ENZYME INDUCTION AND RENAL FUNCTION IN MAN

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1 In a previous study in rats, an increased PAH clearance was found following chronic phenobarbitone administration. These results formed the basis for the present study in which fifteen healthy male volunteers were investigated and the parameters of liver microsomal enzyme activity and renal function were measured.

2 As parameters of liver microsomal enzyme activity, the antipyrine elimination in the plasma, the γ -glutamyl-transpeptidase and the D-glucaric excretion in the urine were measured. Endogenous creatinine clearance, ^{51}Cr -EDTA and ^{125}I -Hippuran clearances were determined as measurements of renal function.

3 No correlation was found between any of the parameters of microsomal enzyme activity and renal function.

4 Of the fifteen volunteers, seven having a mean antipyrine half-life of 13.3 h were given antipyrine (500 mg) daily for 3 weeks. Afterwards all measurements of liver microsomal enzyme activity and renal function were repeated. The antipyrine half-life decreased to 8.5 h, while the antipyrine clearance was increased by about 56%. γ -glutamyl-transpeptidase and D-glucaric acid were also significantly increased, while renal function remained unchanged.

5 Therefore, an increased PAH-clearance, as found in the rat, is not obtained in man following induction of liver microsomal enzyme activity.

Introduction

In a previous study in rats an increased excretion of chlorothiazide was found following the administration of phenobarbitone, 30 mg kg⁻¹ day⁻¹ intraperitoneally for 4 days (Ohnhaus, 1972). This led us to an additional study in rats in which the renal function was investigated. In this study a significant increase in the urine volume and the PAH-clearance was found, while the inulin and the endogenous creatinine clearances did not change (Ohnhaus & Siegl, 1974). Similar results were published by Storch & Bräunlich (1975) who found an increased renal excretion of PAH in rats of different ages following phenobarbitone administration. These changes could be attributed to an increased renal blood flow associated with the increased blood flow the splanchnic organs, especially to the liver, as has been demonstrated following enzyme induction by phenobarbitone and antipyrine (Ohnhaus, Thorgiersson, Davies & Breckenridge, 1971; Branch, Shand, Wilkinson & Nies, 1974). In addition, an active transport system for PAH in the tubular cells brought about by phenobarbitone itself or an increase in the transport proteins by enzyme induction could explain this observation. These findings form the basis for the present experiments in human volunteers in which changes in liver microsomal

enzyme activity and renal function were investigated following enzyme induction by antipyrine.

Methods

Fifteen healthy male volunteers, ranging in age from 24-38 years, were investigated in the present study. All volunteers were informed about the aims of the study and gave their written consent. The health of the volunteers was checked by clinical and laboratory investigations, whereby the following measurements were performed: haemoglobin, haematocrit, white cell count, potassium, chloride, protein, glutamic oxalacetic transaminase, glutamic pyruvate transaminase and alkaline phosphatase. In addition to the chemical urinalysis, sediment examination was performed.

Evaluation of liver microsomal enzyme function

As parameters of liver microsomal enzyme activity, antipyrine elimination and γ -glutamyl-transpeptidase in the plasma and D-glucaric acid excretion in the urine were measured. After an overnight fast, antipyrine (1000 mg) was given

orally. One hour later the volunteers were allowed to have breakfast. Blood for the estimation of the antipyrine concentration in the plasma according to the method of Brodie, Axelrod, Soberman & Levy (1949) was taken at the following time intervals: 0, 3, 6, 9, 12 and 24 h. From these plasma concentrations, the overall elimination rate constant was calculated by means of a linear regression using the method of the least squares of errors. From the overall elimination rate constant, the antipyrine half-life was calculated according to following equation:

$$T_{1/2} = \frac{\log 2}{K_e} \text{ (h)} \quad (1)$$

An approximate value of the apparent volume of distribution, V_d , was calculated from the dose of antipyrine given D and the apparent initial plasma concentration at zero time, C_0 :

$$V_d = \frac{D}{C_0} \text{ (litres)} \quad (2)$$

From the apparent volume of distribution, V_d , and the overall elimination rate constant, K_e , the total body clearance, Cl_A , for antipyrine was calculated according to the following equation:

$$Cl_A = K_e \cdot V_d \text{ (ml/min)} \quad (3)$$

γ -glutamyl-transpeptidase (γ -glutamyl-transferase EC number 2.3.2.2.) in the plasma was measured by the method of Szasz (1969); reference values in males 6-28 U/l, 25°C. For the estimation of D-glucaric acid, three 24 h urine collections were performed at the time when antipyrine elimination was measured, and the mean of these three measurements is given. The determination of D-glucaric acid in urine was carried out as described by March (1963), but using appropriate blanks and highly purified rat liver β -glucuronidase (600'000 Fishman units/g) in the place of the originally described rat liver homogenate.

Measurements of renal function

As measurements of renal function, the glomerular filtration rate and the renal plasma flow were investigated using the endogenous creatinine clearance and the single injection clearance technique as described by Sapirstein, Vidt, Madel & Hanusek (1955), modified by Truniger, Donath & Kappeler (1968). The single injection method is a relatively simple means of simultaneously determining the glomerular filtration rate and the effective renal plasma flow using ^{51}Cr -EDTA (^{51}Cr -ethylendiamino-tetraacetate) and ^{125}I -Hippuran (^{125}I -orthoiodohippuric acid). The ^{51}Cr -EDTA clearance correlates with the inulin clear-

ance, and ^{125}I -Hippuran clearance with the PAH-clearance. After withdrawing blood for the zero value, the two isotopes are injected simultaneously, ^{51}Cr -EDTA in a dose of 3.1 $\mu\text{Ci/kg}$ and ^{125}I -Hippuran in a dose of 0.9 $\mu\text{Ci/kg}$, followed by a flushing dose of approximately 100 ml physiological saline. Blood samples are then taken every 8 min for 88 min. The samples are counted in both the ^{51}Cr and ^{125}I peaks with the appropriate background subtraction and subtraction of the ^{51}Cr activity in the ^{125}I canal (Picker, Autowell nuclear II). The count results are fed into a computer (Digital Equipment Corp. PDP 11/40) which, using a special program (Noelpp, 1974), calculates the glomerular filtration, the effective renal plasma flow and the filtration fraction. The endogenous creatinine clearance was determined using the three 24 h urine collections obtained for the measurement of D-glucaric acid. Creatinine concentrations in the 24 h collections and in the plasma were determined on the Greiner GSA II analyser using a kinetic method with alkaline picrate. From these three determinations a mean endogenous creatinine clearance was calculated. The total renal blood flow (TRB) was calculated according to the following formula:

$$\text{TRB} = \frac{\text{PAH-C}}{1 - \text{HK}} \quad (4)$$

whereby PAH clearance was calculated from the ^{125}I -Hippuran clearance according to the regression published by Tauxe, Maher & Taylor (1971) and the haematocrit estimated in the blood on the day of the experiment. In addition, a constant extraction ratio for ^{125}I -Hippuran was assumed throughout the experiment.

Induction study

Seven volunteers having an antipyrine half-life longer than 10 h participated in this part of the study, in which the liver microsomal enzymes were induced by antipyrine (500 mg daily) for 3 weeks. Afterwards, all investigations mentioned above were repeated.

Statistical analysis

All values obtained were compared by a *t*-test for paired observations, as all variances were found to be homogeneously distributed as tested by an *F*-test. In addition, all results of liver microsomal enzyme activity were compared with those of renal function and the parameters of antipyrine elimination were correlated to the D-glucaric acid excretion both before and after enzyme induction using a linear regression.

Results

The parameters of liver microsomal enzyme activity measured in the fifteen healthy volunteers in the present study are seen in Table 1. The overall elimination rate constant for antipyrine was 0.0623 h^{-1} and the respective half-life 11.9 h. The antipyrine clearance was 44.5 ml/min, and the volume of distribution for antipyrine was 0.59 litres/kg body weight. The mean γ -glutamyl-transpeptidase was $11.4 \mu\text{mol min}^{-1} \text{ litre}^{-1}$, ranging from $6\text{--}28 \mu\text{mol min}^{-1} \text{ litre}^{-1}$ which is within

Table 1 Measurements (mean $\pm$ s.d., $n = 15$) of microsomal enzyme activity

Antipyrine	
Elimination rate constant K_e (h^{-1})	0.062 ± 0.02
Half-life $T_{1/2}$ (h)	11.9 ± 3.5
Volume of distribution V_d (litres/kg)	44.5 ± 13
Total body clearance Cl_A (ml/min)	0.59 ± 0.05
γ -glutamyl-transpeptidase ($\mu\text{mol min}^{-1} \text{ litre}^{-1}$)	11.4 ± 8.4
Glucaric acid ($\mu\text{mol 24 h}^{-1}$)	123 ± 49
($\mu\text{mol mg}^{-1}$ creatinine)	0.079 ± 0.03

Table 2 Measurements (mean $\pm$ s.d., $n = 15$) of renal function

Endogenous creatinine clearance	$120 \pm 27 \text{ ml/min}$
$^{51}\text{Cr-EDTA}$ -clearance	$137 \pm 38 \text{ ml/min}$
^{125}I -Hippuran clearance	$614 \pm 154 \text{ ml/min}$
Renal blood flow	$1138 \pm 261 \text{ ml/min}$

the range of reference values. The mean glucaric acid excretion in the three 24 h urine collections was $123 \mu\text{mol 24 h}^{-1}$ or $0.078 \mu\text{mol mg}^{-1}$ creatinine. The calculated correlation between the overall elimination rate constant of antipyrine, antipyrine half-life and D-glucaric acid excretion in the urine was not found to be significant ($r = 0.38$; $P < 0.1$). In addition, no significant correlation was found between D-glucaric acid excretion in the urine and the total body clearance of antipyrine ($r = 0.34$; $P < 0.1$).

The parameters of renal function are seen in Table 2. The endogenous creatinine clearance was 120 ml/min while the $^{51}\text{Cr-EDTA}$ clearance showed a mean value of 137 ml/min. The ^{125}I -Hippuran clearance, as a measurement of plasma flow, was 614 ml/min, and the calculated renal blood flow 1138 ml/min. All of the values for renal function as measured in the present study were correlated to the results of the measurements of liver microsomal enzyme activity, but no significant correlation was found.

The seven volunteers who took antipyrine (500 mg daily) for 3 weeks had a mean half-life of 13.3 h and showed no side effects from the antipyrine administration. The results of liver microsomal enzyme activity measured following the induction period are shown in Table 3. The overall elimination rate constant of antipyrine increased significantly from 0.053 to 0.081 h^{-1} ($P < 0.005$) and the antipyrine half-life decreased significantly from 13.3 to 8.6 h ($P < 0.005$). In addition, the total body clearance of antipyrine increased from 38.3 to 59.7 ml/min ($P < 0.005$). The γ -glutamyl-transpeptidase was $12.3 \mu\text{mol min}^{-1} \text{ litre}^{-1}$ before and $18.9 \mu\text{mol min}^{-1} \text{ litre}^{-1}$ after induction of the liver microsomal enzyme activity, and this increase was significant in the

Table 3 Evaluation of liver microsomal enzyme function and renal function (mean $\pm$ s.d., $n = 7$) following antipyrine administration

	<i>Before induction</i>		<i>After induction</i>		<i>Significance</i>
Antipyrine					
K_e (h^{-1})	0.053 $\pm$	0.009	0.081 $\pm$	0.006	$P < 0.005$
$T_{1/2}$ (h)	13.3 $\pm$	2.0	8.6 $\pm$	0.6	$P < 0.005$
Cl_A (ml/min)	38.3 $\pm$	6.3	59.7 $\pm$	10.3	$P < 0.005$
V_d (litres/kg)	0.59 $\pm$	0.03	0.6 $\pm$	0.09	NS
γ -glutamyl-transpeptidase ($\mu\text{mol min}^{-1} \text{ litre}^{-1}$)	12.3 $\pm$	8.2	18.9 $\pm$	9.5	$P < 0.005$
D-glucaric-acid ($\mu\text{mol 24 h}^{-1}$)	103 $\pm$	56	161 $\pm$	74	$P < 0.005$
Creatinine clearance (ml/min)	109 $\pm$	30	113 $\pm$	32	NS
$^{51}\text{Cr-EDTA}$ -clearance (ml/min)	141 $\pm$	41	116 $\pm$	38	NS
^{125}I -Hippuran clearance (ml/min)	684 $\pm$	196	658 $\pm$	178	NS
Total renal blood flow (ml/min)	1249 $\pm$	343	1195 $\pm$	326	NS

paired *t*-test ($P < 0.005$). The excretion of D-glucaric acid in the 24 h urine collection was $103 \mu\text{mol } 24 \text{ h}^{-1}$ or $0.0069 \mu\text{mol mg}^{-1}$ creatinine, and these values increased significantly ($P < 0.005$) after the induction period to $161 \mu\text{mol } 24 \text{ h}^{-1}$ or $0.103 \mu\text{mol mg}^{-1}$ creatinine.

In contrast, no parameter of renal function changed after 3 weeks of antipyrine administration, as seen in Table 3. The endogenous creatinine clearance showed nearly identical values of 109 and 113 ml/min before and after the induction period. The ^{51}Cr -EDTA clearance showed a slight decrease from 141 to 116 ml/min, but this decrease was not significantly different. The ^{125}I -Hippuran clearance was also nearly identical on both occasions with values of 684 and 658 ml/min. Accordingly, the calculated values of total renal blood flow were not different (1249 before and 1195 ml/min after antipyrine administration). In comparing the values of the renal function with the values of liver microsomal enzyme activity using a linear regression, no significant correlation was found as in the pre-induction study.

Discussion

The present study in man is based on the observation of an increased PAH-clearance in rats following phenobarbitone administration (Ohnhaus & Siegel, 1974). This was thought to be due to enzyme induction, probably associated with an increased blood flow to the kidneys, since neither increases in protein content nor cytochrome P-450 nor other microsomal enzymes were found in the kidneys following phenobarbitone administration (Jacobsson, Thor & Orrenius, 1970; Feuer, Sosa-Lucero, Lumb & Moddel, 1971). To determine if these changes also occur in man enzyme induction was performed in healthy male volunteers by giving antipyrine (500 mg daily) for 3 weeks. The reason for using antipyrine as the inducing agent is the advantage of its not having such side-effects as are found after giving the doses of phenobarbitone necessary to get an effect on liver microsomal activity. In addition, by stimulating its own metabolism antipyrine avoids other drug interactions, such as competition for enzyme binding sites (Prescott, 1969), which may modify the effects of enzyme induction by one drug on the plasma kinetics of another. However, if the effect on PAH-clearance in the rat kidney is associated with the phenomenon of enzyme induction, the same results should be obtained as both induce an increase in liver blood flow (Ohnhaus *et al.*, 1971), despite the fact that antipyrine and phenobarbitone may be different

types of inducing agents (Orme, Davies & Breckenridge, 1974).

Using the antipyrine dose mentioned above all parameters of liver microsomal enzyme activity were significantly changed, and the changes observed are in agreement with those reported by other authors using antipyrine as an inducing agent, even in higher dosage (Davies, Simmons, Dordoni & Williams, 1974; Whitfield, Moss, Neale, Orme & Breckenridge, 1973). Furthermore, in an additional study in which 1200 mg antipyrine (600 mg two times daily) was given daily for a fortnight, the same results were obtained as in the present study, and the change of the parameters of microsomal enzyme activity measured were to the same extent percentage wise (Ohnhaus, Coninx, Ramos & Noelp, 1976). Similar results in the parameters of microsomal enzyme activity were also obtained using phenobarbitone (180 mg daily) as an inducing agent in healthy volunteers (Hildebrandt, Roots, Speck, Saalfrank & Kewitz, 1975). Therefore, an optimal induction of liver microsomal enzyme activity can be assumed, in spite of the fact that differences in the enzyme inducing capacity were observed in animals and man (Breckenridge, Orme, Davies, Thorgeirsson & Davies, 1972) using different enzyme inducing agents and different dosages. However, the administration of higher doses of enzyme inducing substances in healthy volunteers was not done as the incidence of side effects might be increased.

Despite the fact that an optimal enzyme induction can be assumed, no changes in renal function were found in the present study. This raises the question of whether an enzyme induction or an increased blood flow to the kidneys is responsible for the changes of PAH-clearance observed in rats and whether or not there is an effect of phenobarbitone itself on the tubular transport system which is not associated with enzyme induction. A shortening of the PAH half-life and its accumulation in renal cortical slices is not only observed following phenobarbitone administration in rats but also after administration of other lipid soluble drug which are excreted by the kidneys (Storch & Bräunlich, 1975). These substances are transported by the same carrier as PAH and none have an enzyme inducing capacity on the liver microsomal enzyme activity. Therefore, these observations seem not to be associated with enzyme induction and are probably only specific for rats as no changes in renal blood flow were found in the rhesus monkey after inducing liver microsomal enzyme activity by phenobarbitone and using radioactive microspheres for the measurement of renal blood flow (Branch *et al.*, 1974).

Since increases in the parameters of microsomal enzyme activity were obtained but no changes in

renal function were found following antipyrine administration in the present study, it can be concluded that the findings observed in rats do not occur in man. The results found in rats probably reflect an unspecific effect of phenobarbital itself, which is specific for rats and not associated with

enzyme induction and not even with an increased renal blood flow.

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