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Influence of phenobarbitone pretreatment on disposition of amidopyrine and its metabolites in rat

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Plasma concentration-time profiles for amidopyrine and its primary desmethyl metabolite have been determined in control rats and in rats pretreated with phenobarbitone. The kinetics of both compounds indicate enhanced clearance following pretreatment. Metabolite excretion in urine, bile and breath support the plasma data. It would appear that phenobarbitone pretreatment results in quantitative but not qualitative changes in the metabolism of amidopyrine in rat.

Amidopyrine (AP) is extensively metabolized in vivo by man (Brodie & Axelrod 1950; Goromani et al 1976) and rat (Lockwood & Houston 1979). A high percentage of an administered dose of AP is demethylated to give monomethylaminoantipyrine (MAP) which in turn is demethylated to aminoantipyrine (AA). The latter metabolite undergoes acetylation to form acetylaminoantipyrine (AAA). This pathway (see Fig. 1) accounts for approximately 80% of an AP dose in rat (Lockwood & Houston 1979), but only 20–50% in man (Goromani et al 1976).

A number of in vitro and in vivo tests employing AP have been developed based on the above pathway. These are regarded as sensitive measures of the intrinsic drug metabolizing ability of the liver. Production of formaldehyde from AP by microsomal enzyme preparations is used as an in vitro measure of mixed function oxidase activity (Mannering 1968; Mazel 1972). Breath analysis of $^{14}CO_2$ following demethylation of (*N*-dimethyl-^{14}C)amidopyrine (Lauterburg & Bircher 1976; Hepner et al 1977) is the in vivo equivalent test for AP demethylation. Other investigators have used urinary recovery of AA and AAA as a means of monitoring changes in AP metabolism (Remmer 1962; Roots et al 1977).

Neither the microsomal AP test (which measures formaldehyde production) or the AP breath test (which measures $^{14}CO_2$ production) is specific for AP, both measure the single carbon moieties released from AP and MAP (see Fig. 1). We have investigated the relative rates of demethylation of these two compounds in vivo in the rat. The effect of phenobarbitone pretreatment on the disposition of AP and its primary metabolite MAP has been assessed by plasma kinetic studies and determination of the excretion of AP metabolites in urine, bile and breath.

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MATERIALS AND METHODS

Chemicals

Amidopyrine and acetylaminoantipyrine were obtained from Aldrich Chemical Co. Ltd, and aminoantipyrine, antipyrine and phenobarbitone sodium from BDH. [*N*-dimethyl-^{14}C]amidopyrine was supplied by The Radiochemical Centre, Amersham, and had a specific activity of 25 mCi m mol^{-1}.

Animals and treatment

Male Sprague-Dawley rats, mean weight 300 g, were pretreated with phenobarbitone (100 mg. kg^{-1}, i.p.) once daily for 4 days before experimentation. The last dose of phenobarbitone was given approximately 24 h before the AP dose. AP (30 mg kg^{-1}) was administered i.v. in the plasma and biliary excretion studies and i.p. in the cross-over urinary and $^{14}CO_2$ studies.

Plasma concentration studies

A control (5) and a phenobarbitone-pretreated group (4) of rats were anaesthetized with urethane (1·3 g kg^{-1}) and cannulae (PE 50, Intramedic) placed in the right carotid artery and right jugular vein. AP was administered in aqueous solution via the jugular vein cannula. The cannula was flushed with heparinized 0·9% NaCl (saline) (400 units ml^{-1}), to ensure the entire dose was administered, and kept filled with heparinized saline when samples were not being taken. Plasma was obtained from blood samples (400 μl) taken via the carotid cannula at regular intervals for 240 min in control rats and 120 min in pretreated rats.

Urinary and ^{14}C excretion studies

In these studies 5 rats were used in a balanced cross-over design. Each animal received two doses of [*N*-dimethyl-^{14}C] AP (2μCi kg^{-1}, 30 mg kg^{-1}) one week apart. Three rats were pretreated with phenobarbitone on days 4–7 following the 'control' experiment. The other two rats were pretreated with drug before their first AP dose and the 'control' experiment carried out in the second week.

Rats were individually housed in all glass metabolism cages adapted for CO_2 collection. Air was drawn through the system via a soda lime trap to remove endogenous CO_2. After passing through the metabolism cage, the air was bubbled through a concentrated sulphuric acid trap and two CO_2 traps (40 ml of ethanolamine –methanol, 1:4) in series. The CO_2 trapping fluid was changed every 10 min for the first 2 h and then every 20 min for a total of 7 h. Urine was collected in ice-cooled flasks over 24 h after dosing.

Biliary excretion studies

Both control and phenobarbitone-pretreated animals were anaesthetized with urethane and cannulae placed in the right jugular vein and in the common bile duct (PE 10, Intramedic) 3 cm distal to the liver. [*N*-dimethyl-^{14}C] AP was administered as described above via the jugular cannula.

Chemical analysis

Plasma and urine samples were assayed for AP, MAP, AA, and AAA by h.p.l.c. as described by Lockwood & Houston (1979). An octadecyl silane-bonded silica column (Partisil 10, Whatman) was used with a u.v. detector. Antipyrine was the internal standard.

Trapping fluid, urine and bile samples were assayed for radioactivity using a Packard Tri-carb Scintillation Counter (Model 2405) using internal standard quench correction. The scintillation fluids employed were Bray's dioxan mixture (Bray 1960) for the $^{14}CO_2$ trapping fluid and Unisolve 100 (Koch-Light Laboratories Ltd) for the bile and urine samples.

The importance of alternative metabolite routes for AP and MAP (M2—see Fig. 1) was assessed from total radioactivity after correction for the radioactivity contribution from AP and MAP.

Pharmacokinetic analysis

The clearance (CL) and volume of distribution (V) of AP were calculated from CL = D/AUC and V = CL/β where D is the dose administered, AUC is the area under the plasma concentration-time curve between zero and infinity (calculated by the trapezoidal rule) and β is the rate constant for the terminal phase of the plasma concentration-time curve (calculated by method of least squares).

The clearance of MAP (CL_m) may be calculated from equation 1 (Pang & Gillette 1978).

$$CL_m = (f_m.D.R)/AUC_m \quad .. \quad (1)$$

where f_m is the fraction of the AP dose metabolized to MAP, R is the molar ratio of MAP to AP and AUC_m is the area under the plasma concentration-time curve between zero and infinity for MAP (calculated by the trapezoidal rule). The volume of distribution of MAP (V_m) was calculated by

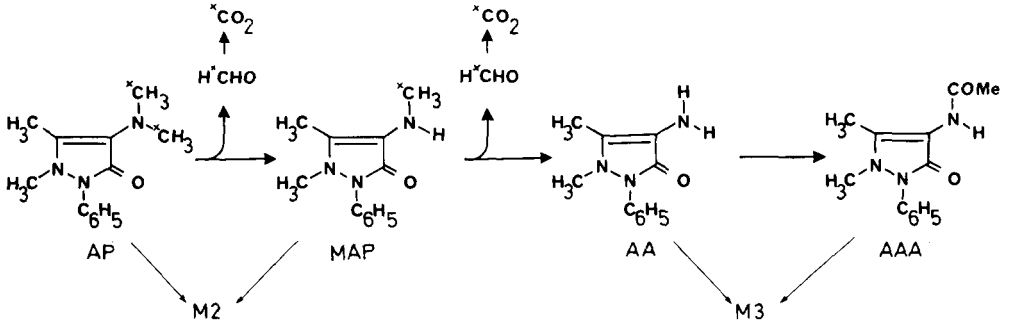

FIG. 1. Amidopyrine metabolism in the rat. Amidopyrine (AP), monomethylaminoantipyrine (MAP), aminoantipyrine (AA), and acetylaminoantipyrine (AAA). The positions of the ^{14}C label is indicated by stars. M2 represents a hypothetical metabolite pool derived from either AP or MAP and hence retains ^{14}C. M3 represents another hypothetical metabolite which is non-radioactive and derived via AA and AAA.

$$V_m = CL_m/k \quad .. \quad .. \quad .. \quad (2)$$

where k is the rate constant for the decline of MAP plasma concentrations with time (calculated by method of least squares).

Parameter values are reported as mean with standard deviation.

RESULTS

Plasma concentration studies

Pretreatment with phenobarbitone resulted in a predictable increase in liver weight-control 0·32 (s.d. 0·04) and treated 0·47 (s.d. 0·03) g kg^{-1} ($P < 0.001$ by t-test). This is accompanied by significant changes in the parameters describing the disposition of both AP (Table 1) and its primary metabolite, MAP (Table 2).

Representative examples of amidopyrine plasma concentration-time profiles in control and phenobarbitone-pretreated rats are shown in Fig. 2. In both cases the semi-logarithmic plots displayed a biphasic decline. In the phenobarbitone-pretreated rats, plasma concentrations were consistently lower than in the control group. The reduction in terminal half-lives is due solely to an increased clearance since the volume of distribution was not altered (see Table 1).

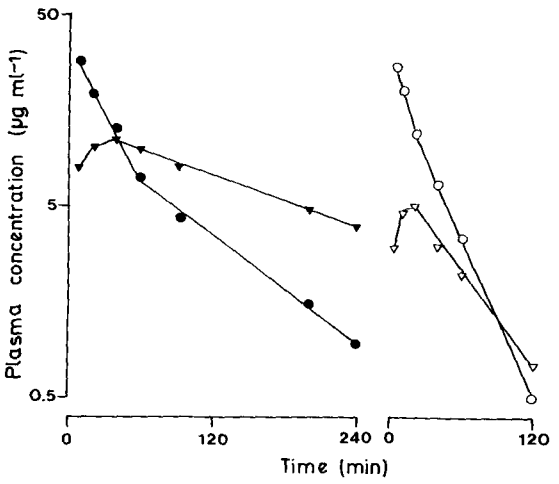

FIG. 2. Plasma concentration-time profiles for amidopyrine (circles) and monomethylaminoantipyrine (triangles) following i.v. administration of amidopyrine to a control rat (open symbols) and a phenobarbitone-pretreated rat (closed symbols). Ordinate: plasma concentrations (μg ml^{-1}). Abscissa: time (min).

Table 1. Pharmacokinetic parameters describing the disposition of amidopyrine (AP) in control and phenobarbitone pretreated rats.

Parameter	Control[a]	Phenobarb. pretreated[b]	Diff. between groups[c]
Clearance (ml min^{-1} kg^{-1})	20·3 s.d. 9·0	36·0 s.d. 5·9	$P < 0.02$
Volume of distribution (litre kg^{-1})	1·39 s.d. 0·35	1·13 s.d. 0·16	N.S.
Terminal half-life (min)	53·6 s.d. 17·8	23·0 s.d. 5·0	$P < 0.02$

[a] Mean with s.d. of 5 rats.
[b] Mean with s.d. of 4 rats.
[c] By t-test, N.S.—not significantly different.

Table 2. Pharmacokinetic parameters describing the formation and disposition of monomethylaminoantipyrine (MAP), a primary metabolite of amidopyrine.

Parameter	Control[a]	Phenobarb. pretreated[b]	Diff. between groups[c] P
Time of maximum plasma concentration (min)	38 s.d. 15	15 s.d. 6	< 0·02
Area under plasma concentration-time curve AUC_m (μg ml^{-1} min)	1737 s.d. 838	373 s.d. 133	< 0·01
Terminal half-life (min)	128 s.d. 32	42 s.d. 13	< 0·005
Clearance of metabolite/fraction formed—Cl_m/f_m (ml min^{-1} kg^{-1})	21·9 s.d. 15·5	82·5 s.d. 26·4	< 0·005
Volume of distribution of metabolite/fraction formed—V_m/f_m (litre kg^{-1})	3·71 s.d. 2·28	5·78 s.d. 2·82	N.S.

[a] Mean with s.d. of 5 rats.
[b] Mean with s.d. of 4 rats.
[c] By t-test.

The plasma concentration-time profile for MAP is also influenced by phenobarbitone pretreatment (Fig. 2). The time taken to achieve maximum concentration is decreased (Table 2), which is consistent with an increased rate of formation. Also a reduction in both the area under the plasma MAP concentration time curve between zero and infinity and the terminal half-life of MAP is apparent (Table 2). Therefore a substantial increase in the ratio, MAP clearance to f_m, results.

Metabolite excretion in urine, bile and breath

The urinary excretion products resulting from the cross-over studies are shown in Table 3. The percentage dose excreted as AP and AAA is significantly reduced following phenobarbitone pretreatment. Four of the animals also show a decrease in the percentage dose recovered as MAP but the large inter-animal variation prevents this reduction achieving statistical significance. No change in AA excretion is apparent. The total dose recoverable as the 4 excretion products is reduced on average by 35% after pretreatment.

Fig. 3 presents the cumulative % $^{14}CO_2$-time plot in a typical rat under control and pretreated condi-

Table 3. Amidopyrine urinary excretion products under control conditions and after phenobarbitone pretreatment.

Urinary excretion product	Percent dose[a] Control	Phenobarb. pretreated	Diff. from control[b] P
Amidopyrine (AP)	6·2 s.d. 3·2	2·9 s.d. 2·2	< 0·05
Monomethylamino-antipyrine (MAP)	4·6 s.d. 4·3	0·7 s.d. 0·3	N.S.
Aminoantipyrine (AA)	15·3 s.d. 8·1	13·8 s.d. 5·0	N.S.
Acetylaminoantipyrine (AAA)	51·8 s.d, 5·9	32·6 s.d. 8·8	< 0·05
Total recovered by hplc	77·5 s.d. 10·5	50·2 s.d. 13·9	< 0·02

[a] Mean with s.d. of 5 rats.
[b] By paired *t*-test.

tions. Exhalation of radioactivity is essentially complete within 5 h. Exhalation rates are faster after drug pretreatment; maximum rate 0·51 (s.d. 0·11)% dose min^{-1} in control and 0·78 (s.d. 0·11)% dose min^{-1} in pretreated ($P < 0.025$ by paired *t*-test). No statistical difference was apparent in the cumulative % radioactive dose recovered in breath (control 61·3 s.d. 3·9, pretreated 58·8 s.d. 6·3) or urine (control 24·8 s.d. 6·4, pretreated 27·8 s.d. 5·1). Calculation of the % radioactivity in the urine as M2 showed no statistical difference between control (20·1 s.d. 8·9) and pretreatment (23·9 s.d. 5·5).

Biliary excretion is a minor route in the elimination of AP and metabolites. Cumulative biliary excretion-time plots for a typical control and pretreated rat is presented in Fig. 4. Most radioactivity is in the form of M2 since the amount of AP and MAP excreted in the bile represents less than 1% of the dose administered. Phenobarbitone increases the rate of biliary excretion from 0·056 (s.d. 0·005) to 0·099 (s.d. 0·018)% dose min^{-1} ($P < 0.025$ by *t*-test) but not the total amount excreted by this route (control 6·2 s.d. 0·8, pretreated 8·4 s.d. 3·2).

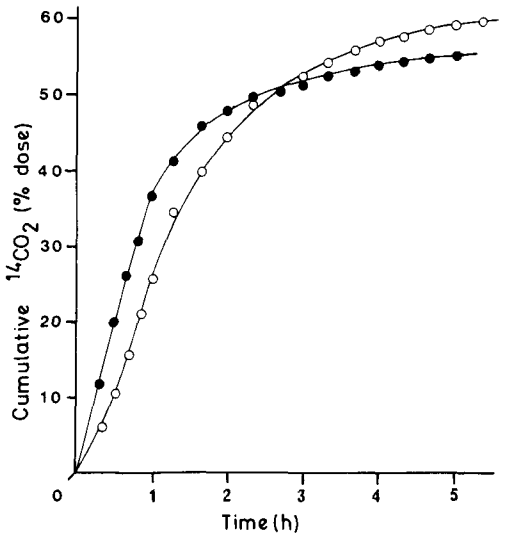

FIG. 3. Cumulative $^{14}CO_2$ exhalation-time curves in a typical rat following i.p. administration of [*N*-dimethyl-^{14}C]amidopyrine under control conditions (○) and following pretreatment with phenobarbitone (●). Ordinate: Cumulative $^{14}CO_2$ (% dose). Abscissa: time (h).

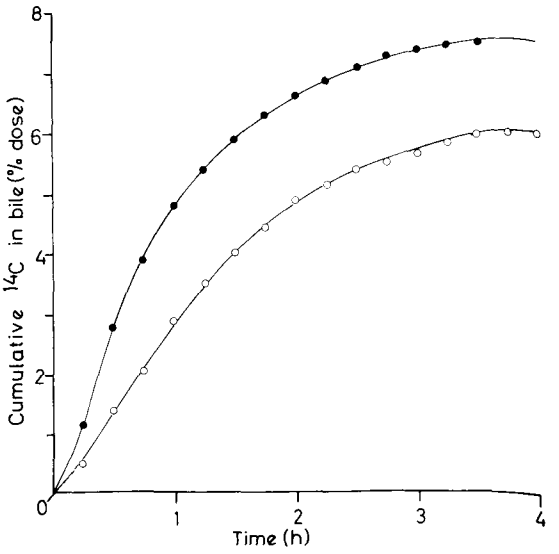

FIG. 4. Cumulative ^{14}C biliary excretion-time curve following i.v. administration of [*N*-dimethyl-^{14}C] amidopyrine to a control rat (○) and a phenobarbitone-pretreated rat (●). Ordinate: cumulative ^{14}C in bile (% dose). Abscissa: time (h).

DISCUSSION

Both AP and its primary metabolite MAP are substrates for *N*-demethylation. Pretreatment with phenobarbitone enhances the clearance of both compounds. These changes are reflected in the plasma half-lives of both compounds and increased rates of excretion of metabolites in both bile and breath results. Enhanced rates of metabolism of AP and MAP cause a decrease in the extent to which these compounds are excreted in the urine. However, since under control conditions the fractions excreted in the urine are small (<10%) the extent of metabolism cannot increase appreciably.

The clearance of MAP may only be calculated from data obtained after AP administration if the fractional conversion of AP to MAP (f_m) is known (see eqn 1). Unfortunately this parameter cannot be calculated directly due to the sequential metabolism of MAP. An estimate of f_m can be obtained by summing the fractions excreted in the urine as MAP,

AA and AAA. For the control studies f_m equals 0·7 by this method. This estimate represents a minimum value for f_m since it does not allow for alternative metabolic pathways for the above 3 compounds (i.e. M2 and M3 in Fig. 1). However, it is consistent with the percentage of the AP dose recovered as $^{14}CO_2$ if a correction is made for the fractional conversion of formaldehyde to CO_2 (Lockwood & Houston 1979). Thus the MAP clearance is approximately 15 ml min^{-1} kg^{-1} in the control situation.

After phenobarbitone pretreatment, calculation of MAP clearance presents more of a problem. The relative importance of demethylation (based on $^{14}CO_2$) and the alternative (M2) metabolic routes for AP and MAP (based on urine and bile recoveries) are not significantly altered by the inducing agent, suggesting no change in f_m. However, a significant reduction in the urinary excretion of AAA occurs after phenobarbitone treatment. This apparent paradox would be resolved if alternative routes of metabolism of AA or subsequent metabolism of AA (i.e. M3 in Fig. 1) exist. Under control conditions M3 must be a minor route, but may become important under induced conditions. If f_m is not altered by phenobarbitone treatment then MAP clearance increases to 58 ml min kg^{-1}. Alternatively, using a f_m of 0·5 (calculated from the sum of MAP, AA and AAA) the MAP clearance is 42 ml min kg^{-1}. In either case the percentage increase over the control MAP clearance (280% for $f_m = 0·7$, 150% for $f_m = 0·5$) is considerably greater than the change in AP clearance (80%).

The results of the effects of phenobarbitone on AP and MAP demethylation are consistent with published in vitro reports. Microsomal studies have shown that formaldehyde production from AP (Gram et al 1968; Sultatos et al 1979) and MAP (Gillette 1963) is enhanced by pretreatment. In the case of the former substrate, at least, this is due to an increase in V_{max} and K_m is not altered. Unfortunately direct in vivo—in vitro correlations by the procedures outlined by Rane et al (1977) could not be attempted. In vitro rates of formaldehyde production are not unique to AP demethylation, some contribution from MAP must occur. Thus in vitro intrinsic clearance (Rane et al 1977) may not be calculated. The in vitro studies of Gram et al (1968) demonstrated that AA production was more susceptible to phenobarbitone pretreatment than formaldehyde production. This suggests that the two demethylation are subject to different regulatory systems. Our in vivo observations support this hypothesis, since the clearance of MAP is enhanced to a larger degree than that of AP.

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