism. An analogous system was used by Hildebrandt et al. [10] who measured changes in H_2O_2 production during ethylmorphine metabolism.

We used a method that evaluates changes in H_2O_2 production in vivo by measuring the catalase activity which remains after inhibition of the catalase-H_2O_2 complex, compound I, by 3-amino-1,2,4-triazole (AT) in combination with methanol [11,12].

This allowed us to demonstrate increases in H_2O_2 production in vivo during metabolism of aminopyrine (AP) and phenobarbital (PB) induction in the guinea pig and rat [13].

We now report on the influence of allylisopropylacetamide (AIA), a destructive inhibitor of cytochrome P-450 in vivo [14,15] on AP- and PB-dependent H_2O_2 production.

2. MATERIALS AND METHODS

Male DHPL guinea pigs (400–700 g) and male Wistar rats (250–350 g) were used. All animals received standard laboratory diet and water ad libitum. PB (80 mg/kg) was given by intraperitoneal (i.p.) injection for 3 days preceding the day of the experiment.

AT (1 g/kg) was given by i.p. injection in the guinea pig 4 h and in the rat 2 h before killing. Methanol was always given simultaneously with AT (guinea pig, 3 mmol/kg; rat, 3.5 mmol/kg). AP (Sigma, FRG) was administered orally, suspended in water (dose 1 g/kg). Control animals received water by the same manipulation. AIA was given (200 mg/kg) by an i.p. injection 2 h before administration of AP.

After killing livers were quickly removed and homogenized as described [13]. Catalase activity was assayed in this liver homogenate, by the

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titanium oxysulfate method [16]. Residual catalase activity (RCA) is the catalase activity that remains after inhibition by AT. A lower RCA reflects higher H_2O_2 production [11]; an increase in RCA reflects a decrease in H_2O_2 production [17,18].

Cytochrome P-450 was determined according to Omura and Sato [19] as modified by Matsubara et al. [20] using a Pye Unicam SP 1750 spectrophotometer. An extinction coefficient of 104 $mM^{-1} \cdot cm^{-1}$ was used in the calculations.

For statistical analysis, the Mann-Whitney test was used [21].

3. RESULTS

In agreement with earlier results [13], AP metabolism decreases the RCA in non-induced guinea pigs and in induced guinea pigs and rats reflecting stimulation of H_2O_2 production. Secondly, it is shown that PB induction alone causes a decrease in RCA in the guinea pig (table 1).

Next we examined the direct involvement of cytochrome P-450 in H_2O_2 production using pretreatment with a destructive inhibitor of cytochrome P-450, AIA. Cytochrome P-450 concentrations were measured as a control of the AIA effect in vivo within the time of the experiment.

In the rat, the influence of AP on the RCA is completely abolished by AIA, while cytochrome P-450 concentration returns to the level of non-induced rats. In non-induced rats, AP has no effect on the RCA [13]. In rats receiving water orally, we measured no difference in RCA due to AIA inhibition.

In the non-induced guinea pig, AIA not only prevents the effect of aminopyrine but even raises the RCA to the level of the total catalase activity. This means that beside the AP-dependent H_2O_2 production, another source of H_2O_2 has been inhibited. This source might correspond to a basic cytochrome P-450-dependent H_2O_2 production from endogenous substrates.

To exclude the possible interference of AIA with the formation of catalase compound I, or with AT inhibition, AIA and AT were given without methanol (table 2). AT inhibition is not diminished by AIA.

In induced guinea pigs without AP, AIA raises RCA to the level of non-induced animals (table 1). In induced animals with AP, AIA raises RCA to a level between that of induced animals with H_2O

Table 1

Residual catalase activity and cytochrome P-450 concentration in non-induced and PB-induced guinea pigs and rats

	Guinea pig				Rat	
	Non-induced		3 days PB		3 days PB	
	RCA	Cyt.P-450	RCA	Cyt.P-450	RCA	Cyt.P-450
AT + Me + H_2O	171 ± 11[a] (9)	31 ± 5 (3)	60 ± 8[c] (9)	57 ± 3 (2)	56 ± 4[h] (10)	49 ± 4 (2)
AT + Me + AP	49 ± 16[b] (5)	31 ± 4 (5)	16 ± 5[d] (8)	–	35 ± 3[i] (7)	–
AT + Me + H_2O + AIA	277 ± 18[e] (4)	30 ± 3 (4)	155 ± 30[f] (5)	26 ± 2 (5)	54 ± 1 (6)	23 ± 1 (6)
AT + Me + AP + AIA	313 (1)	38 (1)	107 ± 8[g] (5)	18 ± 1 (5)	53 ± 3[j] (5)	20 ± 1 (5)
Total catalase activity	265 ± 20 (4)	21 ± 2 (2)	206 ± 9 (7)	–	94 ± 2 (9)	51 ± 1 (4)

Values represent residual catalase activity (RCA) (U_B) and cytochrome (cyt.) P-450 concentration (nmol) per g liver wet wt after an i.p. injection of AT + methanol (AT + Me) and an oral administration of H_2O or AP 4 and 2 h before killing for guinea pig and rat, respectively. AIA was given by an i.p. injection 2 h before the injection of AT + Me.
Statistical significance of differences: $p < 0.01$: [a] vs [b,c]; [c,g] vs [d]; [e] vs [a]; [f] vs [c]; [h,j] vs [i]

Table 2

RCA (U_B/g liver) in non-induced guinea pigs measured 4 h after the i.p. injection of AT

	RCA (U_B/g liver)
AT	6.9 ± 2.5 (4)
AT + AIA	3.4 ± 0.9 (2)

AIA was given by i.p. injection 2 h before AT administration

and that of non-induced animals with H_2O. In this last case, both the AP and PB effects are inhibited but not by 100%. Cytochrome P-450 levels are decreased to that of non-induced animals.

4. DISCUSSION

Our results provide evidence that cytochrome P-450 is involved in the production of H_2O_2 during AP metabolism or after PB induction.

During metabolism of AP, AIA, an inhibitor of cytochrome P-450, leads to a significant decrease of H_2O_2 bound to catalase. This effect is paralleled by a decrease in cytochrome P-450 concentration.

Also in the absence of an exogenous substrate, AIA and PB behave as antagonists, in parallel with the cytochrome P-450 concentration. Even without PB induction AIA causes a dramatic fall of H_2O_2 in guinea pigs; under the circumstances of the experiment our method no longer demonstrates the presence of any H_2O_2. This argues for significant and continuous H_2O_2 production from endogenous substrates of the basal type of cytochrome P-450. However, this is not observed in the rat.

The question should be asked whether H_2O_2 is an 'inborn error' of the cytochrome P-450 activities or whether the cytochrome P-450-dependent system is continuously metabolizing endogenous substrates that act as 'uncouplers'. The broad range of chemical reactions catalyzed by the cytochrome P-450 system could implicate a less effective performance of some reactions, i.e. the incorporation of oxygen into an organic molecule.

Our results also show that catalase is active in decomposing peroxide produced in the endoplasmic reticulum where cytochrome P-450 is localized. In the guinea pig this is not unexpected as the major part of catalase is localized in the cytosol [22]. Thus catalase, even in basal circumstances, successfully competes with glutathione peroxidase; by this action the energy-consuming regeneration of more glutathione is avoided. In the rat, AP-dependent H_2O_2 also follows the catalase pathway, but not H_2O_2 from endogenous substrates (table 1). This could point to a mainly peroxisomal localization of catalase in this species and an overflow of H_2O_2 from the cytosol when production is enhanced only or to very low endogenous cytochrome P-450-dependent H_2O_2 production.

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