J. Pharm. Pharmacol. 1989, 41: 388–393
Received September 20, 1988

Interaction of Nefopam and Orphenadrine with the Cytochrome P-450 and the Glutathione System in Rat Liver

R. LEURS, D. DONNELL*, H. TIMMERMAN AND A. BAST

Department of Pharmacochemistry, Faculty of Chemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands
**3M Riker, Morley Street 1, Loughborough, Leicestershire LE 11 1EP, UK*

Abstract—Nefopam, a cyclic analogue of orphenadrine, exhibits a type I (substrate) and a type II (ligand) interaction with ferri-cytochrome P-450 in control and phenobarbitone induced rat hepatic microsomes respectively. In-vitro metabolism of nefopam in phenobarbitone-induced microsomes leads to the production of a reactive metabolite which complexes with cytochrome P-450. In contrast to the known complexation of orphenadrine, complexation by nefopam can be inhibited by glutathione (GSH, 0·1–1·0 mM). However, in-vivo administration of nefopam to rats does not diminish the GSH content of liver cytosol nor increase oxidized glutathione levels nor alter the activities of GSH transferase and GSH peroxidase. In-vivo administration does not lead to cytochrome P-450 induction nor cytochrome P-450 complexation as has been shown for orphenadrine. Finally, nefopam inhibits the NADPH dependent endogenous H_2O_2 production in both control and phenobarbitone-induced microsomes.

The anti-Parkinson agent orphenadrine has been shown to produce higher steady state plasma levels in man after chronic administration than were predicted on the basis of single dose pharmacokinetics (Labout et al 1982). This increased half life could be explained by the inhibitory effects of a cytochrome P-450 metabolic intermediate (MI) complex, formed during orphenadrine metabolism (Bast et al 1983). In a previous study we showed that NADPH catalysed biotransformation of nefopam, a cyclic analogue of orphenadrine, in phenobarbitone induced rat liver microsomes also leads to MI complex formation via *N*-demethylation to *N*-desmethylnefopam (Leurs et al 1987).

In the present study we investigated the effects of nefopam on the rat hepatic cytochrome P-450 system in more detail; in-vitro we studied the binding characteristics towards cytochrome P-450, MI complexation and effects on the microsomal H_2O_2 production. After in-vivo administration we investigated MI complexation, cytochrome P-450 induction, some cytochrome P-450 catalysed reactions and effects on the hepatic glutathione (GSH) system. In the case of the GSH-system we measured effects on the GSH- and oxidised glutathione (GSSG)-levels and on the activities of the GSH-consuming enzymes, GSH-peroxidase and GSH-transferase in rat liver.

Materials and Methods

Chemicals

GSH, glucose-6-phosphate-dehydrogenase, 1-chloro-2,4-dinitrobenzene (CDNB), NADPH, and catalase were purchased from Sigma Chemical Co. (St. Louis, USA). GSSG, NADP and glucose-6-phosphate were obtained from Boehringer Mannheim (West Germany). *N*-ethyl-maleimide, t-butylhydroperoxide and *o*-phthalicdicarboxyaldehyde (OPT) were obtained from Aldrich (Milwaukee, USA). H_2O_2 was purchased from Merck (Darmstadt, West Germany). Orphenadrine hydrochloride and tofenacine hydrochloride were gifts from Gist-Brocades (Delft, The Netherlands). Nefopam hydrochloride, nefopam-*N*-oxide and *N*-desmethyl-nefopam fumarate were gifts from 3M Riker (Loughborough, UK). All other reagents were of analytical grade purity.

Correspondence to: A. Bast, Department of Pharmacochemistry, Faculty of Chemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.

Preparation of microsomes and pretreatment of animals

Liver microsomes were prepared from male Wistar rats (250–300 g, TNO, Zeist, the Netherlands) as described previously (Bast & Noordhoek 1980). The rats used were either untreated (controls) or received intraperitoneal (i.p.) injections of phenobarbitone, orphenadrine or nefopam dissolved in saline. The phenobarbitone treatment consisted of three daily injections of 80 mg kg^{-1}. Orphenadrine and nefopam were administered for 5 days. On the first day, rats received a single dose of 20 mg kg^{-1} and on the 4 subsequent days 30 mg kg^{-1} daily. The rats were decapitated 3 or 24 h after the last injection.

Spectral measurements

The spectral measurements of the interaction between nefopam and its major metabolites with cytochrome P-450 were performed as described previously (Bast & Noordhoek 1982).

To estimate the concentration of cytochrome P-450 two cuvettes were bubbled with carbon monoxide gas (CO), sodium dithionite was added to the sample cuvette and a difference spectrum was recorded (Estabrook et al 1972).

The metabolic intermediate (MI) cytochrome P-450 complex formation was measured as described (Leurs et al 1987). Ferricyanide oxidation of the microsomes was used to quantitate the cytochrome P-450-MI complex formation after in-vivo administration of the drugs. Microsomes were incubated for 3 min at 37°C with 50 μM potassium ferricya-

nide. Thereafter, CO was bubbled into the cuvette and a dithionite difference spectrum was recorded.

Biochemical assays

The activity of glutathione peroxidase (GSH-Px) was monitored at 340 nm by the disappearance of NADPH (Lawrence & Burk 1978). H_2O_2 (15 mM) was used as a measure of the activity of the Se-dependent GSH-Px and t-butylhydroperoxide (30 mM) was used to establish the activity of both the Se-dependent and Se-independent GSH-Px.

The activity of glutathione transferase was monitored at 340 nm by measuring the formation of the conjugate of glutathione and the substrate CDNB (Mannervik & Guthenberg 1981).

The concentrations of reduced (GSH) and oxidised glutathione (GSSG) were measured by their reaction with OPT at different pH values (Hissin & Hilf 1976). In the case of GSSG the reaction of GSH with OPT was excluded by the addition of the sulfhydryl-group blocking agent, *N*-ethylmaleimide. The OPT-adducts so formed were quantified by the use of fluorescence-spectroscopy.

The effects of nefopam and orphenadrine on the oxidase function of cytochrome P-450 were monitored by measuring hydrogen peroxide formation in liver microsomes. Hydrogen peroxide was determined as formaldehyde, which is formed during the incubation by the peroxidative reaction of catalase (0·25 u mL^{-1}) in the presence of 50 mM methanol (Hildebrandt et al 1978). The amount of formaldehyde was estimated with the Nash reagent (Nash 1953). The incubations were performed in 50 mM phosphate buffer (pH = 7·4), containing 0·1mM EDTA, 4·2 mM $MgCl_2$ and an NADPH-generating system (4·2 mM glucose-6-phosphate, 0·3 u mL^{-1} glucose-6-phosphate dehydrogenase and 0·5 mM NADP). In the following text this buffer will be refered to as buffer A.

N-demethylation of aminopyrine was estimated by measuring the formaldehyde formation, using the Nash reagent. Aminopyrine (0·5 mM) was incubated at 37°C for 5 min in buffer A. The reaction was stopped by the addition of 1 mL 15% trichloroacetic acid (TCA). The concentrations of formaldehyde formed were estimated by determining the absorbance at 415 nm.

Aniline hydroxylation was determined by the amount of *p*-hydroxyaniline formed. Aniline (0·1 mM) was incubated at 37°C for 20 min in buffer A. The reaction was stopped by the addition of 1 mL 15% TCA. After centrifugation, 1 mL supernatant was mixed with 0·5 mL 10% Na_2CO_3 and 1 mL 1% *o*-cresol in 0·1 M NaOH and incubated at 37°C for 30 min. The amount of *p*-hydroxyaniline formed was estimated by determining the absorbance at 640 nm (Mazel 1971).

Protein measurements were carried out as described (Lowry et al 1951), using bovine serum albumin as standard.

Results

Interaction with ferri-cytochrome P-450

Nefopam exhibited a type I (substrate) interaction with ferri-cytochrome P-450 from control hepatic microsomes (Fig. 1A). Analysis of the spectral data, using a Eadie-Hofstee plot (ΔA (peak to trough) divided by the applied substrate concentration versus the ΔA) showed that nefopam produced a biphasic interaction (Fig. 2A). In contrast to the interaction with control microsomes, nefopam exhibits a typical type II (ligand) interaction in phenobarbitone-induced microsomes (Fig. 1B). Transformation of the spectral data into a Eadie-Hofstee plot revealed a linear relationship (Fig. 2B), resulting in a spectral dissociation constant (K_s) of 2·42 ± 0·09 μM and a maximal difference in absorbance (ΔA_{max}) of 0·0078 ± 0·0003 nmol cytochrome P-450^{-1} mL^{-1} (n = 3, ± s.e.m.).

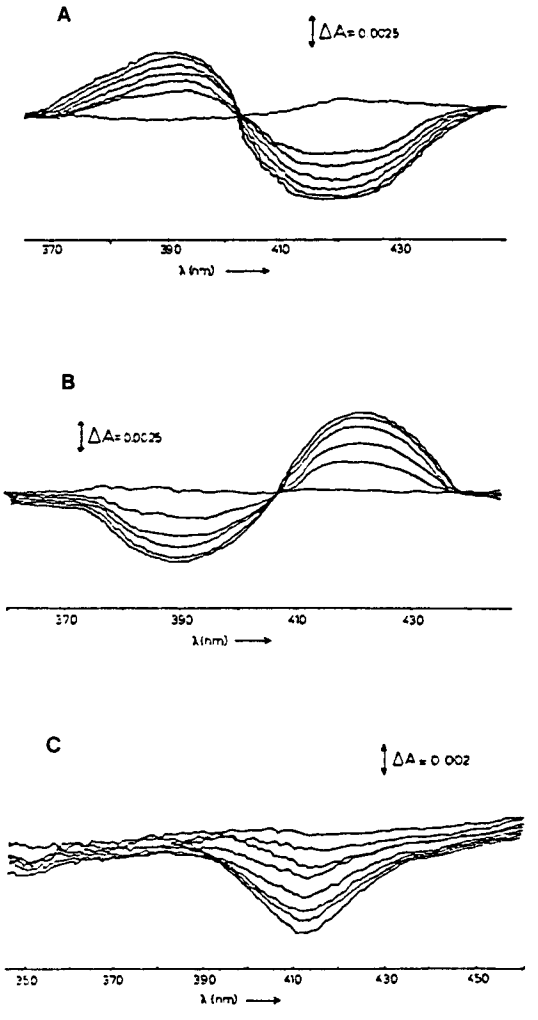

FIG. 1. Difference spectra of several concentrations nefopam (0·8–35 μM) upon interaction with liver microsomes from either control (Fig. 1A) or phenobarbitone-induced rats (Fig. 1B). Fig. 1C shows the interaction of nefopam-*N*-oxide (0·2–6 μM) with microsomes of control rats. In each experiment the concentration of cytochrome P-450 was approximately 2 μM. Representative experiments out of three are shown.

N-Desmethyl-nefopam showed the same phenomenon; a type I interaction with ferri-cytochrome P-450 from control microsomes, but a type II interaction with ferri-cytochrome P-450 from phenobarbitone-induced hepatic microsomes (data not shown). Analysis of the spectral data resulted in both cases in a linear Eadie-Hofstee plot ($r > 0·95$). In the case of control microsomes a K_s-value of 0·34 ± 0·05 μM and a ΔA_{max}-value of 0·0089 ± 0·0005 nmol cytochrome P-450^{-1} mL^{-1} was calculated, whereas in phenobarbitone-induced microsomes a K_s-value of 1·04 ± 0·14 μM and a ΔA_{max}-value

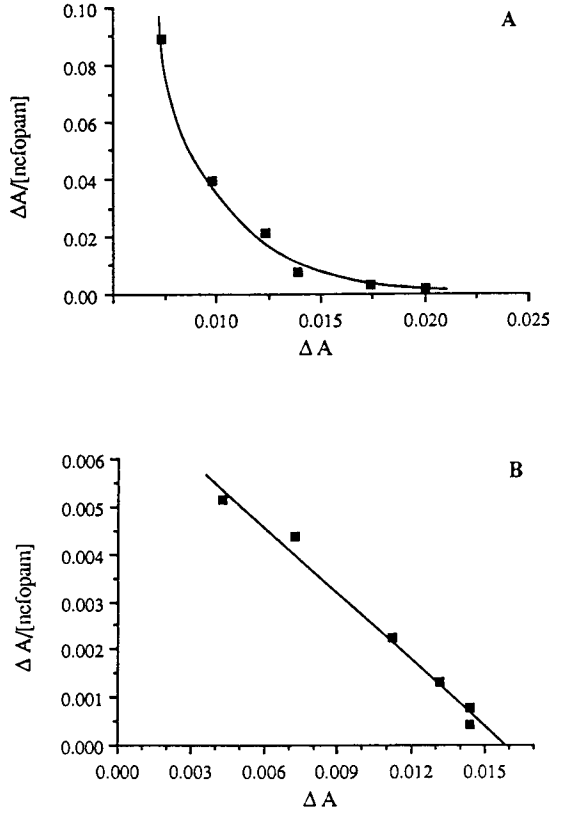

FIG. 2. Eadie-Hofstee plot of the spectral data of nefopam upon interaction with liver microsomes from either control (Fig. 2A) or phenobarbitone-induced rats (Fig. 2B). Representative plots out of three are shown.

of $0{\cdot}0100 \pm 0{\cdot}0003$ nmol cytochrome P-450^{-1} mL^{-1} was found.

In contrast to nefopam and *N*-desmethyl-nefopam, the other metabolite investigated, nefopam-*N*-oxide, did not show either a type I or a type II interaction in control or phenobarbitone-induced microsomes nefopam-*N*-oxide showed a trough in the difference absorbance spectrum at approximately 415 nm (Fig. 1C).

Interaction with ferrous cytochrome P-450

We have shown that a single administration of tofenacine (mono-*N*-demethylated orphenadrine, i.p., 30 mg kg^{-1}) to phenobarbitone-induced rats resulted in metabolites, which sequester cytochrome P-450 in its reduced state (Bast & Noordhoek 1982). This stable inhibitory metabolic intermediate-cytochrome P-450 complex (MI complex) can be detected spectrophotometrically as an absorbance maximum near 455 nm. Incubation of phenobarbitone-induced hepatic microsomes with nefopam also results in the formation of an MI complex, appearing as an absorbance maximum at 459 nm (Leurs et al 1987). In-vivo administration of SKF 525-A, a compound which also forms an MI complex in mice, leads to consumption and depletion of GSH (James & Harbison 1982). Therefore the effect of GSH on the extent of MI complex formation of 33 μM nefopam, 33 μM *N*-desmethylnefopam and 33 μM tofenacine in phenobarbitone-induced microsomes has been investigated. GSH (0·1 mM) reduced the extent of MI complex formation of nefopam by 30%,

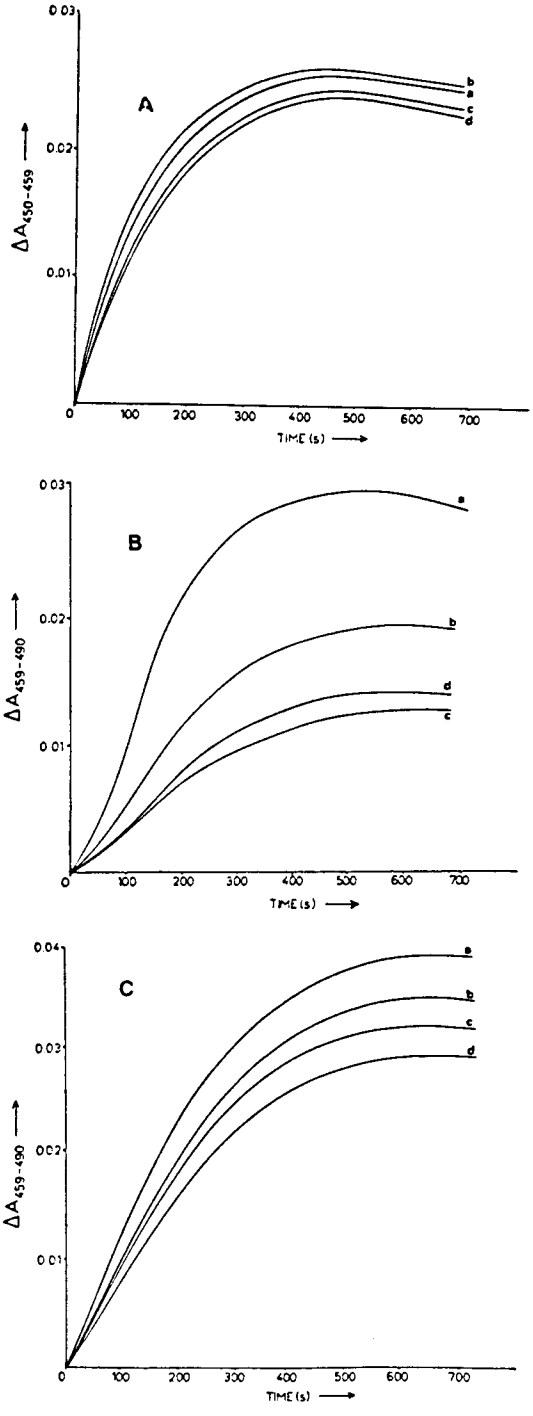

FIG. 3. Time course of the MI complexation elicited during microsomal metabolism of 33 μM of tofenacine (Fig. 3A), nefopam (Fig. 3B) or *N*-desmethyl-nefopam (Fig. 3C) in the presence of 0 mM (a), 0·1 mM (b), 0·5 mM (c) and 1·0 mM (d) GSH. The reaction was started by the addition of NADPH at a final concentration of 400 μM in the sample cuvette. Representive experiments out of four are shown.

whereas 0·5 mM GSH reduced the extent by approximately 50% (Fig. 3B). The same concentrations of GSH also affected the MI complex formation of *N*-desmethylnefopam, although to a much smaller extent (Fig. 3C). However, GSH (0·1–1·0 mM) had no effect on the MI complex formation of tofenacine (Fig. 3A).

Effects on the oxidase function of cytochrome P-450

The effect of nefopam and orphenadrine on the oxidase function of cytochrome P-450 was determined by measuring their effects on basal NADPH-induced endogenous microsomal H_2O_2 production. Fig. 4 shows that both drugs inhibit basal H_2O_2 formation in both control and phenobarbitone-induced rat hepatic microsomes in a concentration dependent manner. Endogenous H_2O_2 formation, differs in control and phenobarbitone-induced microsomes ($2{\cdot}82 \pm 0{\cdot}09$ and $4{\cdot}09 \pm 0{\cdot}09$ nmol min^{-1} (mg protein)$^{-1}$ $\pm$s.e.m., respectively). The greatest inhibition was apparent in phenobarbitone-induced microsomes for both drugs; 50 μM orphenadrine inhibited H_2O_2 formation by 45%, while 50 μM nefopam inhibited the H_2O_2 formation by 35%. The same concentrations were also optimal for inhibition in control microsomes (Fig. 4). H_2O_2 formation was inhibited by 25% and 15%, respectively.

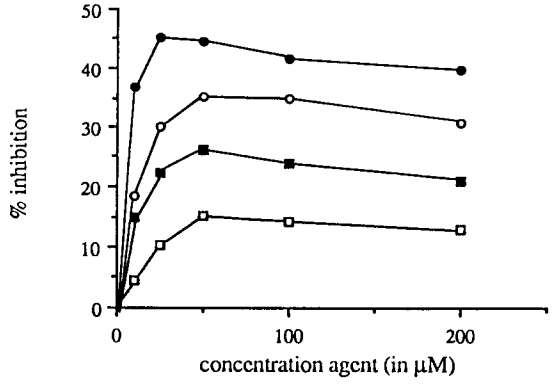

FIG. 4. Concentration dependent inhibition of basal endogenous hydrogen peroxide formation in hepatic microsomes by nefopam (open symbols) and orphenadrine (closed symbols). Liver microsomes of either control (square) or phenobarbitone-induced rats (circle) were used. Each point is the mean of at least three determinations.

Effect of multiple in-vivo administration of nefopam or orphenadrine on cytochrome P-450 and the GSH system of the rat liver

After five daily doses of nefopam as indicated in the Methods section, no increase in the amount of uncomplexed P-450 (CO-reactive) could be detected 24 h after the last administration (Table 1). There was also no change in the total amount of cytochrome P-450 (CO-reactive + MI complex), indicating that administration of nefopam does not result in the formation of an MI complex in-vivo nor does it cause an induction of cytochrome P-450. In contrast to nefopam, orphenadrine administration resulted in an induction of cytochrome P-450 (26%) and the formation of an MI complex in-vivo. Approximately 4% of the total amount of cytochrome P-450 was in the complexed form (Table 1). These results were reflected in the in-vitro *N*-demethylation of aminopyrine, a cytochrome P-450 catalysed reaction. Pretreatment with nefopam had no effect on the biotransformation of aminopyrine, whereas administration of orphenadrine induced aminopyrine metabolism (28%), if the data were expressed per gram of liver (Table 1).

Since the half-life ($t_{\frac{1}{2}}$-value) of nefopam in rat is relatively long ($t_{\frac{1}{2}} = 10{\cdot}5$ h) (unpublished results Riker) compared with orphenadrine ($t_{\frac{1}{2}} = \pm 4{\cdot}5$ h) (Hespe & Kafoe 1970) it was unlikely that the lack of an MI complex formation in-vivo by nefopam was the result of rapid clearance. However, we eliminated this possibility by measuring cytochrome P-450-levels (CO-reactive and total amount) in microsomes, prepared from rats, which had been killed 3 h after the last nefopam administration. At this time plasma levels of nefopam should be higher than after 24 h. Also in this case no

Table 1. The influence of multiple dosage administration of orphenadrine and nefopam to rat (i.p.) on cytochrome P-450 levels, some cytochrome P-450 catalysed reactions (ex-vivo) and on the GSH system of the rat liver 24 h after the last administration.

	Pretreatment		
	Saline	Orphenadrine	Nefopam
Cytochrome P-450			
nmol cytochrome P-450 (total) g^{-1} liver	$18{\cdot}2 \pm 0{\cdot}8$	$24{\cdot}0 \pm 1{\cdot}5$*	$18{\cdot}1 \pm 2{\cdot}0$
nmol cytochrome P-450 (CO reactive) g^{-1} liver	$18{\cdot}3 \pm 0{\cdot}8$	$23{\cdot}0 \pm 1{\cdot}3$*	$19{\cdot}1 \pm 2{\cdot}0$
Aminopyrine			
nmol HCHO g $liver^{-1}$ min^{-1}	$76{\cdot}3 \pm 2{\cdot}7$	$97{\cdot}4 \pm 6{\cdot}1$*	$78{\cdot}7 \pm 1{\cdot}8$
nmol HCHO nmol P-450^{-1} min^{-1}	$4{\cdot}2 \pm 0{\cdot}1$	$4{\cdot}3 \pm 0{\cdot}3$	$4{\cdot}3 \pm 0{\cdot}4$
Aniline			
nmol *p*-OH-aniline g $liver^{-1}$ min^{-1}	$3{\cdot}4 \pm 0{\cdot}3$	$3{\cdot}3 \pm 0{\cdot}2$	$3{\cdot}2 \pm 0{\cdot}2$
nmol *p*-OH-aniline nmol P-450^{-1} min^{-1}	$0{\cdot}18 \pm 0{\cdot}02$	$0{\cdot}17 \pm 0{\cdot}01$	$0{\cdot}18 \pm 0{\cdot}02$
GSH			
μmol reduced GSH g $liver^{-1}$	$3{\cdot}9 \pm 0{\cdot}5$	$4{\cdot}1 \pm 0{\cdot}4$	$4{\cdot}3 \pm 0{\cdot}4$
μmol GSSG g $liver^{-1}$	$0{\cdot}55 \pm 0{\cdot}05$	$0{\cdot}68 \pm 0{\cdot}08$	$0{\cdot}56 \pm 0{\cdot}09$
GSH-peroxidase			
μmol NADPH g $liver^{-1}$ min^{-1} (Se-dependent)	$20{\cdot}1 \pm 1{\cdot}3$	$20{\cdot}1 \pm 0{\cdot}9$	$21{\cdot}4 \pm 2{\cdot}3$
μmol NADPH g $liver^{-1}$ min^{-1} (Se-dep. + non-Se-dep.)	$29{\cdot}3 \pm 2{\cdot}0$	$27{\cdot}8 \pm 0{\cdot}3$	$28{\cdot}3 \pm 1{\cdot}9$
GSH-transferase μg $liver^{-1}$ min^{-1}	114 ± 11	113 ± 9	114 ± 11

All incubation conditions are described in the Materials and Methods section. Data are expressed as the mean of the result of six animals ($\pm$s.e.m.). The results have been calculated per gram of liver (wet weight) and per nmol total cytochrome P-450 (CO reactive + MI complex). The results obtained with liver microsomes of rats, which received (i.p.) orphenadrine or nefopam for 5 days were compared with controls receiving saline (i.p.) for 5 days.

* Significance of differences $P < 0{\cdot}02$ (Student's *t*-test).

changes in the amount of uncomplexed cytochrome P-450 (CO-reactive) or total cytochrome P-450 (CO-reactive + MI complex) levels could be detected in comparison with a control group (data not shown).

Finally, the effect of nefopam or orphenadrine administration on the glutathione system of the liver was investigated 24 h after the last administration. Liver cytosol was examined to determine changes in GSH or GSSG levels, whereas liver homogenates were analysed for glutathione transferase and for selenium- and non-selenium-dependent glutathione peroxidase activities because changes in both these activities may affect the defence against oxidative stress or toxic xenobiotics. However, as shown in Table 1 administration of nefopam or orphenadrine had no effect on the glutathione system of the liver 24 h after the last administration.

However, changes in GSH and GSSG levels could also have been missed when measuring 24 h after the last administration. Therefore we also measured the GSH and GSSG levels 3 h after the last administration. At this time no changes in GSH or GSSG levels could be detected either (data not shown).

Discussion

We show that both nefopam and one of its metabolites, *N*-desmethylnefopam, exhibit a dualistic, concentration dependent binding pattern towards rat hepatic cytochrome P-450. In control microsomes a substrate (type I) interaction occurs, whereas in phenobarbitone-induced microsomes a ligand (type II) interaction becomes apparent. Although great care has to be taken when interpreting the calculated K_s-values because of the possibility that the observed spectra might be the result of two types of interaction occurring at the same time, it is clear that both compounds have high affinities towards ferri-cytochrome P-450 from both control and phenobarbitone-induced microsomes. In contrast, the spectral interaction of another metabolite, nefopam-*N*-oxide, only led to a trough.

Previously we showed that cytochrome P-450 mediated metabolism of orphenadrine lead to the production of a reactive intermediate, probably a nitroxide radical or nitroso compound, which irreversibly interacts with reduced cytochrome P-450 (Bast & Noordhoek 1982). This interaction results in an inhibition of the orpenadrine metabolism (Bast et al 1983) and could explain the observed increased steady state plasma concentration of this agent in man after chronic administration (Labout et al 1982). Such an inhibition of cytochrome P-450 via MI complex formation is known for several drugs, e.g. amphetamines (Franklin 1977) or macrolide antibiotics (Pershing & Franklin 1982) and might lead to drug interactions. Recently, we also showed an in-vitro MI complex formation of the cyclic analogue of orphenadrine, nefopam (Leurs et al 1987). This complex absorbs at 459 nm and is formed to an even greater extent during the metabolism of *N*-desmethylnefopam. Together with the observed lag-time of the MI complex formation of nefopam, this suggests a preliminary *N*-demethylation. Although orphenadrine produces only a small amount of MI complex in-vitro, in-vivo administration still leads to a measurable amount of MI complexation. However, in-vivo administration of nefopam does not lead to the production of an MI complex despite the large extent of MI complex formation in-vitro (Table 1). Inhibitory effects on drug metabolism after chronic nefopam administration are therefore not to be expected. This observation may be explained by the proposed substrate inhibition at higher nefopam concentration (Leurs et al 1987) or by the observed effect of GSH. In contrast to orphenadrine, the MI complex formation of nefopam can be inhibited by GSH. These results suggest different metabolic pathways ultimately leading to the MI complex forming species of orphenadrine and nefopam. For several other MI complex forming compounds (e.g. SKF 525-A) in-vivo administration leads to depletion of GSH (James & Harbison 1982), an effect which is not apparent after nefopam administration (Table 1).

In-vivo administration of orphenadrine gives rise to an induction of cytochrome P-450 (Table 1). This induction leads to an enhanced ex-vivo metabolism of aminopyrine when the data are expressed as product formed per gram of liver (Table 1). However, if the data are expressed as product formed per nmol total cytochrome P-450 no difference was observed. Apparently this cytochrome P-450 catalysed reaction is induced to the same extent as the amount of cytochrome P-450. In the case of nefopam administration, no effect on cytochrome P-450 levels or cytocrome P-450 mediated reactions could be observed (Table 1).

Finally, we studied the oxidase function of cytochrome P-450. The origin of endogenous NADPH-dependent H_2O_2 (without any substrate) is not completely clear (Bast 1986). It has been suggested that H_2O_2 originates from the decay of oxycytochrome P-450 by the dismutation of released superoxide anion radicals. Ligand binding, i.e. interaction of nefopam or orphenadrine with the haem-iron of cytochrome P-450, may prevent oxygen activation and thereby H_2O_2 formation as e.g. is known for several H_2-antagonists (Bast et al 1984). This action reduces the oxidative stress on the liver cells. Firstly, H_2O_2 is the precursor for the reactive hydroxyl radical, which can initiate the process of lipid peroxidation leading to membrane damage and GSH consumption. Secondly, H_2O_2 is eliminated by GSH peroxidase, a process which also consumes GSH and thereby diminishes a defence mechanism of the liver. In both control and phenobarbitone-induced microsomes orphenadrine and nefopam inhibit the NADPH dependent endogenous H_2O_2 production. This characteristic leads to a reduction of the oxidative stress and can therefore be regarded as favourable.

Via its oxidase function cytochrome P-450 could indirectly effect the intracellular GSH levels. Moreover the compounds studied may also directly modulate the GSH system of the liver. However, administration of nefopam or orphenadrine does not affect the GSH system of rat liver when measured 3 or 24 h after the last nefopam administration (Table 1).

The datasheet for the analgesic Acupan (nefopam hydrochloride) states that, in dogs, high doses of nefopam (24 mg kg^{-1}, 6–8 times higher than the average human doses), potentiate the hepatotoxicity of high doses of paracetamol (236 mg kg^{-1}). In paracetamol toxicity a disturbance of the thiol-homeostasis and subsequent changes in the calcium homeostasis are thought to contribute to the observed hepatic necrosis (Moore et al 1985). A potentiation of paracetamol-induced hepatotoxicity can occur via several distinct mechanisms, like induction of cytochrome P-450 or

consumption of GSH, a major detoxification route of paracetamol (Rosen et al 1984). However, multiple administration of nefopam does not have an inducing effect on the total amount of cytochrome P-450 and therefore is unlikely to stimulate the biotransformation of other compounds (like paracetamol) and the endogenous H_2O_2 production, leading to GSH consumption.

Although in-vivo administration of nefopam does not lead to the production of an MI complex, probably due to the effect of GSH on the MI complexation, nefopam does not alter the GSH system of rat liver, one of the main routes of defence against oxidative stress and toxic xenobiotics. This can be explained by assuming that only minute amounts of GSH will be consumed during nefopam induced MI complexation. In-vitro MI complexation by nefopam leads to a complexation of 10% of the total amount of cytochrome P-450 (Leurs et al 1987). Therefore, maximally 10% of 18·1 nmol GSH g^{-1} liver will be consumed via in-vivo MI complexation (Table 1). Since the liver contains approximately 4 μmol GSH g^{-1} liver a reduction with 1·8 nmol g^{-1} liver will not be measured. Finally, nefopam inhibits basal endogenous H_2O_2 production and thereby reduces the overall oxidative stress and GSH consumption of rat liver cells. Therefore, our data cannot explain the reported increase in paracetamol toxicity in dogs.

In summary, in this study we have shown that nefopam has high affinities towards rat hepatic cytochrome P-450 and exhibits a dualistic binding pattern. Secondly, we show that in contrast to orphenadrine, in-vitro nefopam induced MI complexation can be inhibited by GSH. In-vivo nefopam administration does, however, not lead to reduced GSH levels. In-vivo administration also does not lead to an induction of cytochrome P-450 levels, as is found after orphenadrine administration, or an altered GSH system. Finally, both nefopam and orphenadrine inhibit basal endogenous H_2O_2 production and will therefore reduce an oxidative stress on the liver cells.

References

Bast, A., Noordhoek, J. (1980) Calculation of competitive inhibition of substrate binding to cytochrome P-450 illustrated by the interaction of d,l-propanolol with d,l-hexobarbital. Biochem. Pharmacol. 29: 747–751

Bast, A., Noordhoek, J. (1982) Spectral interaction of orphenadrine and its metabolites with oxidized and reduced hepatic microsomal cytochrome P-450 in the rat. Ibid. 31: 2745–2753

Bast, A., van Kemenade, F. A. A., Savenije-Chapel, E. M., Noordhoek, J. (1983) Product inhibition in orphenadrine metabolism as a result of a stable cytochrome P-450 metabolic intermediate complex formed during the disposition of mono-N-desmethylorphenadrine (tofenacine) in the rat. Res. Comm. Chem. Path. Pharmacol. 40: 391–403

Bast, A., Savenije-Chapel, E. M., Kroes, B. M. (1984) Inhibition of mono-oxygenase and oxidase activity of rat hepatic cytochrome P-450 by H_2-receptor blockers. Xenobiotica 14: 399–408

Bast, A. (1986) Is formation of reactive oxygen by cytochrome P-450 perilous and predictable. Trends in Pharmacol. Sci. 7: 266–270

Estabrook, R. W., Peterson, J. A., Baron, J., Hildebrandt, A. (1972) The spectrophotometric measurement of turbid suspensions of cytochromes associated with drug metabolism. In: Chignell, C.F. (ed.) Methods in Pharmacology, vol 2, Appleton-Century Crofts, New York, p 303–350

Franklin, M. R. (1977) Inhibition of mixed-function oxidation by substrates forming reduced cytochrome P-450 metabolic-intermediate complexes. Pharmac. Ther. 2: 227–245

Hespe, W., Kafoe, W. F. (1970) Aspects of the bilary excretion of orphenadrine and its N-demethylated derivative, tofenacine, in the rat. Eur. J. Pharmacol. 13: 113–122

Hildebrandt, A., Roots, I., Tjoe, M., Heinemeyer, G. (1978) Hydrogen peroxide in hepatic microsomes. In: Fleischer, S., Packer, L. (eds) Methods in Enzymology, vol 52, Academic Press, New York, p 342–350

Hissin, P. J., Hilf, R. (1976) A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal. Biochem. 74: 214–226

James, R. C., Harbison, R. D. (1982) Hepatic glutathione and hepatotoxicity. Biochem. Pharmacol. 31: 1829–1835

Labout, J. J. M., Thijssen, C. T., Keyzer, G. G. J., Hespe, W. (1982) Difference between single and multiple dose pharmacokinetics of orphenadrine hydrochloride in man. Eur. J. Clin. Pharmacol. 21: 343–350

Lawrence, R. A., Burk, R. F. (1978) Species, tissue and subcellular distribution of non-Se dependent glutathione peroxidase activity. J. Nutr. 108: 211–215

Leurs, R., Donnell, D., Timmerman, H., Bast, A. (1987) Cytochrome P-450 metabolic intermediate complex of nefopam. J. Pharm. Pharmacol. 39: 835–837

Lowry, O. H., Rosenborough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin Phenol reagent. J. Biol. Chem. 193: 265–275

Mannervik, B., Guthenberg, A. (1981) Glutathione transferase (human placenta). In: Jacoby, W. B. (ed.) Methods in Enzymology, vol 77, Academic Press, New York, p 231–235

Mazel, P. (1971) General principles and procedures for drug metabolism in vitro. In: La Du, B. N., Mandel, H. G., Way, E. L. (eds) Fundamentals of drug metabolism and drug disposition. The Williams & Wilkins Company, Baltimore, p 546

Moore, M., Thor, H., Moore, G., Nelson, S., Moldeus, P., Orrenius, S. (1985) The toxicity of acetaminophen and N-acetyl-p-benzoquinone imine in isolated hepatocytes is associated with thiol depletion and increased cytosolic Ca^{2+}. J. Biol. Chem. 260: 13035–13040

Nash, T. (1953) The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. Biochem. J. 55: 416–421

Pershing, L. K., Franklin, M. R. (1982) Cytochrome P-450 metabolic-intermediate complex formation and induction by macrolide antibiotics: a new class of agents. Xenobiotica 12: 687–699

Rosen, G. M., Rauckman, E. J., Ellington, S. P., Dahlin, D. C., Christie, J. C., Nelson, S. D. (1984) Reduction and glutathione conjugation reactions of N-acetyl-p-benzoquinone imine and two dimethylated analogues. Mol. Pharmacol. 25: 151–157