DIFFERENTIAL INDUCTION OF RAT LIVER MICROSOMAL CYTOCHROME-DEPENDENT MONOOXYGENASE AND UDP-GLUCURONOSYLTRANSFERASE ACTIVITIES BY VARIOUS NARCOTIC DRUGS

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ABSTRACT: Chronic administraction of morphine to adult male rats has long been known to lower hepatic cytochrome p-450 content and its dependent mixed-function oxidase activity. Following the treatme.it of adult male rats with morphine, pethidine pentazocine and codeine and also by concomitant administration of naloxone activities of microsomal electron transfer in the adult male rats were examined. In present study, the acute treatment of mature male rats with a dose of narcotic drugs higher than that used chronically also reduces their hepatic cytochrome p-450. We demonstrate that this acute reduction of cytochrome p-450 in the rat liver is a result of narcotic drug-mediated oxidation and conjugation and is associated with hepatotoxicity of the drug. The blockade of narcotic drug-mediated hepatotoxicity, naloxone significantly inhibited the stimulation of hepatic mixed-function oxidase evoked by narcotic drugs. These narcotic drug-mediated effects are largely prevented by concomitant administration of naloxone, a morphine antagonist.

Key words: UDP-glucuronosyltransferase, Cytochrome p-450 and b_5 mixed-function oxidase, Narcotic drug

INTRODUCTION

The liver plays a major role in the metabolic elimination of drugs and other foreign compounds. In many cases, this involves sequential reaction catalyzed by enzyme located in the endoplasmic reticulum of the hepatocyte which convert lipophilic substrates into water-soluble and, thus, more easily excretable metabolites. "Phase I" reactions involve oxidation of the xenobiotic catalyzed by the cytochrome p-450 and monooxygenase system followed by "Phase II" reaction in which the hydroxylated metabolite is conjugated with a polar ligand, often with glucuronic acid, catalyzed by

the UDP-glucuronosyltransferase (GT) (Kasper, 1980).

A key property of the cytochrome p-450 and the UDP-glucuronosyltransferase (Conny, 1967; Dutton, 1966) and a basis for the classification of these enzymes is that they are selectively inducible by xenobiotics and that their activities may be characterized not only by substrate specificities but also by the profile of their inducers. It is well established that inducing agents of the 3-methylcholanthrene (MC) or phenobarbital-type (Conny, 1967) cause a selective increase of distinct forms of microsomal cytochrome p-450 and thus stimulate different monooxygenase reaction. (Nebert, 1980; Hougen, 1976). Conjugation with glucuronic acid catalyzed by UDP-glucuronosyltransferase is quantitatively the most important phase II reaction (Dutton, 1980). Similar to cytochrome p-450 the GT probably consist of a family of closely related enzyme forms with differing substrate specificity and inducibility (Bock, et al., 1973; Wighart, 1978).

In the present studies, selectivity of various inducers of GT was investigated with regard to multiple enzyme forms and compared with their effects on monooxygenase reactions.

MATERIALS AND METHODS

Chemicals

Morphine, codeine and pethidine were supplied by Dae-Won Pharmaceutical Company. Pentazocine was obtained from Han-ol pharmaceutical company. NADPH, NADH and horse heart cytochrome C (TYPE III) were purchased from Sigma Chemical Company. Other chemicals used were of the highest quality commercially available.

Treatment of Animals and Preparation Liver Microsomes

Male Wister rats (200 g) were used. Inducing drugs were dissolved in distilled water and given once intraperitoneally. Doses: Morphine (50 mg/kg); pethidine (100 mg/kg); pentazocine (75 mg/kg); codeine (100 mg/kg); naloxone (1 mg/kg); naloxone with morphine (1 mg/kg + 50 mg/kg); naloxone with pethidine (1 mg/kg + 100 mg/kg); naloxone with pentazocine (1 mg/kg + 75 mg/kg); naloxone with codein (1 mg/kg + 100 mg/kg). Animals were decapitated 2 hr after the treatment.

Microsomes were prepared as previously described (Lowry et al., 1951). Microsomal preparations were diluted to 10 mg protein/ml with 0.25 M sucrose containing 5 mM Tris-HCl buffer, pH 7.4, and stored at -20°C for up to 4 weeks. No decrease of GT activities was observed during this time. Monooxygenase activities were determined one week after the preparation of microsomes. Protein content was determined according to the method of Lowry et al. (Stewart, 1987) using bovine serume albumin as protein standard.

Assay of UDP-glucuronosyltransferase

Enzyme activity towards p-nitrophenol was determined as described by Stewart (Stewart, 1987). The incubation was carried out in 0.3 ml mixture containing 25 μmole Tris-HCl buffer (pH 7.4) and 0.1 μmole UDP-glucuronic acid. The incubation

was started by adding microsomes (1.0-2.0 ng of protein) and lasted for 10 min. at 37°C. UDP-glucuronosyltransferase activity towards 1-naphthol was also measured.

Monooxygenase Assays

Benzo(a)pyrene monooxygenase activity was determined by the method of Nebert and Gelboin (Stewart, 1987). p-Nitroanisole-O-demethylase was determined by the method of Netter and Seidel (Omura, 1964). Levels of cytochrome p-450 was determined by the method of Omura and Sato (Enomoto, 1973) and cytochrome b_5 was determined by the Enomoto and Sato method (Shenkman, 1972). Cytochrome C reductase assays were performed in 0.1M Tris-HCl pH 7.5, containing 60 μM horse heart cytochrome C per milliter. The reaction was started by adding 0.2 mM NADPH or NADH (final concentration) and the reduction of cytochrome C was monitored at 550 nm minus 541 nm (Yonetani, 1965). An extinction coefficient of 19.1 $mM^{-1} cm^{-1}$ was used to calculate the activity (Kato, 1977).

Lipid peroxidation was measured by determining the amount of malondialdehyde produced in that reaction according to the method of Ohkawa $et\ al.$ (Ohkawa, 1979).

Statistical evaluation of the results was done using student's t-test.

RESULTS

Effects on microsomal enzyme activities and electron transfer system contents Results of the effects of administering the four narcotic drugs on specific activities and contents of hepatic microsomal enzymes involved in O-dealkylation, Benzo(a)pyrene hydroxylation and glucuronidation are shown in Table 1.

The UDP-glucuronosyltransferase activity, determined by using p-nitrophenol, a product of the O-demethylation of p-nitroanisole, was increased nearly 2.5 fold by administration of pethidine, and slightly increased by pentazocine, but it was observed to be declined by morphine treatment. Benzo(a)pyrene monooxygenase activities

Table 1. Effects of morphine, Pethidine, Pentazocine or codeine aministration on rat liver microsomal UDP-glucuronosyltransferase(A) monooxygenase(B) Activities

| Substrate | Control | Enzyme activities (nmoles/min/mg protein) | | | |
		Morphine	Pethidine	Pentazocine	Codeine
(A) p-Nitrophenol	4.03± 0.44	2.25± 0.18*	9.52± 0.60*	4.87± 0.36***	4.08± 0.34
(B) Benzo(a)pyrene	3.33± 0.48	2.41±0.45	15.83± 1.76*	7.05± 2.39*	7.66± 1.94*
p-Nitroanisole-O-demethylase	2.15± 0.46	0.71± 0.02	1.29± 0.44	1.58± 0.48	1.71± 0.30
Cytochrome P-450	1.02± 0.07	0.80± 0.05**	0.97±0.09***	0.95± 0.05	0.83± 0.04**
Cytochrome b_5	0.92± 0.008	0.084±0.004	0.064±0.007	0.072±0.012	0.05± 0.003
NADPH P-450 reductase	44.02± 1.75	83.91± 5.54*	69.90± 2.95*	94.05±11.21*	152.09± 1.85*
NADH b_5 reductase	267.35±18.93	467.27±12.62*	382.77±31.63*	333.82±27.40***	738.18±44.13*

Values are the mean ± S.D. of 6 experiments.
* Significantly different from control value p<0.001.
** Significantly different from control value p<0.01.
*** Significantly different from control value p<0.05.

were increase by administrations of pethidine, pentazocine and codeine, but it was declined by morphine. The concentration of cytochrome p-450 were decreased by the four narcotic drugs. Also the concentrations of cytochrome b_5 were markedly declined by the four narcotic drugs. However, NADPH cytochrome p-450 reductase and NADH cytochrome b_5 reductase activities were preferentially enhanced by the four compounds.

Upon treatment with naloxone, a morphine antagonist, the glucuronidation of p-nitroanisole was declined when compared with control group. Benzo(a)pyrene monooxygenase activity was markedly stimulated, whereas the O-demethylase activity was slightly increased and the concentration of cytochrome p-450 was increased by naloxone alone. And cytochrome b_5, NADH cytochrome p-450 reductase and NADH cytochrome b_5 reductase were enhanced by naloxone. After concomitant treatment with naloxone following i.p. administration of morphine, pethidine, pentazocine and codeine, the activities of microsomal UDP-glucuronosyltransferase, cytochrome p-450 dependent monooxygenase, and electrone transport system contents were compared with those of only naloxone pretreated rats (Table 2). The liver microsomal UDP-glucuronosyltransferase activities were increased significantly by morphine, pentazocine and codeine followed with naloxone when compared with those which were pretreated with naloxone only. Especially the activity of UDP-glucuronosyltransferase was altered by the morphine plus naloxone treatment, but it was stimulated as much for the control groups. Benzo(a)pyrene monooxygenase activities were markedly enhanced by the four compounds investigated with naloxone, but it was not stimulated higher than by only naloxone administration. In contrast to O-demethylase activity were not stimulated but even decreased by all induced compounds with naloxone. The concentrations of

Table 2. Effects of morphine, pethidine, pentazocine or codeine by concomitant administration of naloxone on rat liver microsomal UDP-glucuronosyltransferase(A) and monooxygenase(B) activities

Substrate	Enzyme activity(nmoles/min/mg protein)					
	Control	Naloxone	Naloxone + Morphine	Naloxone + Pethidine	Naloxone + Pentazocine	Naloxone + Codeine
(A) p-Nitrophenol	4.03 ± 0.44	3.20 ± 0.18**	4.05 ± 0.17#	5.36 ± 0.31#	6.83±0.24#	4.13 ± 0.16
(B) Benzo(a) pyrene	3.33 ± 0.48	22.18 ± 1.00	10.99 ± 0.46#	11.68 ± 0.57#	11.36±1.69##	8.80 ± 2.24##
p-Nitroanisole-O-demethylase	2.15 ± 0.46	2.33 ± 0.05	1.26 ± 0.21#	1.69 ± 0.21##	1.95 ±0.06#	1.96± 0.54
Cytochrome P-450	1.02 ± 0.07	1.13 ± 0.09**	1.85 ± 0.11#	1.83± 0.09#	1.60 ±0.05#	1.20 ± 0.06
Cytochrome b_5	0.129±0.008	0.158± 0.019**	0.133± 0.009	0.159± 0.011	0.147±0.004	0.164±0.02#
NADPH P-450 reductase	44.02 ± 1.75	92.18 ± 2.03*	91.98 ±3.62	115.05 ± 3.76#	90.98 ±3.82	60.01 ± 6.45
NADH b_5	267.35 ±18.93	412.42 ± 9.79*	490.03 ±15.92#	469.62±20.47###	507.04±6.01#	577.11 ±39.64#

Values are the mean ± S.D. of 6 experiments.
* Significantly different from control value p<0.001.
** Significantly different from control value p<0.05.
# Significantly different from Naloxone value p<0.001.
Significantly different from Naloxone value p<0.01.
Significantly different from Naloxone value p<0.05.

Table 3. Lipid peroxide content of rat liver microsome

	Malondialdehyde (nmoles/min/mg protein)
Control	1.566 ± 0.051
Morphine	2.344 ± 0.007
Pethidine	$1.466 \pm 0.039^{**}$
Pentazocine	$2.411 \pm 0.092^{*}$
Codeine	$1.986 \pm 0.053^{*}$
Naloxone	1.439 ± 0.139
Naloxone + Morphine	$0.586 \pm 0.001^{\#}$
Naloxone + Pethidine	1.539 ± 0.033
Noloxone + Pentazocine	$1.769 \pm 0.049^{\#\#}$
Naloxone + Codeine	$0.789 \pm 0.091^{\#}$

Values are the mean $\pm$ S.D. of 6 experiments.
* Significantly different from control value $p < 0.001$.
** Significantly different from control value $p < 0.05$.
# Significantly different from Naloxone value $p < 0.001$.
Significantly different from Naloxone value $p < 0.01$.

cytochrome p-450 and cytochrome b_5 were induced by all compounds investigated with naloxone. NADPH cytochrome p-450 and NADH cytochrome b_5 reductase activitiers were stimulated by all four compounds investigated.

Microsomal lipid peroxidation was shown Table 3. Lipid peroxide contents were increased preferentially by four compounds with naloxone but, not by morphine and codeine with naloxone.

DISCUSSION

The enzyme activity is controlled by several factors including the administration of drugs (Conny, 1967) and hormonal imbalances (Kato, 1977; Yamazoe, 1987, 1988; Morgan, 1985). In contrast to a large number of drugs which induce drug metabolism, Kato and Gillette (1965) have shown that the administration of morphine to male rats depressed hepatic drug oxidation. Sladek *et al.* (1974) and Amzel and Van der Hoeven (1978) have reported a decline in cytochrome P-450 and phospholipid contents in the liver microsomal fraction of morphine-treated male rats. In marked contrast to mature male rats, similar treatment of immature male rats or of mature and immature female rats has failed to depress their hepatic cytochrome P-450 content and cytochrome P-450-associated parameters of drug metabolism (Kato, 1965, 1977). Rat hepatic cytochrome P-450 and its dependent enzymes are apparently subject to regulation not only by androgens but also by neurohumoral factors controlled by the hypothalamicpituitary-adrenal axis(Sladek, 1974), it has been suggested that a concerted interaction of these neurohumoral factors may be critical in the regulation of morphine-mediated impairment of hepatic cytochrome P-450.

The hepatic content of cytochrome P-450 is regulated by its turnover, *i.e.* formation and degradation. Formation of cytochrome P-450 requires synthesis of the apocytochrome and of heme, followed by coupling of these two moieties to assemble the

holohemoprotein. Impairment of one or more of the above processes could effectively cause a decrease in the content of cytochrome P-450 in the liver. The observed loss in cytochrome P-450 could be due to accelerated degradation of this hemoproteins. Accelerated degradation of cytochrome P-450 appears to precede stimulation of hepatic microsomal heme oxygenase (MHD) (Bissell, 1974, 1976; Correia, 1978), the rate-limiting enzyme in heme catabolism. This occurs ostensibly because turnover of the cytochrome leaves its heme moiety unscathed, there by reslting in a relative excess of unutilized of "free" heme in the liver.

Recently, biochemical studies have focussed on the balance between the formation of metabolites and the elimination of reactive species. Therefore, we have compared of narcotic drug-dependent oxidation and glucuronidation observed in rat hepatic microsomes. In the present studies, effects of administering a single dose of morphine, pethidine, pentazocine and codein on rat hepatic cytochrome P-450, NADPH-cytochrome P-450 reductase, mixed function oxidase activity and UDP-glucuronosyltransferase activity were examined. The findings described earlier indicate that morphine-treatment of male adult rats elicited decrease in hepatic cytochrome P-450. That the decrease in cytochrome P-450 content was due to reduced mixed function oxidase activity, O-demethylase activity and UDP-glucuronosyltransferase activity. The oxidation and conjugation reactions are tightly coupled, and consequently, for compunds requiring oxidation by mixed function oxidases prior to being conjugated, the rates of their glucuronide formations would depend on the activities of oxidases (Lilienblum, 1982; Yokoda, 1988). Therefore, the greater decrease in p-nitroanisole-O-demethylation activity by the morphine is responsible for the decrease of hepatic cytochrome P-450. These results were observed despite the fact that UDP-glucuronosyltransferase. Notably, the morphine antagonist naloxone, when administered in combination with morphine, effectively increased hepatic P-450, O-demethylase and UDP-glucurnosyltransferase activity. Although antagonistic effects of naloxone have been well documented in the CNS and the peripheral nervous system (Jaffe, 1985), such demonstraction had not been reported for naloxone in the liver. A report essentially confirming the antagonistic effects of naloxone on morphine-mediated increase in

Table 4. Summary of differential effects of various narcotic drugs on rat liver microsomal UDP-glucuro-nosyltransferase and monooxygenase activities

Drug	UDP-glucuronosyltransferase p-Nitrophenol	Monooxygenase p-Nitroanisole	activities B(a)P
Control	4.03 ± 0.44	2.15 ± 0.46	3.33 ± 0.48
Morphine	2.25 ± 0.18	0.72 ± 0.02	2.41 ± 0.45
Pethidine	9.52 ± 0.60	1.29 ± 0.44	15.83 ± 1.76
Pentazocine	4.87 ± 0.36	1.58 ± 0.48	7.05 ± 2.39
Codeine	4.08 ± 0.34	1.71 ± 0.30	7.66 ± 1.94
Naloxone	3.20 ± 0.18	2.33 ± 0.05	22.18 ± 1.00
Naloxone + Morphine	4.05 ± 0.17	1.26 ± 0.21	10.99 ± 0.46
Naloxone + Pethidine	5.36 ± 0.31	1.69 ± 0.21	11.68 ± 0.57
Naloxone + Pentazocine	6.83 ± 0.24	1.95 ± 0.06	11.36 ± 1.69
Naloxone + Codeine	4.13 ± 0.16	1.96 ± 0.54	8.80 ± 2.24

the activities of rat hepatic microsomal cytochrome P-450 and also increased monooxygenase activities and UDP-glucuronsyltransferase activities administered morphine only. Indeed, naloxone, when included in the reaction mixture, significantly protected from the NADPH-dependent lipid peroxidative degradation(Table 3). This protection may be ascribed to successful diversion of NADPH-reducing equivalents into oxidative metabolism of naloxone rather than concurrent microsomal lipid peroxidation as has been observed with other drugs (Wills, 1969, 1971). Alternatively, naloxone binding to cytochrome P-450 may yield a stable complex, less susceptible to lipid peroxidative degradation. These observations argue for a specific naloxone-morphine antagonism in the liver. It has been observed, however, that morphine depletes hepatic glutathione, and that the narcortic antagonists naloxone and naltrexone block this depletion (Amzel, 1980; Gurantz 1981). Therefore, it is tempting to speculate that the observed antagonism between narcotic drugs and naloxone might be merely chemical in nature and occur at the active site of an enzyme converting morphine to a reactive hepatotoxic metabolite. Thus substantial metabolic activation of narcotic drugs to a reactive intermediate would be critically required for expression of its hepatotoxicity and consequent reduction of hepatic cytochrome P-450.

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