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Divergent Regulation of Cytochrome P450 Enzymes by Morphine and Pethidine: A Neuroendocrine Mechanism?

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SUMMARY

We have studied the effects of opiates on cytochrome P450 (CYP) expression in rats and, intriguingly, found that these compounds have divergent effects on this system. Morphine induced a selective down-regulation of hepatic 16α-hydroxylation of androstenedione, which was associated with a reduction in the expression of the 16α-hydroxylation-associated CYP2C11. In addition, other enzymes from the CYP2C, CYP3A, and CYP4A gene families were also suppressed, whereas CYP1A2, CYP2B1, and CYP2E1 were induced. These changes were reflected in the mRNA levels, indicating that they were due to alterations at the transcriptional level. α_2-Adrenoceptor blockade with yohimbine augmented the effects of morphine on proteins identified by antibodies against CYP2C11 and CYP3A, indicating an involvement or influence of the α_2-adrenergic receptor system. In contrast, pethidine had completely different effects. No CYPs were suppressed by this compound. CYP1A2, CYP2B1, CYP2C6, CYP2C7, CYP3A, and CYP4A1 were all induced by pethidine treatment, whereas CYP2C11 and CYP2E1 were unchanged. Our results demonstrate for the first time the divergent effects of pethidine and morphine on the hepatic CYP system, indicating that an opioid receptor mechanism is not involved. These differences are probably mediated by different influences on growth hormone secretion. The mechanisms by which this may occur are discussed.

The CYP superfamily catalyzes the oxidation of numerous drugs and other xenobiotics and a variety of endogenous substrates, such as steroids, fatty acids, and prostaglandins (1). The expression of some of these proteins is constitutive and subject to tissue-specific and developmental regulation, whereas that of others is dependent on environmental factors (1).

The CYP system functions as an adaptive response to environmental challenges and, as a consequence, exposure to a specific chemical often results in the induction of an enzyme that is active in its metabolism. A vast number of compounds have been cataloged for their ability to induce specific CYP enzymes. However, to date there has been little work on opiate compounds such as morphine, which is still one of the cornerstones in the treatment of severe pain.

In a previous report we showed that, in contrast to inducing CYP activity, morphine selectively down-regulates its own *N*-demethylation, whereas glucuronidation is unaffected (2). Subsequent studies revealed an analogous reduction in the *N*-demethylation of its chemical congeners codeine and ethylmorphine, without the corresponding *O*-dealkylation reactions being affected (3). More importantly, a significant suppression of hepatic steroid oxidations, including 16α-hydroxylation of DHA and 17α-hydroxylation of progesterone, was observed.

The mechanisms by which morphine exerts the effects described above are unclear. However, this agent modulates GH secretion patterns and we have observed that administration of GH to male rats causes changes in hepatic drug metabolism similar to those induced by morphine (4, 5). The importance of sex differences in the GH secretion pattern and hepatic metabolism has been thoroughly investigated (6, 7).

The aim of the present work was to determine the selectivity of the effects of two important opiates, morphine and pethidine, on the expression of different CYP species, to establish the mechanisms of regulation. Pethidine was included to determine whether the response to morphine is shared by other members of the opiate group of drugs.

Materials and Methods

Animals and Tissue Handling

Male Sprague-Dawley rats (ALAB, Sollentuna, Sweden), initially weighing 170 g, were used. Body weights were recorded both before the treatment and before sacrifice. All rats were housed under standardized light/dark conditions. Animals were sacrificed on the morning of day 5, 10, or 15, and the livers, kidneys, and adrenal glands were processed

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ABBREVIATIONS: CYP, cytochrome P450; GH, growth hormone; DOCOL, 11-desoxycortisol; DOC, 11-desoxycorticosterone; DHA, dehydroepiandrosterone; bp, base pair(s); SDS, sodium dodecyl sulfate.

immediately for the isolation of microsomes (2) or RNA (8). Protein determination was performed according to the method of Lowry *et al.* (9).

Drug Treatments

Intermittent dosing. Groups of male Sprague-Dawley rats were given single daily intraperitoneal injections of saline solution or escalating daily doses of morphine HCl for 4 days (20–30 mg/kg of body weight daily) or 9 days (20–80 mg/kg of body weight daily), in increments of 10–20 mg/kg every other day. The doses indicate the amount of free morphine and were chosen because they were well tolerated, did not affect the behavior of the rats, and had been used previously by us (2, 4). Other groups were treated for 4, 9, or 14 days with single daily intraperitoneal doses of pethidine, escalated every other day in the following daily steps: 11.5, 17.3, 25.9, 33.7, and 44.0 mg/kg of body weight daily. These doses of pethidine were titrated and found to be the highest tolerable doses that did not cause behavioral symptoms or seizures.

Continuous dosing. In another series of experiments, morphine, clonidine, yohimbine, and combinations thereof were administered with osmotic mini-pumps (Alzet) for 4 days. The daily doses of morphine and yohimbine were 38.75 and 6.0 mg/kg body weight, respectively. Clonidine HCl was administered for 4 days via the drinking water, which contained 2.28 μg/ml. Control animals were sham-operated.

Metabolic Assays

The microsomal metabolism of CYP substrates was measured under conditions of linearity with respect to time and protein concentration. The assays were performed at 37° for 10 min and were stopped by placing the samples on dry ice. The frozen samples were stored at −80° until extraction and analysis. The steroid substrates were dissolved in methanol (2 μl/incubation). Larger volumes of that solvent inhibited the reactions. The *N*-demethylation and *O*-dealkylation of ethylmorphine and codeine were assayed as described previously (10, 11). The 16α-hydroxylation and 5α-reduction of 4-androstene-3,17-dione (androstenedione) were assayed with tritiated substrate and thin layer chromatographic separation of metabolites, as described previously (4). The 16α-hydroxylation of DHA was measured using the method of Taylor *et al.* (12), with small modifications. Briefly, 0.1 ml of the incubation mixture or standards in aqueous solution was diluted with 1.0 ml of distilled water and extracted with 5 ml of dichloromethane by slow shaking for 15 min. The samples were then centrifuged at 2000 rpm for 5 min at 4°. The aqueous phase was aspirated, and 4 ml of the dichloromethane phase were transferred to new tubes and evaporated to dryness under nitrogen at 35–40°. The residues were either analyzed immediately or stored dry at −20°. The samples were dissolved in 200 μl of 65% methanol in water, and 50 μl were taken for high performance liquid chromatographic analysis. Chromatography was performed with a Nova-Pak, phenyl, 4-μm, Radial-Pak cartridge (Millipore), at a flow rate of 0.7 ml/min. Detection was carried out at 205 nm (detection limit, 5–10 ng/ml).

The analysis of 16α-OH- and 17α-OH-progesterone, DOCOL, and DOC was based on the method of Taylor *et al.* (12), with minor modifications. The incubation mixture (0.1 ml) or standards in aqueous solution were diluted with 1 ml of distilled water and extracted with 5 ml of dichloromethane by shaking for 15 min. The samples were then centrifuged at 2000 rpm for 5 min at 4°, the aqueous phase was evaporated under nitrogen at 35–40°, and the residue was stored dry at −20° until analysis. The residue was then dissolved in 200 μl of 65% (v/v) methanol, and 50 μl were injected onto a Nova-Pak, phenyl, 4-μm, Radial-Pak cartridge (Millipore). The flow rate was 0.7 ml/min and the metabolites were detected at 243 nm. Detection limits for all four metabolites were 2.5–5 ng/ml, and the retention times were about 10.5 min, 11 min, 17 min, and 20 min for DOCOL, 16α-OH-progesterone, 17α-OH-progesterone, and DOC, respectively.

Immunoblotting Experiments

Liver and kidney microsomes from individual rats were pooled on an equal-protein basis, and 10 and 20 μg of protein, respectively, were separated by 9% SDS-polyacrylamide gel electrophoresis (13). Adrenal microsomes were from pools of organs from each treatment group, and 15 μg of protein were used for SDS-polyacrylamide gel electrophoresis. After transfer to nitrocellulose membranes, the proteins were probed with polyclonal and/or monoclonal antibodies against the various CYPs, as described previously (13). The protein bands were visualized by ^{125}I-Protein A labeling (13) and semiquantitated using an LKB laser densitometer. The antibodies used were against CYP1A2, CYP2B1, CYP2C6, CYP2C11, CYP2E1, and CYP3A1, as referred to by Forrester *et al.* (13), CYP2C7 (14), and CYP4A1 (15).

Northern Blotting

RNA was isolated according to the method of Chomczynski and Sacchi (8), from pooled liver specimens (about 1 g) from each treatment group. The concentration, purity, and integrity were checked spectrophotometrically and by electrophoresis on a denaturing formaldehyde gel. RNA was separated in 1% agarose gels and transferred to Hybond membranes. Hybridization with CYP cDNA probes was performed according to the method of Meehan *et al.* (16). cDNA probes were labeled by the random priming method (17, 18). After washing in 2× standard saline citrate (0.3 M NaCl, 0.03 M trisodium citrate, pH 7.4) at 65°, without and then with 0.1% SDS and 0.1% sodium tetrapyrophosphate, the membranes were subjected to autoradiography.

The cDNA probes used in this study were as follows. The full length rat cDNAs for CYP2B1 and CYP2C7 and a partial human CYP2E1 cDNA were generous gifts from Dr. M. Adesnik (New York University, New York, NY), Dr. D. W. Russell (University of Texas Southwestern Medical Center at Dallas, Dallas, TX), and Dr. F. J. Gonzalez (National Cancer Institute, Bethesda, MD), respectively. The CYP3A4 cDNA probe was prepared as described previously and consisted of a 727-bp *Eco*RI fragment (19). CYP2C11 mRNA levels were estimated using an antisense oligonucleotide to the 3′ noncoding region (positions 1524–1554) of CYP2C11 (5′-ATGAAGTAGACAGGACCCTCAGAAGAGACA-3′). A specific probe for CYP2C7 (approximately 220 bp) was generated from the 3′ noncoding region of the CYP2C7 cDNA clone, as described previously (20).

Equivalence of loading was checked by ethidium bromide staining of the denaturing formaldehyde-agarose gel and also by the use of a rat glyceraldehyde phosphate dehydrogenase cDNA, as described previously (21). Autoradiographs with hybridized mRNAs were quantitated by use of an LKB laser densitometer.

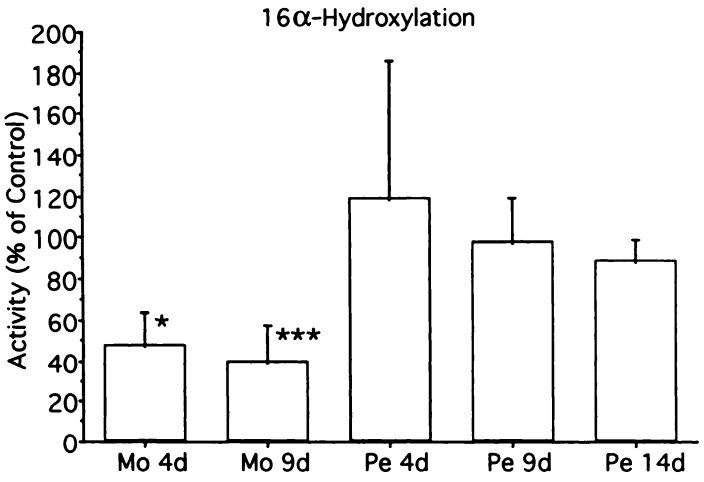
All results are expressed as means ± standard deviations for each group of rats, based on data from five to eight individual liver microsomal preparations or liver specimens. Statistical analysis was performed using Student's *t* test, and the level of significance was either $p < 0.05$, $p < 0.01$, or $p < 0.001$.

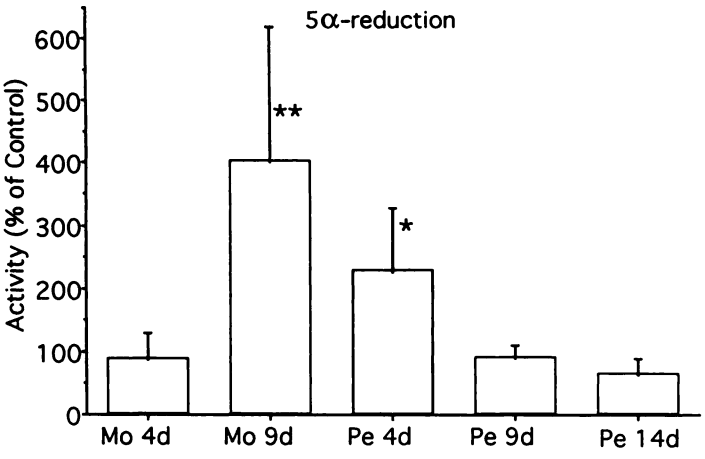
Results

Steroid Metabolism in the Liver

Morphine treatment. The 16α-hydroxylation of androstenedione, a marker for the male-predominant CYP2C11, was decreased to 47% ($p < 0.05$) and 40% ($p < 0.001$) of controls after treatment with morphine for 4 and 9 days, respectively (Fig. 1, *top*). Androstenedione 5α-reductase, a female-predominant activity (6), was increased 4-fold ($p < 0.01$) after 9 days of treatment (Fig. 1, *middle*). These changes represent 2-fold ($p < 0.05$) and 10-fold ($p < 0.01$) increases in the 5α/16α metabolic ratio after 4 and 9 days of treatment, respectively (Fig. 1, *bottom*). The DHA 16α-hydroxylase was also reduced to 42% ($p < 0.05$) and 39% ($p < 0.001$) of controls after 4 and 9 days of treatment with morphine, respectively (Fig. 2).

Pethidine treatment. Pethidine had no effect on the 16α-





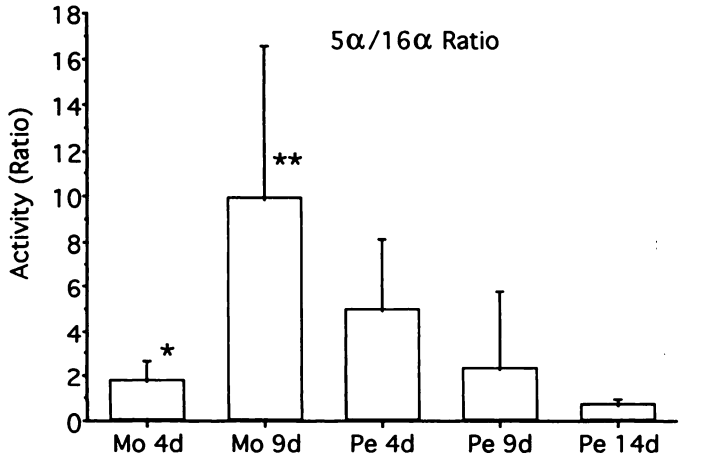


Fig. 1. Androstenedione 16α-hydroxylation and 5α-reduction in liver microsomes from rats treated with morphine (*Mo*) or pethidine (*Pe*). *Abscissa*, treatment times, in number of days; *error bars*, standard deviation; *asterisks*, level of significance of the differences (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). *Top*, average control activities (on day 4, 9, or 14) for the 16α-hydroxylation were 0.60, 1.39, 1.45, 2.38, and 2.99 nmol/min/mg (from *left* to *right*); *middle*, average control activities for the 5α-reduction were 2.72, 1.53, 1.01, 1.25, and 1.92 nmol/min/mg (from *left* to *right*); *bottom*, ratio. The values are based on the results from experiments with five to eight animals in each group.

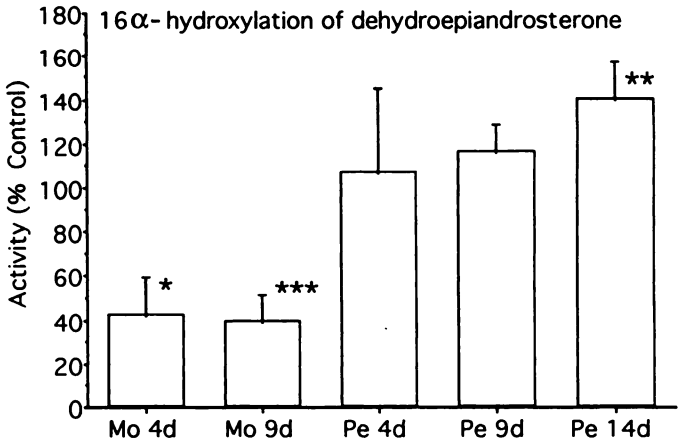


Fig. 2. DHA 16α-hydroxylation in rats treated with morphine (*Mo*) or pethidine (*Pe*). Incubations with hepatic microsomal fractions were carried out as described in Materials and Methods. *Error bars*, standard deviation; *asterisks*, level of significance of the differences (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Control activities were 2.02, 2.62, 2.66, 3.16, and 3.21 nmol/min/mg (from *left* to *right*). The values are based on the results from experiments with five to eight animals in each group.

hydroxylation of androstenedione (Fig. 1, *top*). The 5α-reductase was initially increased (2-fold) at 4 days but then returned to normal at 9 and 14 days (Fig. 1, *middle*). This was not sufficient to cause a significant change in the 5α/16α ratio (Fig. 1, *bottom*). The 16α-hydroxylation of DHA was not affected by pethidine after 4 or 9 days of treatment. However, after 14 days a significant ($p < 0.01$) increase was observed (Fig. 2).

Morphine treatment in combination with yohimbine or clonidine. Clonidine alone did not significantly affect the 16α-hydroxylation or 5α-reduction of androstenedione. Yohimbine produced a 59% decrease ($p < 0.01$) in the 16α-hydroxylation of this steroid (Table 1) but had no significant effect on the 5α-reductase activity or the 5α/16α ratio. The use of these compounds with morphine did not significantly alter the effects caused by morphine alone except for the 5α-reductase activity, which was increased by the morphine/yohimbine combination, although significantly less than by morphine alone.

Effects on Drug Metabolism

Morphine treatment. Morphine caused a marked decrease in the *N*-demethylation of codeine, to 39% ($p < 0.001$) and 58% ($p < 0.01$) of control values after 4 and 9 days of treatment, respectively (Table 2). The *O*-demethylation was significantly reduced only after 4 days (to 63%, $p < 0.001$). Ethylmorphine *N*-demethylation, catalyzed by CYP3A (22) as well as CYP2C11 enzymes (3, 23), was significantly reduced by morphine after 4 days of treatment but returned to normal at 9 days (Table 2).

Pethidine treatment. This did not suppress the *N*-demethylation of either codeine or ethylmorphine (Table 2). Indeed, pethidine treatment significantly increased both of these activities ($p < 0.05$ and $p < 0.05$, respectively), although only after 14 days. The *O*-deethylation of ethylmorphine was significantly reduced after 4 and 9 days of treatment. The *O*-demethylation of codeine was unaffected.

Morphine treatment in combination with yohimbine or clonidine. The suppression by morphine of the *O*-demethylation of codeine (Fig. 3), but not the *N*-demethylation, was potentiated by concomitant administration of yohimbine. On the other hand, clonidine attenuated the effects of morphine

TABLE 1

Effects of yohimbine and clonidine in combination with morphine on androstenedione 16α-hydroxylation and 5α-reduction in liver microsomes

Morphine and yohimbine were administered via osmotic mini-pumps (Alzet) for 4 days, at daily doses of 38.75 and 6.0 mg/kg of body weight, respectively. Clonidine HCl was administered via the drinking water for 4 days; the concentration in water was 2.28 μg/ml.

Treatment	Enzyme activity[a]		Activity ratio, 5α/16α
	16α-Hydroxylation	5α-Reduction	
	nmol/mg/min		
Sham-operated	1.91 ± 0.56	2.05 ± 0.94	1.13 ± 0.48
Morphine	0.27 ± 0.08[b]	7.28 ± 1.35[b]	30 ± 12[b]
Morphine + yohimbine	0.27 ± 0.04[b]	3.50 ± 1.12[c]	22 ± 6.7[b]
Morphine + clonidine	0.33 ± 0.14[b]	7.66 ± 2.82[c]	25 ± 8.7[b]
Yohimbine	0.79 ± 0.19[c]	3.64 ± 2.29	5.4 ± 4.8
Clonidine	1.37 ± 0.56	2.44 ± 1.12	2.09 ± 1.52

[a] All activities are given as nmol of metabolite formed/mg/min (mean ± standard deviation).
[b] $p < 0.001$, compared with sham-operated animals and based on the results from five to eight animals in each group.
[c] $p < 0.01$.

TABLE 2

Effect of morphine or pethidine treatment on codeine and ethylmorphine metabolism

The range of average control activities for the *N*-demethylation of codeine and ethylmorphine and the *O*-dealkylation of same drugs were 3.1–12.4, 8.1–15.2, 2.29–12.8, and 4.3–8.6 nmol/mg/min, respectively. Values for each group represent the average of five to eight individual rats.

	Metabolism				
	Morphine (4 days)	Morphine (9 days)	Pethidine (4 days)	Pethidine (9 days)	Pethidine (14 days)
	% of control				
Codeine *N*-demethylation	39[a]	58[a]	131	126	172[b]
Ethylmorphine *N*-demethylation	41[a]	90	119	120	140[b]
Codeine *O*-demethylation	63[a]	95	83	83	96
Ethylmorphine *O*-deethylation	60[b]	104	73[b]	60[b]	64

[a] $p < 0.001$.
[b] $p < 0.05$.

alone on the *N*-demethylation of codeine but made no difference in the *O*-demethylation, compared with morphine alone. Yohimbine alone reduced the formation of both metabolites.

Effects on Hepatic CYP Enzymes

To establish whether the complex changes in enzyme activity could be associated with changes in the expression of specific CYP enzymes, immunoblots were carried out with liver microsomes pooled from the animals of each treatment group (Fig. 4A). Treatment with morphine decreased the expression of proteins detected with antibodies against the CYP enzymes CYP2C6, CYP2C11, CYP3A1, and CYP4A1 (only 4 days), whereas CYP1A2, CYP2B1, and CYP2E1 were increased. Two bands were seen in the CYP2B blots. They probably represent CYP2B1 and CYP2B2. Our antibody could not distinguish the two isoforms. Similarly, the presence of a doublet for the CYP4A blots is consistent with the experience in our laboratory and other laboratories that polyclonal antibodies often fail to differentiate between CYP4A1, CYP4A2, and CYP4A3. In addition, our antibody against CYP3A1 may be cross-reactive with CYP3A2. Because CYP3A1 is not usually expressed in adult male rat liver (7), the observed band most likely represents CYP3A2. No change was observed for CYP2C7 after 4 or 9 days of morphine treatment. Although in this experiment no change in CYP3A1 was observed at 9 days of morphine treatment, in repeat experiments using a monoclonal antibody to this protein a reduction was observed. A clear reduction of CYP3A1 is also observed in Fig. 4B.

None of the CYP enzymes was suppressed by pethidine. However, there was a marked increase in the levels of CYP2B, CYP2C6, and CYP3A. A moderate increase of CYP1A2, CYP2C7, and CYP4A1 was also seen, with the other enzymes, CYP2C11 and CYP2E1, remaining unchanged.

For most of the enzymes the mobility of the standard did not exactly correspond to that of the studied proteins. We do not understand the reason for this systematic difference, which has been observed before in our laboratory.

The effect of yohimbine and clonidine on the enzyme protein expression was studied for CYP2C11 and CYP3A. In this experiment, morphine was administered continuously for 4 days via osmotic mini-pumps (Fig. 4B). The suppression of these enzymes by morphine alone was markedly potentiated by yohimbine. The morphine-induced suppression of CYP3A was attenuated by clonidine.

Hepatic CYP mRNA Levels

The levels of mRNAs encoding the affected CYP enzymes were measured in rats treated intermittently for 4 days with escalating doses of morphine or pethidine (Fig. 5). Table 3 gives the levels of the CYP-specific mRNAs, normalized on the basis of glyceraldehyde phosphate dehydrogenase-specific mRNA. The levels of mRNAs encoding CYP2C11 and members of the CYP3A gene family, but not that for CYP2B, were markedly decreased as a result of treatment with morphine. CYP2E1- and CYP2C7-specific mRNAs were increased by morphine treatment (Fig. 5). Pethidine did not have any effect on CYP mRNA levels, except for the CYP2B and CYP2C11 mRNAs, which were slightly increased.

Studies in Adrenal Glands and Kidneys

Morphine or pethidine treatment for 4 days did not produce any consistent effects on the 16α-, 17α-, or 21-hydroxylation

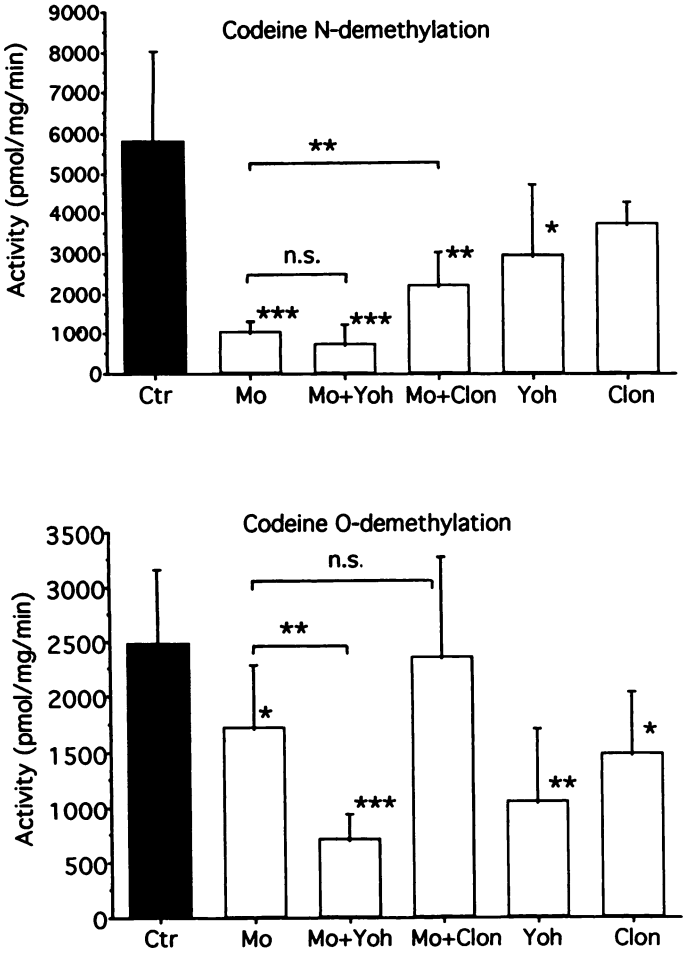


Fig. 3. Codeine *N*- and *O*-demethylation in rats treated with morphine (*Mo*) alone or in combination with yohimbine (*Yoh*) or clonidine (*Clon*). Morphine and yohimbine were administered via mini-osmotic pumps, and clonidine was administered via the drinking water, as described in Materials and Methods. The activities are compared with sham-operated animals. *Error bars*, standard deviation; *asterisks*, level of significance of the differences (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) from control groups (*Ctr*) or from other groups of animals (*brackets*). *n.s.*, not significant. The values are based on the results from experiments with five to eight animals in each group.

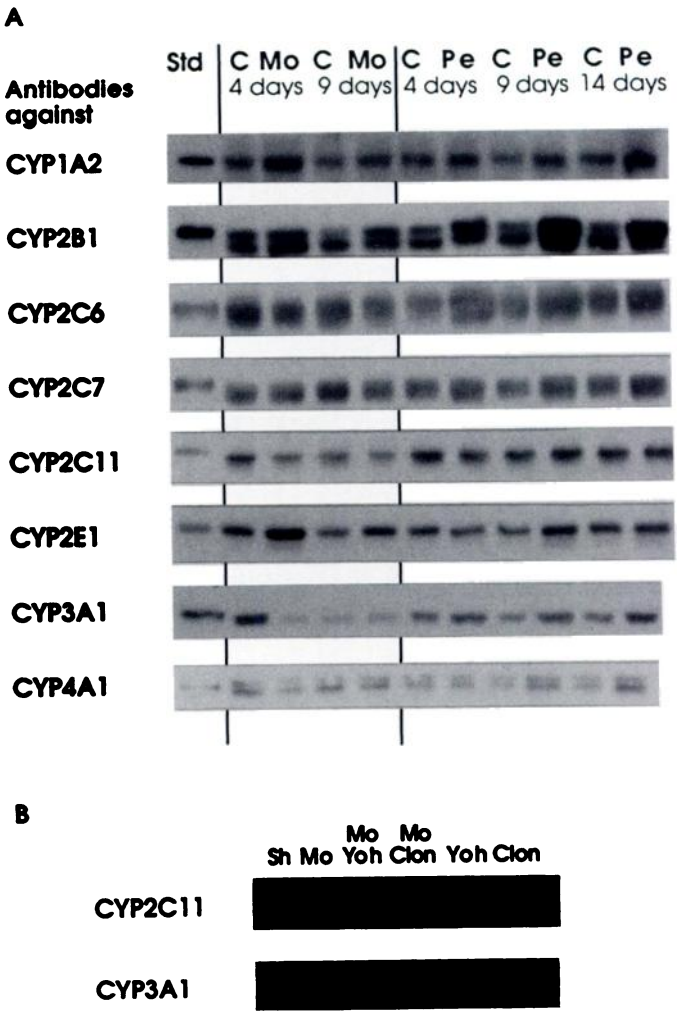


Fig. 4. Effect of morphine or pethidine on hepatic CYP enzyme expression. A, Morphine (*Mo*) (4 or 9 days) or pethidine (*Pe*) (4, 9, or 14 days) administered intraperitoneally, compared with controls (*C*); B, morphine without or with yohimbine (*Yoh*) or clonidine (*Clon*), or yohimbine or clonidine alone, administered for 4 days, compared with sham-operated animals (*Sh*), as described in Materials and Methods. *Std*, purified CYP standards. The two bands in the CYP2B blot probably represent CYP2B1 and CYP2B2. Our antibody could not distinguish the two isoforms. The protein identified by an antibody against CYP3A1 is probably CYP3A2.

of progesterone (formation of 16α-OH progesterone, 17α-OH progesterone, and DOC/DOCOL, respectively) in microsomes from adrenal glands that were pooled for each treatment group (data not shown). Microsomes from pooled adrenal glands for the groups treated with saline solution or morphine for 4 or 9 days were also analyzed by immunoblotting. Of the CYPs studied, only CYP2C7 and CYP2C11 could be detected. No difference in band intensity between treated and untreated rats was observed (data not shown).

Microsomes from kidneys were also analyzed by immunoblotting. No difference in CYP2B1 and CYP4A1 expression between the treated and untreated groups was observed (data not shown).

Discussion

The suppression of selected CYP enzymes by morphine has a variety of important implications. Firstly, it demonstrates the potential of this drug to perturb metabolic pathways such as the endogenous metabolism of steroids. Secondly, if extrapolated to humans, this will be relevant to patients treated with either morphine or congener drugs that are transformed to morphine *in vivo*, such as codeine and ethylmorphine. Such effects may also occur in opiate addicts and in healthy individuals through natural (dietary) morphine exposure. Indeed, various endocrine effects of morphine, such as suppression of testosterone plasma levels and decreased libido, have been described both in animals (24) and in humans (25).

The metabolic effects of morphine appear to be directly attributable to effects on the CYP system. The expression of several CYP enzymes, particularly in the CYP2C gene family, e.g., CYP2C6 and CYP2C11, was down-regulated by morphine given in an intermittent, escalating, dose regimen. Other enzymes (CYP1A2 and CYP2B1) were, however, induced. These results demonstrate the complexity of the morphine effects observed in previous work (4), where we showed that the effects could be mimicked or augmented by GH. This supported the contention that the effects of morphine are mediated via the

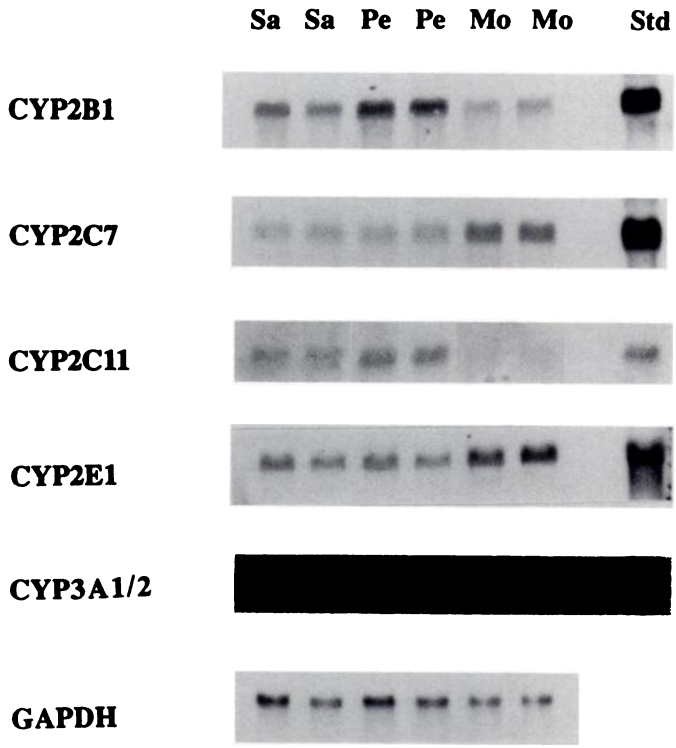


Fig. 5. Comparison of hepatic CYP-specific mRNAs obtained from rats treated for 4 days with saline (*Sa*) or escalating doses of morphine (*Mo*) or pethidine (*Pe*), as described in Materials and Methods, and probed with CYP-specific cDNAs. Duplicate wells were loaded with mRNA from each group of animals. Equivalence of loading was checked with glyceraldehyde phosphate dehydrogenase (*GAPDH*) cDNA.

TABLE 3

Effect of morphine or pethidine treatment on CYP-specific mRNA levels

Rats were treated for 4 days with daily injections (dose escalated after 2 days) of pethidine (11.5 and 17.3 mg/kg of body weight/day) or morphine HCl (20 and 30 mg/kg of body weight/day). Values are given as percentage of values for saline-treated control animals (average of two determinations).

	mRNA levels	
	Pethidine	Morphine
	% of control	
CYP2B1	144	103
CYP2C7	91	320
CYP2C11	215	37
CPY2E1	82	281
CYP3A1/2	50	39

pituitary by a modulation of GH secretion. Because morphine does not seem to exert a direct effect on the pituitary (26), this would appear to be through a hypothalamic mechanism (27). The requirement for an intact pituitary to observe these effects was demonstrated by us in experiments with hypophysectomized rats (4). In such rats there were no effects of morphine treatment on the androstenedione metabolism. It is therefore possible that morphine affects the secretion of GH-releasing factor and/or somatostatin, resulting in changes in the GH secretion pattern. Evidence to support this possibility was provided by Martin *et al.* (28), who demonstrated that morphine stimulates the release of GH-releasing factor.

The induction of CYP2B1 and CYP1A2 does not require pituitary hormones (29, 30) and can result from a direct effect on hepatocytes. However, there is some evidence that hypophysectomy increases the levels of CYP2B1 (31). It is therefore possible that the induction of these enzymes could be either direct or indirect.

Morphine may act not directly on the GH-releasing factor but by a monoaminergic mechanism. Even though dopamine promotes the release of GH (27), it could be speculated that morphine acts by decreasing dopamine levels (27, 32), just as for the morphine induction of prolactin release, which has been ascribed to a decrease in dopamine turnover (33). Several reports indicate that this is possible. Thus, it has been shown that the pituitary stalk plasma dopamine concentration is markedly reduced by morphine (34) and, consistent with this, dopamine has also been shown to enhance the release of somatostatin (35). It is also possible that other neurotransmitters are involved. For example, serotonin, whose release is stimulated by morphine, promotes the release of GH. Because morphine stimulates the turnover and release of serotonin (32), the effects on GH may also be mediated by this monoamine.

Other monoaminergic mechanisms of GH release also need to be considered. Studies on the role of the postsynaptic α_2-adrenergic receptors in the GH-releasing effects of morphine have shown that these receptors are essential for the physiological secretion of GHT. A perturbation of the peripheral effects of morphine by pharmacological manipulation of the α_2-adrenergic receptor system would therefore be anticipated. To test this hypothesis, we coadministered the α_2-adrenoceptor antagonist yohimbine or the corresponding agonist clonidine concomitantly with morphine. As seen in Fig. 3, suppression of the *O*-demethylation of codeine was more pronounced with morphine in combination with yohimbine than with morphine alone ($p < 0.01$). Yohimbine seemed to augment the suppressive effect of morphine on the CYP2C11 and CYP3A proteins, as seen in the Western blots (Fig. 4B). Clonidine in combination with morphine significantly attenuated ($p < 0.01$) the down-regulation of codeine *N*-demethylation produced by morphine alone. These data indicate that α_2-adrenoceptors are involved in the changes observed. Yohimbine, being an α_2-adrenoceptor antagonist, may cause a pharmacological denervation of the α_2-adrenoceptors after chronic administration. Such a denervation is accompanied by an up-regulation of receptor responsiveness (36). This increased responsiveness of the α_2-adrenoceptors may explain the enhanced biochemical effects of the morphine/yohimbine combination.

As a consequence of the down-regulation of α_2-adrenoceptors, a reverse effect of clonidine would be predicted. However, an attenuation of the morphine effects was observed for only some of the effects (Figs. 3 and 4B). Results consistent with down- and up-regulation of α_2-adrenoceptor sensitivity by clonidine and yohimbine, respectively, have been described (36). Taken together, our results support the view that the biochemical modulation of the CYP enzymes by morphine involves or is affected by an α_2-adrenergic mechanism.

There was no consistent similarity in the pattern of effects exerted by morphine and pethidine. This would suggest that the effects observed, particularly the reduction in CYP enzyme expression, are not mediated by the opioid receptors. This assumption is inconsistent with the findings of Meites *et al.* (27) that naloxone causes a fall in serum concentrations of GH and prolactin and that it attenuates the morphine-induced rise in serum GH and prolactin levels when the drugs are given together. However, data by Spiegel *et al.* (37) are supportive of a non-μ_1-opioid receptor mechanism for the morphine effects

on GH. It is still conceivable that an opioid receptor mechanism is operative, if effects on gene expression are concealed by a secondary inducing effect of pethidine on the liver enzymes. Subsequent studies[1] in our laboratory have demonstrated an increase in the serum GH levels by morphine administered for 14 days. Pethidine administered under similar conditions did not elevate the GH concentration. This gives additional support to the contention of a suprapituitary mechanism of action.

In these experiments we tried to administer pethidine at dose levels that would be equianalgesic to the morphine doses used here and in previous reports (2, 4). The pethidine doses used were the highest tolerable doses that did not affect the behavior of or cause neurological symptoms in the rats.

Other drugs have been reported to decrease levels or activities of CYP enzymes. Waxman *et al.* (38) found a marginal or moderate suppression of the male-specific CYP2C11 by some classical inducers, including phenobarbitone and β-naphthoflavone. No effect on the female-specific CYP2C12 was noted. The androstenedione 16α-hydroxylase activity of CYP2C11 was, however, not suppressed by phenobarbitone. This is at variance with our findings of a parallel down-regulation of CYP2C11 and the 16α-hydroxylation of androstenedione. More recently, various cytokines were also reported to down-regulate several CYP enzymes by direct effects on isolated human hepatocytes (39).

The effects of morphine on the metabolism of DHA, codeine, and ethylmorphine seemed to be reduced with time. This may be due to tolerance development, because tolerance towards the GH-releasing effects of morphine has been reported to occur during development of addiction (40). Yarbrough *et al.* (32) also showed that tolerance developed, resulting in an increase in serotonin turnover, when large morphine doses were given chronically. Such tolerance to the effects of pethidine was not observed, again supporting the contention that nonopioid receptor mechanisms are involved in these processes.

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