Studies on the different metabolic pathways of antipyrine as a tool in the assessment of the activity of different drug metabolizing enzyme systems in man

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Antipyrine plasma half-life or clearance values are widely used to assess changes in the activity of hepatic mono-oxygenases in man. However these parameters do not represent an absolute measure of an individual's capacity to metabolize drugs, since poor correlations have been observed with the clearances of many other drugs that are mainly eliminated by oxidation. It has been suggested that these discrepancies are due to the qualitative and quantitative heterogeneity of the mixed function oxidase system. On the other hand antipyrine metabolism is rather complicated since at least four phase I metabolites have been identified: 4-hydroxy-antipyrine, norantipyrine, 3-hydroxymethyl-antipyrine and 3-carboxy-antipyrine. Methods were developed to determine these compounds quantitatively in urine by high-pressure liquid chromatography or gas chromatography (Danhof, de Groot-van der Vis & Breimer, 1979; Danhof, de Boer, de Groot-van der Vis & Breimer, 1979). Following oral administration of antipyrine (500 mg) to healthy volunteers 3.3% of the dose was excreted as unchanged drug, 28.5% as 4-hydroxy-antipyrine, 16.5% as norantipyrine, 35.1% as 3-hydroxymethyl-antipyrine and 3.3% as 3-carboxy-antipyrine (mean values; 52 h urine). These cumulative amounts of excretion products were not significantly different at 250 and 1000 mg dose levels in the same panel of volunteers (Danhof & Breimer, unpublished). The major part of the metabolites was excreted as glucuronides.

Evidence was obtained in rats that different types of hepatic monooxygenases are involved in the formation of antipyrine metabolites: after 3-methylcholanthrene treatment a significant increase in 4-hydroxy-antipyrine formation occurred, whereas 3-hydroxymethyl-antipyrine formation was significantly decreased (Danhof, Krom & Breimer, 1979). Phenobarbitone pretreatment did not result in a change of antipyrine metabolite ratios in rats. In healthy volunteers, however, pentobarbitone treatment (100 mg for 8 days) resulted in a selective increase in norantipyrine formation and a decrease in 3-hydroxymethyl-antipyrine formation, whereas the excreted amount of 4-hydroxy-antipyrine remained almost unchanged. Preliminary results in patients with liver disease indicate that antipyrine metabolite ratios are quite different in individual cases compared to healthy men.

Studies on the different metabolic pathways of antipyrine may be quite important as a tool in the assessment of the activity of different oxidative drug metabolizing enzyme systems in man.

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Inhibition of aminopyrine demethylation and binding to cytochrome P-450 by its main metabolites in rat liver microsomes

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Aminopyrine (DMAP) is demethylated by two successive *N*-demethylations to monomethyl-4-aminoantipyrine (MMAP) and 4-aminoantipyrine (AAP), respectively. This reaction can be estimated *in vitro* by measuring formaldehyde formation and is frequently used to determine the hepatic monooxygenase activity. It is possible that aminopyrine metabolites will inhibit DMAP demethylation, if they are formed in sufficient quantities. Goromaru, Matsuyama, Noda & Iguchi (1978) demonstrated that, in man, the MMAP plasma concentration achieved a level equal to or higher than the DMAP plasma concentration.

In our study, microsomes of livers from male Wistar rats (250 g) were suspended in a phosphate buffer (50 mM and pH 7.4) containing EDTA (0.1 mM). Incubation (5 min at 37°C) of dimethyl-[^{14}C]-aminoanti-

pyrine ([^{14}C]-DMAP) in microsomes (2 mg protein/ml; 0.5 mM NADP; 4.2 mM $MgCl_2$; 4.1 mM glucose-6-phosphate; 0.3 I.U./ml glucose-6-phosphate dehydrogenase) resulted in [^{14}C]-formaldehyde, the latter being determined according to Poland & Nebert (1973).

[^{14}C]-DMAP *N*-demethylation was competitively inhibited by MMAP (K_i = 0.85 mM) and AAP (K_i = 2.43 mM). Furthermore, it was demonstrated, by means of HPLC determination of DMAP and its metabolites, that MMAP accumulates in the microsomal reaction mixture during DMAP demethylation, confirming results of Gram, Wilson & Fouts (1968).

These phenomena may be due to a mutual competition between aminopyrine and its metabolites for binding to cytochrome P-450.

In this context, we have studied the spectral interaction of DMAP, MMAP and AAP with cytochrome P-450. Spectra were recorded at 37°C in a microsomal suspension (3 nmol P-450/ml). Using hexobarbitone (3 mM), a relatively pure type I compound, as a modifier, we were able to block the type I binding sites. In this way we distinguished between type I and type II binding of DMAP and its metabolites. It will be noticed from Table 1, that the affinity for type I binding to cytochrome P-450 decreases in the order DMAP > MMAP > AAP, while the affinity for type II binding follows the reverse order. From the Lineweaver-Burk plot for DMAP binding, two different K_s and ΔA_{max} values could be calculated (Table 1). No type I binding component could be detected for AAP, in the concentration range studied.

It follows from the ΔA_{max} (Table 1), that not all type I binding loci of DMAP to cytochrome P-450, can be occupied by MMAP or AAP.

This conclusion is supported by the finding, that the spectral change caused by DMAP could not be completely reduced to zero by MMAP or AAP. Furthermore, DMAP and MMAP show a competitive interaction for the cytochrome P-450 binding sites common to both substrates (K_i for MMAP = 1.59 mM). Consequently, DMAP may inhibit MMAP demethylation (Gram *et al.*, 1968) and MMAP may cause product inhibition.

AAP may inhibit DMAP demethylation by ligand binding to cytochrome P-450. However AAP concentrations both *in vitro* and *in vivo* (Goromaru *et al.*, 1978) are relatively low and the K_i (*in vitro*) is relatively high.

References

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Table 1 Binding of aminopyrine and its metabolites to cytochrome P-450

	Type I		*Type II*	
	K_s (*mM*)	ΔA_{max}/*nmol P-450/ml*†	K_s (*mM*)	ΔA_{max}/*nmol P-450/ml*§
Aminopyrine	0.17	0.0084	7.79*	0.0120
	0.92	0.0167		
Monomethyl-4-aminoantipyrine	1.26	0.0064	1.49	0.0096
4-Aminoantipyrine	—	—	0.48	0.0060

Results were obtained by the method of Lineweaver-Burk in the concentration range 0.3 mM–3.0 mM (* in the range 0.3 mM–7.0 mM), and are the average of three experiments. † Difference in absorbance between 385 nm and 420 nm (type I) § and between 392 nm and 427 nm (type II).