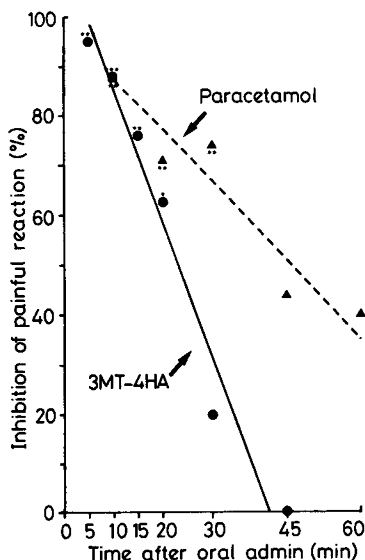

FIG. 1. Activity kinetics for paracetamol (187·5 mg kg^{-1} oral) and 3MT-4HA (500 mg kg^{-1}, oral). Each point represents the mean percentage value for 6 animals calculated from the mean writhing response per group for each time. *, ** and *** indicate $P < 0{\cdot}05$, $P < 0{\cdot}01$ and $P < 0{\cdot}001$ for differences between these values and the values of corresponding control animals (Student's *t*-test).

Jollow (1980), but show that in mice its duration of action is much shorter than the parent compound even at twice the dose.

Since 3MT-4HA is a minor metabolite of paracetamol in man (Klutch et al 1978) and because of its apparent shorter duration of activity shown by the present study, its contribution to the analgesic effect of the parent compound appears to be negligible. The shorter duration of action of this *S*-methyl metabolite of paracetamol requires further investigation.

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Interaction of uridine 5′-diphosphoglucuronic acid (UDPGA) with cytochrome P 450

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It has been well established that many xenobiotics and endogenous compounds are hydroxylated and subsequently conjugated, for example with glucuronic acid, in the endoplasmic reticulum of the liver cell (Dutton 1980). For several substrates glucuronidation plays a decisive role in the extent in which these compounds display toxic properties.

Nemoto & Takayama (1977) reported that during metabolism of benzo[a]pyrene in rat liver microsomes, the addition of UDPGA selectively decreased the amounts of benzo[a]pyrene phenols. Bock (1978) established that the overall rate of benzo[a]pyrene monooxygenase is markedly increased by subsequent glucuronidation. In contrast, Von Bahr & Bertilsson (1971) did not find an effect of UDPGA on the hydroxylation rate of desmethylimipramine. Two tentative explanations have been put forward to explain these data. UDPGA has been thought to provide relief of product inhibition, which is sometimes caused by hydroxylated metabolites. As a result specific cytochrome P 450 catalysed oxygenation reactions can be enhanced considerably by subsequent glucuronidation (Bock 1978; Von Bahr & Bertilsson 1971; Levy & Ashley 1973). A selective inhibition of certain forms of cytochrome P 450 by UDPGA might also be involved (Nemoto & Takayama 1977). The latter has not been studied thus far and is subject of the present investigations.

Methods

Adult Male Wistar rats (approx. 250 g TNO, Zeist) were used. Pretreated animals received either an i.p. injection of 80 mg kg^{-1} phenobarbitone 24, 48 and 72 h before killing. Liver microsomes were prepared as described previously (Bast & Noordhoek 1980), pooled and stored at −70 °C.

Spectral measurements in microsomal suspensions were carried out as described by Bast & Noordhoek (1981). Microsomal protein was assayed according to

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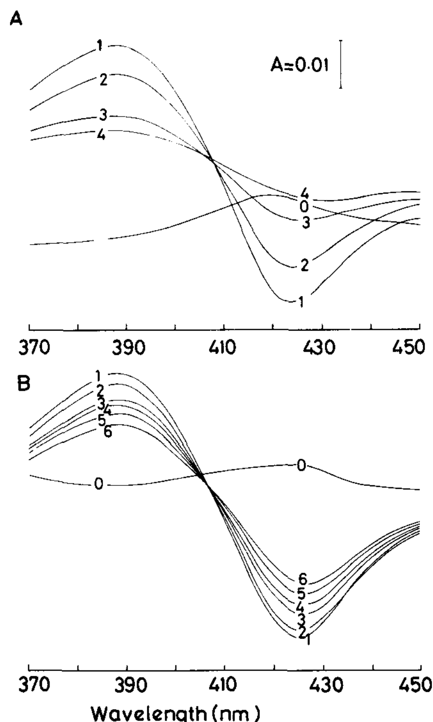

FIG. 1. A. The observed difference spectrum of liver microsomes obtained from phenobarbitone-pretreated rats. After establishing the baseline (0), 4 mM UDPGA was added to the sample cuvette (1). Subsequently hexobarbitone was titrated in the sample and reference cuvette (2–4 are respectively 0·1 mM, 0·5 mM ans 1·0 mM). The cuvettes contained 1.8 nmol cytochrome P 450 ml^{-1}.

B. The observed difference spectrum of liver microsomes obtained from phenobarbitone pretreated rats. After establishing the baseline (0) 1·0 mM aminopyrine was added to the sample cuvette (1). Subsequently UDPGA was titrated in the sample and reference cuvette (2–6 are respectively 0·33, 1·33, 2·0, 3·0 and 4·5 mM). The cuvettes contained 1·8 nmol cytochrome P 450 ml^{-1}.

the method of Lowry et al (1951), with crystalline bovine serum albumin as a standard. UDPGA and UDP were purchased from Boehringer Mannheim. All other chemicals used were of analytical grade purity.

Results and discussion

Binding of UDPGA to cytochrome P 450 in hepatic microsomes of phenobarbitone-pretreated rats causes a conversion in spin state of the haemprotein iron. This is reflected by the type I (substrate) difference spectrum, with a peak at 390 nm and a trough at 425 nm (Fig. 1A). Using difference spectroscopy and titrating the sample cuvette with UDPGA an increase in magnitude of the type I spectrum resulted. By plotting the reciprocal of the peak-to-trough absorbance (ΔA 390 nm–425 nm) versus the reciprocal of the UDPGA concentration, the maximal absorbance (ΔA_{max} μM^{-1} P 450) and the spectral dissociation constant (K_s) are obtained (Schenkman et al 1967) A K_s for UDPGA of 1·2 mM was determined. However, ΔA_{max} was shown to be rather variable. The example in Fig. 1A shows a ΔA μM^{-1} P 450 of 0·03 with 4 mM UDPGA. Moreover Fig. 1A shows that the UDPGA binding spectrum can be decreased by addition of the relatively pure type I compound hexobarbitone. Likewise, aminopyrine (1 mM) is displaced from its type I binding sites by UDPGA (Fig. 1B).

A type I spectrum upon addition to liver microsomes of phenobarbitone pretreated rats was also observed with UDP (data not shown). This suggests that the UDP moiety is responsible for binding to cytochrome P 450. Competition of compounds for the same type I binding sites on cytochrome P 450, generally leads to a mutual inhibition of the metabolism each of the other (Bast & Noordhoek 1981). In this context, conjugation with glucuronic acid is frequently studied in microsomes using very high concentrations (up to 12·5 mM) of UDPGA as cofactor (e.g. Nemoto & Takayama 1977). In studying the effect of subsequent glucuronidation on cytochrome P 450 catalysed oxygenations, a potential inhibitory effect should be considered.

In conclusion, as shown in literature, UDPGA may stimulate oxygenase activity by relief of product inhibition (Bock 1978). In addition UDPGA can interact with type I binding loci of cytochrome P 450. If therefore high UDPGA concentrations are used in-vitro in combination with other substrates one should be mindful of an inhibitory effect of cytochrome P 450 catalysed drug metabolism by UDPGA. A regulatory role of UDPGA in cytochrome P 450 catalysed oxidations of endogenous compounds can also be anticipated, since UDPGA has been found in concentrations of about 0·3 mM in hepatocytes (Singh & Schwarz 1981).

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