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INHIBITION OF ANTIPYRINE METABOLISM BY β-ADRENOCEPTOR ANTAGONISTS

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1 The effects of two β-adrenoceptor antagonists (propranolol and metoprolol), and of the β-adrenoceptor agonist, terbutaline, on the plasma kinetics of antipyrine were studied in five normal subjects. In addition, the influence of propranolol on the clearance of antipyrine to three of its major metabolites was investigated.

2 At the same level of β-adrenoceptor blockade, assessed by lowering of exercise tachycardia, propranolol decreased antipyrine clearance by 37.3 ± 9.9 s.d. % ($P < 0.001$) and metoprolol decreased it by 18.0 ± 4.7 s.d. % ($P < 0.01$). Terbutaline had no effect on antipyrine clearance. The volume of distribution of antipyrine was unchanged following treatment with all three drugs.

3 Only the metabolic clearance of antipyrine to its 3-hydroxymethyl product was impaired to a statistically significant degree by propranolol. However, four of the five subjects also showed impaired clearance to 4-hydroxyantipyrine and three of the five to norantipyrine after propranolol treatment. In four of the five subjects propranolol lowered the renal clearance of antipyrine.

4 Inhibition of the metabolism of antipyrine by β-adrenoceptor antagonists may be related to their lipid-solubility and extent of metabolism and is independent of their effect on β-adrenoreceptors.

Introduction

Propranolol has been shown to lower the plasma clearance of antipyrine in man (Greenblatt, Franke & Huffman, 1978). The mechanism of this interaction is not clear but is unlikely to be related to changes in hepatic blood flow as antipyrine has a very low hepatic extraction ratio.

We have extended the study of Greenblatt *et al.* (1978) by comparing the effects of propranolol (a non-selective β-adrenoceptor antagonist), metoprolol (a selective β_1-adrenoceptor antagonist) and of terbutaline (a β_2-adrenoceptor agonist) on the kinetics of antipyrine. In addition, the effect of propranolol on the clearance of antipyrine to three of its major metabolites, (4-hydroxyantipyrine, 3-hydroxymethylantipyrine and norantipyrine), was investigated to determine any selectivity of inhibition.

Methods

Subjects

The subjects studied were five healthy males (aged 24–37 years; weight 62–77 kg). They were all non-smokers and were taking no other drugs.

The study was approved by the Hospital Ethics Committee and the nature and purpose of the investigation was explained to each subject.

Experimental design

Each subject took part in two studies.

In Study 1 the subjects received four i.v. infusions of antipyrine, each one separated by a 2–3 week interval. One infusion served as a control and the others followed oral pre-treatment with propranolol, metoprolol and terbutaline, respectively. The order of treatments was randomised.

In Study 2 the control and propranolol pre-treatment antipyrine infusions were repeated in random order. Unlike Study 1, this study included measurements of the urinary metabolites of antipyrine.

Drug dosage

(a) *Antipyrine* Shortly after a light breakfast, 750 mg antipyrine was infused into an antecubital vein at a constant rate over 30 min. The antipyrine solution was prepared in the Hospital Pharmacy and was delivered with an accuracy of ± 0.5% using a syringe pump (Sage Instruments).

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(b) *Propranolol* Propranolol hydrochloride, 80 mg, was given every 12 h for 6 days. In both studies dosing started 3 days prior to antipyrine dosing and continued until all blood samples had been taken.

(c) *Metoprolol* Metoprolol tartrate, 100 mg, was given every 12 h for the same time period as propranolol.

(d) *Terbutaline* Terbutaline sulphate, 5 mg, was given every 8 h for the same time period as propranolol.

Blood and urine sampling

In Study 1 blood samples were taken immediately before and at 0.25, 0.5, 1, 3, 9, 12, 27 and 30 h after the start of the antipyrine infusion. A similar protocol was followed in Study 2 with additional blood sampling at 48 and 72 h and urine collections from 0–12 h, 12–24 h, 24–48 h and 48–72 h.

All blood samples were taken by venepuncture and were collected into heparinised tubes. Plasma was separated and stored deep-frozen at −20°C until assayed. Sodium metabisulphite was added to urine samples in a final concentration of about 1 mg ml^{-1}, to stabilize antipyrine metabolites (Danhof, de Groot-van der Vis & Breimer, 1979).

Assay of antipyrine and its metabolites

Plasma antipyrine was measured by h.p.l.c. according to the method of Eichelbaum & Spannbrucker (1977), with the modification of using a reverse-phase column (C_{18} μBondapak, Waters Associates Ltd, Northwich, Cheshire) and a mobile phase of methanol:water, 50:50. The coefficient of variation of the assay was 2.5% at a concentration of 10 μg ml^{-1}. Urinary antipyrine and its metabolites (free and conjugated) were measured by h.p.l.c. using the method of Danhof *et al.* (1979). The coefficients of variation for the assays were antipyrine, 3% at 10 μg ml^{-1}; 4-hydroxyantipyrine, 1% at 100 μg ml^{-1}; 3-hydroxymethylantipyrine 2% at 50 μg ml^{-1} and norantipyrine 7% at 25 μg ml^{-1}.

Antipyrine was obtained from BDH Chemicals Ltd, Poole, and 4-hydroxyantipyrine from Aldrich Chemical Co. Ltd., Gillingham, Dorset. The latter was purified by recrystallization before use. Norantipyrine was synthesized by the method of Koike *et al.* (1954). 3-hydroxymethylantipyrine was isolated from rat urine by the method of Yoshimura, Shimeno & Tsukamoto (1968) with additional purification by preparative h.p.l.c.. The identity and purity of the metabolites was verified by N.M.R. spectroscopy, mass spectroscopy, elemental analysis and melting point determinations.

Conjugated metabolites were hydrolysed with β-glucuronidase/sulphatase (*Helix pomatia*, type H-1, Sigma, London) under previously optimised conditions.

Pharmacokinetic analysis

Antipyrine clearance was calculated from the dose divided by the area under the plasma drug concentration-time curve extrapolated to infinity (AUC).

The terminal elimination half-life of antipyrine was calculated from the elimination rate constant obtained by least squares regression analysis of plasma drug concentrations from 3 h onwards. An apparent volume of distribution was calculated by dividing the dose by the plasma antipyrine concentration extrapolated to zero time using the terminal elimination constant. The error in calculation arising from neglect of the infusion and distribution phases was negligible.

The plasma clearance of antipyrine associated with the formation of a metabolite (CL_m), was calculated from:

$$CL_m = \frac{Ae\,[m(72)].CL}{fe\,(m).D} \qquad (1)$$

where Ae [m(72)] = the amount of free plus conjugated metabolite excreted up to 72 h in the urine, at which time excretion was virtually complete; CL = total plasma antipyrine clearance; fe(m) = the fraction of metabolite (free plus conjugate) available for urinary elimination (assumed to be unity); and D = the dose of antipyrine. The renal clearance of antipyrine was calculated from the amount excreted unchanged in the urine after 72 h divided by the AUC.

Assessment of β-adrenoceptor blockade

The percentage decrease in exercise heart rate following a 1 min step test performed before and on the third day of treatment with β-adrenoceptor blocker was used to assess the extent of β-adrenoceptor blockade. In the control periods the exercise heart rates were between 145–155 beats/min.

Statistical analysis

Group means from Study 1 were compared by two way analysis of variance in which treatment and order of drug administration were the variables. Group means from Study 2 were compared using Student's paired *t*-test. Correlations were tested using Spearman's analysis. A value of $P < 0.05$ was taken to indicate statistical significance.

Table 1 Parameters describing the plasma kinetics of antipyrine: Study 1

Parameters	*Subject*	*Control*	*Propranolol*	*Metoprolol*	*Terbutaline*
Clearance ($l\,h^{-1}$)	1	2.44	1.45	2.00	2.12
	2	3.04	1.89	2.27	2.72
	3	3.18	1.59	2.60	2.89
	4	2.27	1.76	1.99	2.42
	5	3.56	2.30	2.98	4.17
	Mean	2.90	1.80*†	2.37**	2.86
	s.d.	0.48	0.29	0.38	0.70
Volume of distribution (l)	1	42.1	37.7	39.8	39.2
	2	39.7	48.9	46.3	46.5
	3	48.1	46.5	47.6	47.7
	4	36.4	49.4	42.0	35.0
	5	49.0	42.7	41.1	43.6
	Mean	43.1	45.0	43.4	42.4
	s.d.	5.4	4.9	3.4	5.3
Elimination half-life (h)	1	12.3	19.0	14.3	13.7
	2	9.7	17.3	13.0	12.5
	3	10.7	20.6	13.2	11.5
	4	11.4	17.7	14.2	10.7
	5	9.2	13.6	9.6	7.4
	Mean	10.7	17.6*+	12.9**	11.2
	s.d.	1.3	2.6	1.9	2.4

* Significantly different from control $P < 0.001$
** Significantly different from control $P < 0.01$
\+ Significantly different from metoprolol $P < 0.001$
† Significantly different from metoprolol $P < 0.01$

Results

Study 1

Propranolol decreased antipyrine clearance by 37.3 ± 9.9 s.d. % and metoprolol decreased it by 18.0 ± 4.7 s.d. % (Table 1). The difference in the effect of the two agents was statistically significant ($P < 0.01$) yet the mean percentage decrease in exercise heart rate that they produced was similar (propranolol 28 ± 7 s.d. %; metoprolol 30 ± 4 s.d. %). Terbutaline had no effect on antipyrine clearance (Table 1). The volume of distribution of antipyrine was unchanged following all treatments (Table 1). Therefore, the changes in antipyrine clearance produced by propranolol and metoprolol were also reflected in increased antipyrine elimination half-lives (Table 1).

Treatment order was without influence on the changes in antipyrine clearance.

Study 2

Propranolol treatment decreased antipyrine clearance by 32.3 ± 17.0 s.d. %, increased its elimination half-life by 35.0 ± 6.1 s.d. % and did not alter its volume of distribution (Table 2). Although mean values of antipyrine clearance were lower in Study 2 compared to Study 1 for both control and propranolol treatments, the differences were not statistically significant.

Only the metabolic clearance of antipyrine to its 3-hydroxymethyl product was impaired to a statistically significant degree by propranolol (Figure 1). However, four of the five subjects also showed impaired clearance to 4-hydroxyantipyrine and three of the five to norantipyrine after propranolol treatment. In four of the five subjects propranolol lowered the renal clearance of antipyrine (Figure 2). Also shown in Figure 1 is the hypothetical change in the clearance of antipyrine to unmeasured products derived by subtracting the sum of measured metabolic clearances and renal drug clearance from total clearance. This change was not statistically significant.

The mean percentage of the antipyrine dose recovered in the urine as unchanged drug and metabolites was 44.8 ± 8.9 s.d. in the control study and 39.7 ± 5.7 s.d. following propranolol treatment. This difference was not statistically significant. The only significant differences in cumulative urinary recoveries were those of 4-hydroxyantipyrine at 12 h ($P < 0.01$) and at 24 h ($P < 0.05$) and of 3-hydroxymethylantipyrine at 24 h ($P < 0.05$), (Figure 2).

Plasma clearance of antipyrine correlated with its metabolic clearance to 4-hydroxyantipyrine (r_s = 0.74; $P < 0.05$) and to 3-hydroxymethylantipyrine (r_s

Table 2 Parameters describing the plasma kinetics of antipyrine: Study 2

Subject	*Clearance* ($l\,h^{-1}$)		*Volume of distribution* (l)		*Elimination half-life* (h)	
	C	*P*	*C*	*P*	*C*	*P*
1	1.64	1.37	35.1	34.3	14.8	17.7
2	2.55	1.65	40.3	36.1	10.8	15.3
3	2.10	1.74	38.5	34.0	12.5	15.2
4	2.42	1.74	34.1	34.5	9.8	13.8
5	3.24	1.77	40.7	33.1	8.8	13.3
Mean	2.39	1.65	37.7	34.4	11.3	15.1
s.d.	0.59	0.17	3.0	1.1	2.4	1.7
P	< 0.025		NS		< 0.001	

C = control; P = propranolol

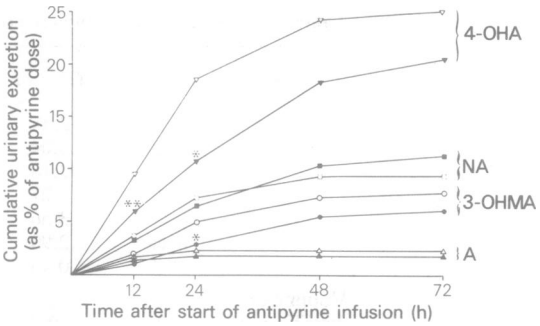

Figure 2 The cumulative urinary excretion of antipyrine and its metabolites (free and conjugated). (Open symbols = control; closed symbols = propranolol treatment. 4-OHA = 4-hydroxyantipyrine; NA = norantipyrine; 3-OHMA = 3-hydroxymethylantipyrine; A = antipyrine. * = $P < 0.05$ and ** = $P < 0.01$ compared with control.)

$= 0.95$; $P < 0.01$) but not to norantipyrine ($r_s = 0.58$; NS) ($n = 10$ in each case).

The percentage decrease in antipyrine clearance caused by propranolol in Studies 1 and 2 was directly related to the control antipyrine clearance (Figure 3).

Discussion

The results confirm the original observation of Greenblatt *et al.* (1978) that propranolol inhibits the activity of the enzymes responsible for the metabolism of antipyrine. In addition we have shown that this phenomenon is not peculiar to propranolol but also occurs to a lesser extent with metoprolol. The observed inhibition is unlikely to be a result of interaction with β-adrenoceptors since despite significant differences in their inhibitory effect on antipyrine clearance, propranolol and metoprolol produced comparable degrees of β-adrenoceptor blockade and terbutaline was without effect on antipyrine clearance. Furthermore, Hermansen (1969) has shown that the optical isomers of propranolol are equipotent in increasing hexobarbitone sleeping time in mice (an indirect measure of drug metabolism),

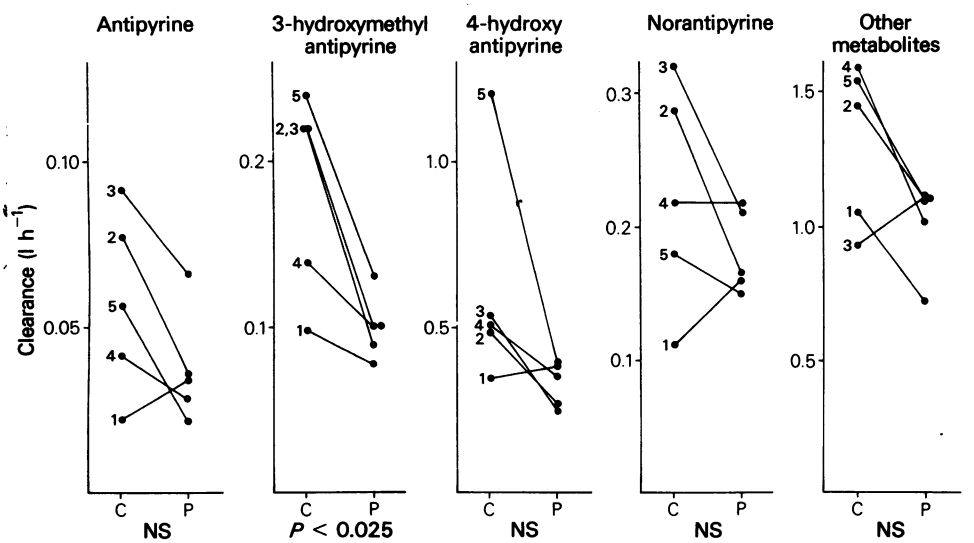

Figure 1 The effect of propranolol on the renal clearance of antipyrine and its clearance to metabolic products. Subject numbers are shown by the control values. Note changes in scales of ordinates. (C = control; P = propranolol treatment.)

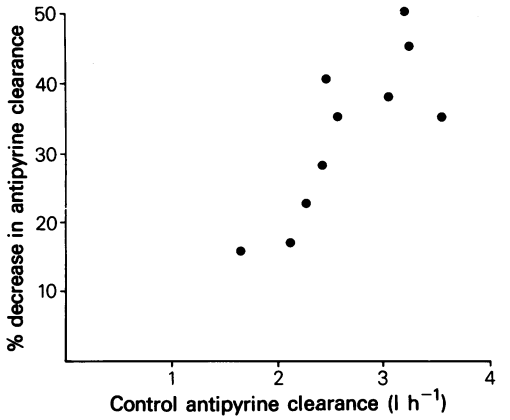

Figure 3 The relationship between the percentage decrease in antipyrine clearance caused by propranolol and the control antipyrine clearance ($n = 10$).

despite differences in their β-adrenoceptor blocking potency.

The difference in the effect of propranolol and metoprolol on antipyrine clearance may reflect the difference in their lipid-solubilities (octanol/water, pH 7 partition coefficients: propranolol 5.39; metoprolol 0.18—Smith & Tucker, 1981), and, thus, affinities for cytochrome P-450 (Facino & Lanzani, 1979). In support of this hypothesis, studies in progress in this laboratory indicate a direct relationship between lipid-solubility in a series of β-adrenoceptor antagonists and their ability to inhibit lignocaine metabolism in rat liver microsomes (Deacon *et al.*, 1981).

The decrease in renal clearance of antipyrine caused by propranolol, observed in all but one of the subjects, is consistent with the known effects of this agent on renal function (Wilkinson *et al.*, 1980).

It has been suggested that the production of the 4-hydroxy- and 3-hydroxymethyl-metabolites of antipyrine is mediated by different forms of cytochrome P-450 (Danhof, Krom & Breimer, 1979; Kahn *et al.*, 1979; Inaba, Lucassen & Kalow, 1980). Our findings do not indicate any clear selectivity in the inhibition of the metabolic pathways of antipyrine and, therefore, of different forms of cytochrome P-450.

Apart from inhibiting the formation of 4-hydroxyantipyrine, norantipyrine and 3-hydroxymethyl antipyrine, propranolol also lowered the metabolic clearance of antipyrine to other unmeasured products in some subjects. This was particularly evident in subject 1, who showed paradoxical rises in the clearance to the 4-hydroxy- and nor-metabolites and only a small fall in the clearance to 3-hydroxymethylantipyrine following propranolol treatment (Figure 1). The fate of the fraction of antipyrine dose unaccounted for as unchanged drug, 4-hydroxyantipyrine, norantipyrine and 3-hydroxymethylantipyrine is only partially known (Zeitz *et al.*, 1978). Also the assumption that the value of fe(m) is unity remains to be established. The 3-hydroxymethyl metabolite is known to be further metabolised to 3-carboxyantipyrine, but this appears to be only a minor pathway (Danhof & Breimer, 1979).

Apart from inhibiting the intrinsic metabolism of antipyrine, propranolol, and to a less extent metoprolol, have also been shown to inhibit that of theophylline (Conrad & Nyman, 1980). Propranolol also inhibits the metabolism of chlorpromazine (Peet, Middlemass & Yates, 1980), but not that of morphine (Brunk, Delle & Wilson, 1974) or quinidine (Ochs *et al.*, 1978).

In view of the widespread clinical use of β-adrenoceptor antagonists, a propensity to inhibit the metabolism of other drugs may be an important consideration in predicting response to drug therapy. This may be of greatest relevance when the more lipid soluble agents are prescribed for patients who are rapid drug metabolisers.

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